Optima and Limiting Factors.

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With two Diagrams in the Text.

IN this article it is proposed to subject to critical consideration the conception of the 'optimum' as a primary general relation between physiological processes and the external or internal conditions which affect them.

In treating physiological phenomena, assimilation, respiration, growth, and the like, which have a varying magnitude under varying external conditions of temperature, light, supply of materials, &c., it is customary to speak of three cardinal points, the *minimal* condition below which the phenomenon ceases altogether, the *optimal* condition at which it is exhibited to its highest observed degree, and the *maximal* condition above which it ceases again.

As the maximum temperature for most metabolic processes is very near to the death point, exact location of it is attended with considerable experimental uncertainty and precise data are generally wanting. In practice, attention is usually concentrated upon the optimum of the condition and upon the general form of the middle part of the simple curve, which is usually accepted as a satisfactory graphic expression of the relation between the function and the condition.

In the treatment of the assimilation of carbon dioxide in all text-books we find mention of optima of temperature, of light, and of carbon dioxide-supply for this process. After some years of experimental study of the effect of external conditions upon carbon-assimilation the writer has demonstrated that much of this treatment is quite incorrect, and from this position has passed to the general conviction that there is much that is misleading in that treatment of the effect of an external condition which involves giving definite values to its cardinal points.

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I.

We will at present confine our attention to the condition of temperature, and will begin with certain *a priori* considerations derived from chemical dynamics.

The rate at which all normal chemical change takes place is increased by a heightened temperature condition.

Most reactions *in vitro* take place so quickly that it is impossible to measure their rate, but with all that go slowly in aqueous solution and resemble the processes of the organism, such as the saponification of esters, the inversion of sugar by acids, and others, it has been found that the acceleration produced by increased temperature is about the same. This has been generalized by van't Hoff[1] into the rule that for every rise of 10° C. the rate of reaction is about doubled or trebled.

If this rule of chemical dynamics does not hold good for chemical reactions within the organism it is the duty of the physiologist to attempt, at any rate, to explain the aberration. Now it is interesting to note that this relation has actually been found to hold, as regards medium temperatures, say from 10° C. to 27° C., for quite a number of cases in animal and vegetable organisms so diverse in nature that the law clearly is *primarily* applicable to chemical change in the cell as well as the test-tube. Thus the respiration numbers of Clausen[2], for lupine seedlings and for *Syringa* flowers, show between 0° and 20° C., an increase of two and a half times for a rise of 10° C., the assimilation numbers obtained by Miss Matthaei and the writer for cherry-laurel leaves a coefficient of 2·1, and for sunflower leaves 2·3, while to come to more complex metabolic changes, the times required for spore-formation in *Saccharomyces pastorianus* (Herzog[3]), and for the development of frogs' eggs (as calculated by Cohen from Hertwig's data by Rohde[4]), at different medium temperatures both proceed within the limits of this rule.

As regards the rate of metabolic chemical change in the organism at high temperatures, this law clearly does not express the whole truth. If it did we should expect, with increasing temperature, all vital processes to proceed with ever-increasing velocity till the fatal temperature was reached at which some essential proteid coagulated or some other connexion was dislocated, and the whole metabolic machinery came suddenly to a standstill.

What then does happen as we approach the upper temperature-limit of the working of the organism? An important new factor, the *time-factor*, comes into play.

[1] Vorlesungen ü. theoretische Chemie, 1898, Pt. i, English translation, p. 227.
[2] Landwirtschaftliche Jahrbücher, 1890, Bd. 19, p. 893.
[3] Zeitschrift f. physiologische Chemie, 1903, Bd. 37, p. 396.
[4] Physikalische Chemie der Zelle u. der Gewebe. Leipzig, 1902.

In later years this factor hardly receives the attention that it deserves. Sachs[1], however, clearly pointed out that the higher the temperature the more quickly the fatal effect ensues, and that short exposure to a very high temperature may not kill, when a prolonged exposure to a slightly lower temperature is fatal; see also Pfeffer, Physiology, Section 65.

