ON BIOLOGICAL OXIDATIONS AS FUNCTION OF TEMPERATURE.

BY W. J. CROZIER.*

(From the Zoological Laboratory, Rutgers University, New Brunswick.)

(Accepted for publication, September 28, 1924.)

I.

The use of the critical thermal increment (μ of Arrhenius, 1889) for the characterization of biological processes whose velocities are a function of temperature has been discussed in several preceding papers (Crozier, 1924-25; Crozier and Federighi, 1924-25, a and b). It was pointed out (Crozier, 1924-25) that in the case of oxidative phenomena critical increments of the orders 11,500 and 16,700 were repeatedly encountered. As reason for the occurrence of both these increments in connection with a single phenomenon (tissue respiration), one on either side of some median temperature (usually found to be near 15°), it was suggested that at least two processes might be concerned in biological oxidations. Thus the virtual velocities of two catenary reactions

\[ A \rightarrow B \rightarrow C \]

\[ k_1 \quad k_2 \]

with velocity coefficients \( k_1 \) and \( k_2 \), and having different temperature characteristics, might be so related that at a certain temperature these actual velocities are dynamically identical, while below that temperature \( A \rightarrow B \) would be the "slow reaction," and above that temperature \( B \rightarrow C \) (or vice versa).¹ In discussions of "temperature

* Research Associate, Carnegie Institution of Washington.

¹ There are a few hints obtainable from reactions in vitro to the effect that this situation may be duplicated in simpler two-stage reactions, but such phenomena have been very incompletely investigated. In certain enzymatic processes this change of \( \mu \) may be shown, as in connection with saccharase (von Euler and Laurin,
coefficients" the rôle of the slowest process of a catenary set as the master process of the whole is sometimes insufficiently appreciated; two simple illustrations of it are given in Figs. 1 and 2. It follows, on this view, that in a catenary or other complex system in which several reactions are involved, the critical increment may be different above and below a certain temperature. It is assumed that the critical increment refers to, and is characteristic of, the formation of active molecules or ions of a catalyst.

This general view-point serves to make it comprehensible, as Rice (1923) has strikingly shown for catalyses by the ions of water, that quite diverse phenomena (provided they have the same catalyst) should yield the same critical thermal increment. The empirical fact is otherwise quite mystifying. It has been known for some time that the same reaction exhibits different temperature coefficients when activated by different catalysts (cf. Slator, 1903; Plotnikow, 1907).

The temperature characteristic, \( \mu \), is independent of the amount of a catalyst (cf. Lamble and Lewis, 1914, etc.). The constant \( \mu \) is subject to slight variations with temperature, but these changes, and those due to ion effects (cf. Rice, 1923; Rice and Kilpatrick, 1923; Rice and Lemkin, 1923) are of so small an order as to interfere but slightly with the analysis of biological systems. The chemical composition of living matter is so nearly uniform, and operates under the control of such efficient regulatory mechanisms, as to be distinctly favorable for the application of the concept of critical increments. On the other hand, the processes measured are bound to be far from simple. It will be 1920); and there possibly is indication of it in the data of Hudson and Paine (1910) upon the destruction of invertase by HCl. For biological systems Kanitz (1915) has suggested that a different relation to temperature may obtain in different portions of the temperature range; but he failed to make precise systematic use of the idea, since he was mainly concerned to justify the "\( Q_{10} \)" rule.

It is not a simple matter to illustrate this rule in detail by examples from general chemistry. Perhaps the nearest approach to a suitable case is found in the stepwise hydrolysis of polyesters. As worked out by Meyer (1909, a and b) and Yamasaki (1920) the relations between the velocity coefficients for the removal of successive symmetrical ester groups bear, however, an integral relation to one another, and they have the same temperature characteristic (which for the action of acid and of alkali are usually those associated with processes respectively catalyzed by \( H^+ \) and \( OH^- \), or with acid hydrolyses).
pointed out that under different conditions one and the same activity may indeed show quite different critical increments. From the standpoint that such a resultant as the frequency of heart beat, for example, is determined by the action of a catenary chain, this finding is not at all unreasonable.

Fig. 1. The four lower curves, derived from the measurements of Loeb and Northrop (1917) with aseptic animals, give the velocity of development and of death for egg + larva, pupa, and imago of Drosophila, and for the total life span; Krafka's (1919–20) figures on ordinary culture flies (not aseptic) show quantitative agreement. In the egg + larva curve two sets of measurements (different symbols) have been brought together by multiplying the velocities in one set by a factor; this has also been done with the two uppermost curves, so that they might be conveniently included in the same figure. In the case of Drosophila temperatures above 30° have a destructive effect. The critical increments for the stages involving growth and differentiation are the same in all cases, and reappear in Krogh's (1914) data on the rate of pupal development in Tenebrio. The increment for the reciprocal of imaginal life duration is different from those involved in the control of embryonic and larval stages; the velocity of the