Miss Matthaei's experiments on Carbon-assimilation[2] show, for a given case, precisely how important this same time-factor is in the relation of a single function to temperatures which are high but not fatal. The facts observed indicate the following three laws:—

(1) At high temperatures (30° C. and above for cherry-laurel) the initial rate of assimilation cannot be maintained, but falls off regularly.

(2) The higher the temperature the more rapid is the rate of falling off.

(3) The falling off at any given temperature is fastest at first and subsequently becomes less rapid.

This falling off makes it experimentally impracticable to attain the highest possible assimilation value at any given temperature. The theoretical initial value can, however, be arrived at indirectly by continuing back the curve of falling values actually attained, taking of course due account of the time that elapsed between the initial heating and the earliest actual estimation. Besides this first method there is also a second method available for arriving at these initial theoretical values, which is based on the law of uniform acceleration of reaction mentioned above. At low temperatures, below 25° C., the assimilation-rate does not fall off appreciably with successive estimations—no time-function is involved—therefore at these temperatures the first actual estimations made give an exact measure of the initial values of the function, and by the relation between them we determine the coefficient of increase of rate for a rise of 10° C. In the case of the cherry-laurel leaf this coefficient is 2·1. We can then obtain the theoretical values for higher temperatures by calculation.

We have now to apply these two methods to the case of assimilation, as to which function alone we have adequate numerical data, and to see how far they indicate similar theoretical initial values.

This is carried out graphically in Diagram I. By the van't Hoff rule, starting from the ascertained values of the assimilation at 9° C., and at 19° C., which were 3·8 mg. CO_2 (*A*), and 8·0 mg. CO_2 (*B*) respectively, giving the coefficient of 2·1, we continue the series, and by interpolation on the calculated curve obtain the initial value for any desired temperature. The values for 30·5° C. (*C*), 37·5° C. (*D*), 40·5° C. (*E*), 45° C. (*F*), 50° C. (*G*), are represented by dots in the interrupted line which forms the calculated hypothetical curve of the initial relation of assimilation to temperature.

[1] Handbuch der Experimental-Physiologie, 1865, p. 52 ff.

[2] Phil. Trans. Roy. Soc., vol. 197 B, p. 85, 1904.

We have now to apply the other method. Prolonged assimilation-estimations were made at high temperatures, the rate of falling off being determined at 30·5° C., at 37·5° C., and at 40·5° C.[1]. In each case the

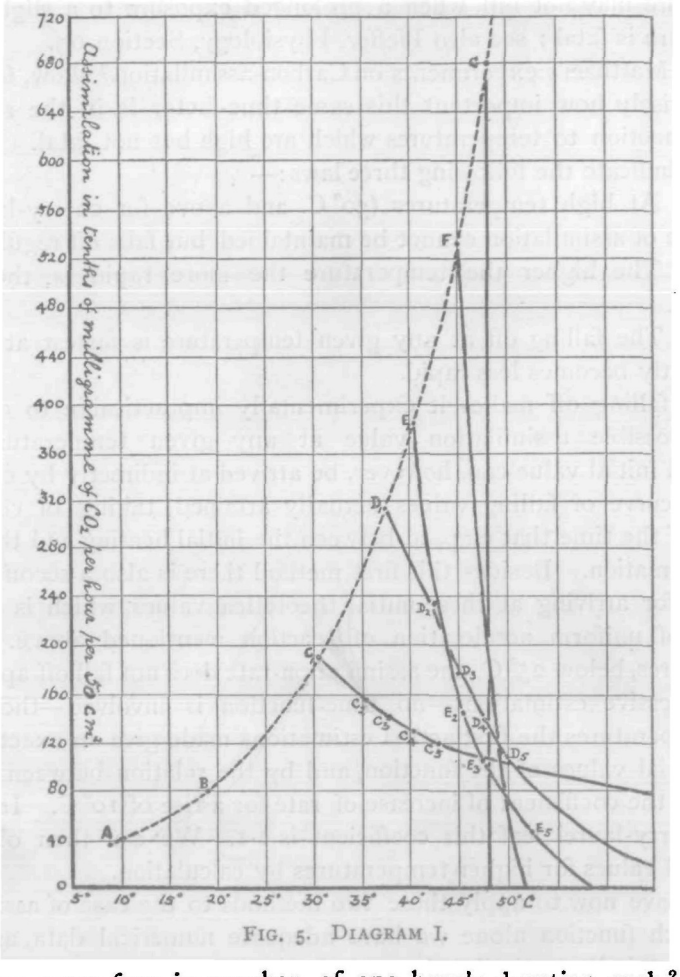

FIG. 5. DIAGRAM I.

estimations were four in number, of one hour's duration each[2], preceded by one and a half hours' preliminary between the initial heating and the beginning of the first estimation. To plot these on the diagram we regard the base line as having only a time significance, each division

[1] See the experiments 58, 59, and 60 in Miss Matthaei's paper.

[2] The numbers obtained for the 'real' assimilation (assimilation corrected for contemporaneous respiration) were as follows, in tenths milligramme CO_2 per hour per 50 cm^2. of leaf-area.

$C_2 = 157$	$D_2 = 237$	$E_2 = 147$
$C_3 = 140$	$D_3 = 176$	$E_3 = 108$
$C_4 = 129$	$D_4 = 139$	$E_4 = 98$
$C_5 = 120$	$D_5 = 109$	$E_5 = 48$

The value for E_4 is clearly out of place, and the curve has not been made to pass through it.

representing two hours, and plot out the falling series of readings obtained at 30·5, 37·5, and 40·5° C. in curves starting from the initial values indicated by method I.

It then becomes at once obvious that the calculated initial value and the observed subsequent values fall into one fairly harmonious curve for each temperature. We thus attain a graphic demonstration that both methods indicate practically identical initial values, and this affords, it seems to me, satisfactory evidence for a preliminary acceptance of the theory that such values actually occur, though it is not possible to measure them.

At low temperatures—up to 25° C.—the assimilation-values keep up to the initial value for a long period, but at these higher temperatures the fall continues and in time brings the assimilation down to zero, and that the more rapidly the higher the temperature. The points at which the respective curves reach zero on the base line are partly outside the limits of the diagram, but the various curves have been continued by freehand beyond the actual estimations in the way that is indicated by other experiments.

Extending the schema that we have arrived at to higher temperatures than 40·5° C., we find at 45° C. a still more rapid fall of the assimilation, for which, however, we have no suitable numerical data, and this declines to zero in a comparatively short time. This is represented in the diagram by the curve starting from *F*.

Finally, to conclude the series we ought to find a temperature at which the earliest estimation that could be actually made would give no measurable assimilation. The lowest temperature to give this result might be called the 'extinction temperature,' and here we should hypothecate that, for the first few seconds after attaining it, each chloroplast would give a higher assimilation rate than at any lower temperature, but that the rate would immediately fall, and that so rapidly that it would become *nil* almost at once (say in 100 seconds, for the accepted specific extinction temperature would of course have to be arbitrarily defined in time-units).

In estimating carbon-assimilation in the presence of over-balancing respiration, it is almost impossible that this temperature should be determined directly, but we are accumulating evidence as to the location of it. I have placed it at 48° C. in the diagram. We thus arrive at a complete theoretical schema of the primary relation between the rate of the photosynthetic process, different temperatures, and time.

Now, without going into details again, it may be stated that for cherry-laurel leaves the process of respiration, although it contrasts powerfully with assimilation as to its metabolic significance, also shows quite the same kind of relation to temperature.

I therefore venture to suggest that, making suitable changes in the

coefficients of temperature and of time, this schema may possibly exhibit the *hypothetical primary relation of all metabolic processes whatever to temperature.*

It has been mentioned that before taking the assimilation estimations at high temperatures a 'preliminary' of one and a half hours was allowed after the initial heating up to the experimental temperature. Hardly any investigators have allowed a shorter preliminary time, so that it is clear that published values, for physiological processes generally, at high temperatures are too low, and special experiments designed to get trustworthy estimations as near the initial effect as possible should give higher values.

Now it is most important to note that not only would the value at each high temperature be thus increased, but that these values would be increased *in different ratios.*

Such special experiments would give values—to take the case of assimilation—that were no higher for temperatures up to 25° C., slightly higher values for 30·5° C., and very much higher for 40·5° C. The important consequence follows that the observed 'optimum' temperature will be raised by such special procedure. Compare, for instance, the two extreme cases on the diagram, in one of which the investigator may be supposed to have taken our set of actual first readings, i. e. those at one and a half hours after heating up, B, C_2, D_2, E_2, and in the other to have allowed four and a half hours' preliminary and so to have obtained our set of fourth readings, B, C_5, D_5, E_5.

The first set[1] will give the 'optimum' at 37·5° C. with a steep falling off to 40·5° C., while the other set will give an optimum at 30·5° C. with a gentle falling off to 37·5° C. A more adventurous investigator whose method would work with only a quarter of an hour preliminary should get an experimental optimum over 40° C., and possibly so close to the extinction point that he would decide that a real optimum was absent. Now it is in this contradictory state that knowledge really stands as regards the relation of *respiration* to temperature. Clausen[2] has recorded a well-marked optimum for a variety of plants at 40° C., while Kreusler[3] finds for *Rubus* no optimum, certainly up to 46° C.

It is not to be supposed that these differences in regard to respiration optima depend on different lengths of *actual* preliminaries, rather no doubt they are to a greater extent dependent on different degrees of high-temperature-impermanence of the function with different plants.

Physico-chemical finality is not to be attained in this matter, but special research might at least show how far the recorded optima for assimilation and respiration are real metabolic truths and how far they are illusions of experimentation.

[1] See numbers in footnote, p. 284. [2] loc. cit. [3] Landwirthschaftliche Jahrbücher, 1887.

A further experimental complication lies in the rate of heating up, which must depend partly on the method and partly on the conductivity of the organ investigated. If heating up is slow, then the falling off produced by passing slowly through the temperatures 40°–44° C. would lower the value obtained for 45° C., to take a concrete example.

The optimum has by some investigators been regarded as the highest temperature which can be permanently sustained without depression of function, but more usually a real optimum is held to be characterized by this, that the retardation produced by exposure to super-optimal temperature must not be of the nature of permanent injury, and that therefore on cooling again to the optimum temperature there must be a return of the function to its highest value.

There has been little attempt to apply this principle experimentally, and it looks as if everything would depend on the *time* of exposure to the super-optimal temperature. Rather than by direct experiment, it is probable that the high transient values will in future have to be estimated by the convergence of the lines of evidence that we have already indicated.

Only respiration and assimilation have been yet mentioned, and they are conditioned by comparatively simple factors, or rather by factors which can be kept under control so that these two processes might be expected to show the primary relation to temperature not greatly masked.

In the case of such a complex process as growth one cannot start analysis with any such expectation.

The available published data as to growth are of very little use for our inquiry. Those that deal with a wide range of temperatures mostly consist of single readings, and these after a long preliminary—in one classical set as long as forty-eight hours (Köppen)[1]; the more detailed studies by Askenasy[2] and Godlewski[3] are concerned only with medium temperatures: True[4] has a couple of not very significant experiments at a 'super-optimal' temperature. It is, however, the universally held opinion that growth exhibits a well-marked optimum in its temperature relation. This optimum is placed by Sachs[5] at 34° C. for seedlings of flowering plants, and in many other cases it is lower, so that the optimum is so far removed from the fatal temperatures as to make it impossible that the phenomenon should be wholly an illusion of experimentation. Were it possible to make critical sets of growth-readings fairly close to the hypothetical initial values, the position of the optimum should be found to be higher than after long preliminaries, but it could hardly be pushed upwards to such an extent as to become uncertain by reason of its nearness to fatal temperatures.

[1] Cf. Pfeffer, Physiology of Plants, vol. ii, p. 77. [2] Ber. d. deut. Bot. Ges., 1890.
[3] Bot. Centralb., Bd. 47. [4] Annals of Botany, vol. ix, 1895. [5] Pfeffer, l. c.

Admitting, then, the existence of a 'real optimum,' not to be broken down by the most ideal experimentation, we have to seek a clue to its causation.

In vitro, a few chemical processes are known which take place *more slowly* as temperature rises, but it is agreed that this abnormality is not a primary effect of the temperature, but that secondary causes are at work.

The rate of hydrolytic action of isolated enzymes, however, always shows a marked optimum temperature effect. Thus Kjeldahl[1] showed that malt-diastase hydrolysed increasing quantities of starch up to about 63°C., after which the action fell off quickly, becoming *nil* at 86°C. The interpretation of this is quite simple, and the diminished effect at super-optimal temperatures is due to an actual destruction of the enzyme by the heat. It has been clearly proved that the destruction is the faster the higher the temperature, and that the apparent optimum is the effect of the two opposed processes at work. If the supply of enzyme could be kept up constantly to counterbalance its destruction, then the true rate of its hydrolytic action at high temperatures could be determined. This result could be also indirectly arrived at by the method adopted for calculating the initial values of assimilation, but to my knowledge neither of these experiments has yet been carried out.

Should not the optimum for growth be interpreted in some similar way? I think we may regard it as certain that it is due to some *secondary* cause working against the causes that have made for increasing growth as the temperature has risen from 0°C., and not due to failure of these causes as a direct effect of the increased temperature.

It is possible that the destruction of an enzyme, or the action of some anti-enzyme, plays a part in causing the 'real optimum' which growth exhibits, but in the absence of any analytical study of the behaviour of growth at high temperatures it is idle to put forward any explanatory hypothesis.

Growth is indeed the finished product of the metabolic loom, and, in order that the weaving of the specific pattern of the individual plant shall continue for a measurable time at the racing speed of metabolism which high temperature enforces, extraordinary co-ordination is required.

It may well be that, soon, co-ordination will fail in some question of supply of material, and it is significant that high temperature does not distort the specific pattern of the plant as do negative or positive extremes of light or of moisture; the machinery slackens and the output is less, but the pattern is the same.

It is therefore easy to conceive that falling off of growth-rate may be due to a variety of causes, and what is really required is a careful investiga-

[1] Compt. rend. Carlsberg Lab., 1879; cf. Czapek, Biochemie der Pflanzen, Bd. 1, p. 345.

tion of the separate factors that are involved in growth to see whether or not in some simple case it is merely the inadequate working of a single one of them that is checking growth. Peradventure food reserves cannot be translocated fast enough, or oxygen cannot quickly penetrate to the deep-lying growing cells.

This analytical attitude brings us naturally to the second part of this study, to the consideration of those influences which I propose to call 'limiting factors.'

II.

We start this section with the following axiom.

When a process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor.

I think one may fairly express surprise at the extent to which this principle has been overlooked by those who have proposed to work out the relation between a function and some *single* one of the various factors that control it.

This desirable end often cannot be really accomplished without taking deliberate thought to the other factors, lest surreptitiously one of *them*, and not the factor under investigation, becomes the real limiting factor to an increase of functional activity.

We will consider in some detail the application of this axiom to assimilation, and briefly its application to respiration and growth.

Carbon assimilation furnishes the most instructive case for the consideration of the inter-relation of conditioning factors, because these factors are largely external ones, whereas in growth they are internal and less under control.

Let us then consider first the case of assimilation. We can recognize five obvious controlling factors in the case of a given chloroplast engaged in photosynthesis.

(1) The amount of CO_2 available,
(2) the amount of H_2O available,
(3) the intensity of available radiant energy,
(4) the amount of chlorophyll present,
(5) the temperature in the chloroplast.

In theory any one of these five might be the limiting factor in the total effect, and it is comparatively easy to experiment with (1), (3), or (5) successively as limiting factors.

Many experimenters have indeed done this without premeditation. The experiments of Reinke[1], in which with increasing light the rate of assimilation (as measured by the bubbling of *Elodea*) suddenly ceased its proportional increase and remained stationary while the light increased yet

[1] Bot. Zeit., 1883.

another tenfold, I interpret as probably a case in which the supply of carbon dioxide was the limiting factor: its limit of arrival by osmosis being once reached no further increase of assimilation was possible.

The experiments of Kreusler[1] on the effect of temperature on the assimilation of a shoot of *Rubus* gave, as higher and higher temperatures were used, at first a steady rise of assimilation up to 15° C., but after this the assimilation practically never rose further. This state of things has been shown by Miss Matthaei[2] to be a case in which inadequate illumination limited the assimilation to that obtained at 15° C., and so further heating produced no increase. There are also contemporary examples of such misinterpretation which will be discussed elsewhere.

When the rate of a function exhibits, in experiment, a sudden transition from rapid increase to a stationary value, it becomes at once probable that a 'limiting factor' has come into play. The form of curve obtained is then like the curve *A B C* in diagram II, where the limiting factor has soon come into play. If the factor in question only becomes 'limiting' when the function is near its high values, then the curve *A B F G* represents the result attained. If the factor only 'limits' when the function is close to its highest values we may get a curve recalling the conventional optimum curve with the top cut off.

The relation of assimilation to intensity of illumination is shortly to be treated elsewhere, but something may be said here with advantage about the relation of assimilation to the supply of carbon dioxide. The willingness to believe in an 'optimum amount of CO_2 for assimilation' is almost universal, and the belief is quite general that Godlewski[3] showed it to be about eight per cent. In my opinion there is no justification whatever for speaking of an optimum at all in this connexion.

Suppose a leaf in a glass chamber to have enough light falling upon it to give energy equal to decomposing 5 c.c. of carbon dioxide per hour. Then, as one gradually increases the carbon dioxide in the air current through the chamber from the amount (or pressure) that causes 1 c.c. to diffuse into the leaf through its stomata up to five times that pressure, so steadily the assimilation will increase from 1 c.c. to fivefold. After that, further increase of the carbon dioxide will produce no augmentation of the assimilation, but will give continually an effect of 5 c.c. of carbon dioxide assimilated—the light being now the limiting factor. The curve obtained will be of the form *A B C*. Ultimately, if the supply of carbon dioxide in the air current be increased up to 30, 50, or 70 per cent., the carbon dioxide will have a general depressing effect on the whole vitality, and before suspension of all function a diminution of assimilation undoubtedly occurs; this is, however, quite a separate process. Now, secondly,

[1] l. c.; see also Pfeffer, Physiology, vol. i, sect. 58. [2] l. c., p. 69.
[3] Arb. bot. Inst. zu Würzburg, Bd. 1, 1873.

suppose the light falling on the leaf to be sufficient for the decomposition of 10 c.c. of carbon dioxide per hour, then twice the external pressure of carbon dioxide will be required to reach the limit and the angle of the curve, which will now be *A B D E*. With still stronger light we should get *A B D F G*. Those who would be prepared to admit that a curve like *A B C* shows an optimum, only with a very long drawn-out top, would have to further admit that for *each intensity of light falling on a leaf there is a different optimum amount of carbon dioxide.* This is not to be entertained.

The light-energy available fixes an upper limit to the carbon dioxide that can be decomposed, and when that amount is attained, which even for direct sunlight could be provided with a current of air containing less than 1 per cent. if the current were sufficiently fast, the limit of effect of carbon

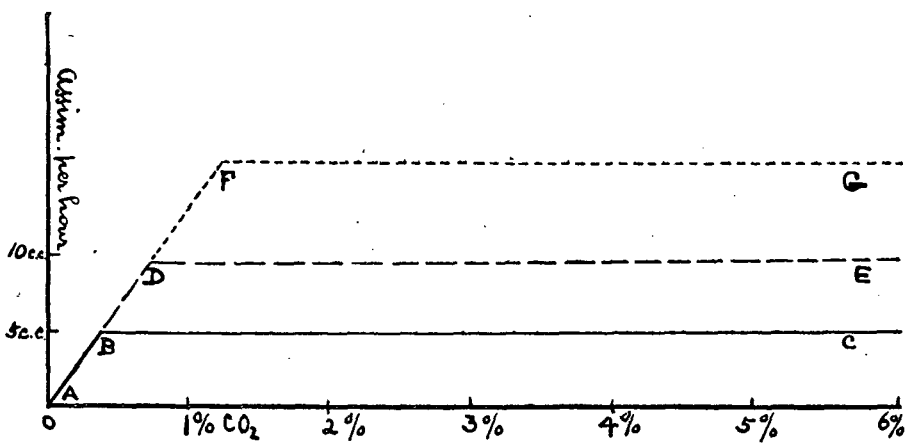

FIG. 6. DIAGRAM II.

dioxide is reached: any more provided is wasted, and has no further effect till many times that concentration is reached and a general depressing effect comes in. Just as little can one speak of an optimum amount of carbon dioxide required to use up a fixed amount of radiant energy (i.e. a given intensity of light) as one can speak seriously of the 'optimum amount' of water required to fill a litre flask, while to attempt to speak of an optimal amount of carbon dioxide for assimilation in general is like speaking of 550 c.c. as the optimal amount of water *to fill flasks*, when the two flasks in question happen to be the one a litre flask and the other a 100 c.c. flask.

With regard to the function of respiration, a factor that sometimes comes markedly into play as a limiting factor is the amount of plastic material available for oxidation, and it is by no means easy to separate analytically the effects of this factor and those of the temperature factor.

Further consideration of this question would, however, involve the quotation of definite examples, and must be postponed.

In the case of growth, besides the more subtle factors, there are two fairly obvious factors which must, each in its turn, and under different conditions, play the part of a limiting factor. These are the temperature and the water supply. To variations in either of these growth is very susceptible, and indeed a vigorous transpiration in dry air has been observed by Frank Darwin[1] to cause actual temporary *loss* of weight in a growing *Cucurbita* fruit, water being drawn off to supply the leaves.

In a quite recent paper by R. H. Lock[2] we meet with what I take to be a most interesting example of the action of limiting factors in growth.

The author made series of measurements of the daily, and sometimes of the hourly, rate of growth of Bamboos growing in the open at Peradeniya, and at the same time noted the temperature and the relative humidity of the air, and the rainfall. The conclusion at which he arrives is that the daily rate of growth is almost entirely a question of the water supply, and that the hourly growth during the day follows very closely the curve of humidity of the air. It is striking that temperature does not appear to come into the causation of the fluctuations at all, and indeed in the fifteen conclusions given temperature is not mentioned. Now during the months of June, July, and August, when the measurements were taken, the temperature was always high, and it is recorded that the extreme range of temperature for these months was between 19° C. and 30° C. Here, then, temperature is favourable to growth, and would presumably permit more growth than actually takes place, so the amount of water attainable is the factor which limits the growth of the bamboo stems. Hence the close correlation between observed growth and water supply.

Now suppose the temperature had been much lower and the water supply not diminished in proportion. Then the temperature would have kept the growth-rate down and would have been the limiting factor, and we should have expected the daily growth to correspond to the fluctuations of temperature, and not to those of water supply, which is now in excess.

I think it abundantly justifies this way of looking at the phenomena when we find that Shibata[3] in 1900, measuring the daily growth-rate of Bamboos growing in the open in Japan, obtained numbers which showed the closest agreement with the fluctuations of temperature and very little relation with the humidity. The temperatures in Japan fluctuated between 11·6° C. and 20·7° C. during the measurements, so that the whole range is quite below the Peradeniya range of temperature and the case entirely fits our hypothesis.

Shibata's measurements are analysed by Lock in his paper, but

[1] Annals of Botany, vol. vii. [2] Annals of R. Botanic Garden, Peradeniya, vol. ii, August, 1904.
[3] Journal Coll. Sci., Tokyo, 1900; cf. Lock, l. c., p. 215.

he has not our clue to harmonize them with his own results. The interpretation here provided harmonizes both sets of observations as examples of the effects of contrasted limiting factors.

Internal factors, such as rate of translocation of plastic material, may no doubt play the part of limiting factors, but clear examples of this correlation are not to hand. One cannot, however, help suspecting that insufficiency of plastic material may play some part in the falling off of growth at high temperatures. As the temperature rises above the 'optimum' for growth, respiration goes on still increasing enormously, and large quantities of carbon are compulsorily lost to the growing plant in this way. Presumably in some cases, possibly quite generally, the insufficient residue of available plastic material in the vigorously respiring part would limit growth.

To take the hypothetical case that translocation could just bring in, per unit-time, enough carbonaceous material for the growth at the optimal temperature *plus* the respiration at the same temperature. Then, as the temperature rose further and the respiration increased *faster and faster*, so necessarily there would be *less and less* carbon material available for growth. The falling curve of growth would become the complement of the rising curve of respiration, and it is of interest that to a large extent, whatever be its significance, the two curves actually have this appearance.

To conclude this section on limiting factors, it seems to me instructive to point out that in the equality of all conditions except one—which is the essence of the 'control-experiment' method of investigation—may lurk a dangerous pitfall if either of the equalized conditions becomes a limiting factor in the result.

Suppose that it were proposed to test the effect upon assimilation of some specific factor that should have an augmenting effect. What more natural than to place two similar leaves side by side under similar medium conditions for assimilation, one subject to this factor and the other not? This would be the typical 'control experiment.' Yet the assimilation of the two leaves might be equally limited by the small amount of carbon dioxide in the air, or, if this were augmented, by the moderate light or by the low temperature; thus equal assimilations might be obtained, and a negative result announced though the specific factor might really show an augmenting effect were another factor not limiting the assimilation.

III.

In conclusion, it is desirable to make some reference to a particular class of agents that complicate the problems of the magnitude of metabolic phenomena which we have been seeking to simplify by analysis.

So far we have spoken of conditions that affect the rapidity of vital chemical processes, whether these be (1) conditions of supply of material or of energy, or (2) tonic conditions that affect only the rate of metabolism. The former act in proportion to the quantity of matter or energy available, but the latter, of which temperature is the type, act by altering the velocity of chemical change. The further class of agents to be just mentioned here is that sometimes known as 'chemical stimuli'—substances of which small traces may produce large alterations of the rate of metabolism, noticeably of the rate of respiration.

This susceptibility to the presence of small traces of unessential substances is so easily manifested that respiration is often justifiably spoken of as exhibiting stimulation effects, and as being controlled as to its magnitude by stimuli as well as by tonic conditions.

Variations of this order would naturally be most disturbing in investigating the effect of tonic conditions, and it is therefore important to have as exact an idea as possible before one of their causation, significance, and possibilities.

It is only within the last two or three years that our conception of the chemical organization of the cell has acquired sufficient solidarity to allow the investigator to face such facts without flinching.

Regarding the cell, as we now may[1], from the metabolic point of view, as a congeries of enzymes, a colloidal honeycomb of katalytic agents, as many in number as there are cell-functions, and each capable of being isolated and made to do its particular work alone *in vitro*, we look for light on the action of chemical stimuli in the cell to their effect on the action of isolated enzymes *in vitro*. Here, too, law and order is now known to reign, and while enzymes only 'accelerate' reactions without being incorporated in their end products, yet the acceleration produced is proportional to the mass of the enzyme present, minute as it is, and the effects of 'activators' and 'paralyzators' of this action are also in proportion to their masses.

Thus all these effects belong to the province of chemical dynamics, and the accelerating effects of ferments and activators upon respiration fall into the same category as the accelerating effect of increase of temperature.

The analytical treatment of metabolic phenomena which is outlined here is then not made any the less certain in its procedure, though it is made more complex by the interaction of those metabolic effects which have been described by their investigators as stimulatory.

These phenomena need not be considered further at present, and the essential quantitative laws of metabolic 'velocity of reaction' may no doubt

[1] For recent views on these points see Fr. Hofmeister, Die chemische Organisation der Zelle, Braunschweig, 1901; G. Bredig, Anorganische Fermente, Leipzig, 1901; F. Czapek, Biochemie der Pflanzen, Zweites Kapitel, 1904; E. F. Armstrong, Proc. Roy. Soc., vol. lxxiii, 1904.

be arrived at without the disturbing effect of introduced 'chemical stimuli.'

On the conclusion of this survey it will be generally conceded, I think, 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. In this latter department of investigation, I think it may be fairly said that at present our science entirely lacks data that will stand critical analysis from the point of view indicated in this article.

Several preliminary analytical investigations in this field are now in progress here.

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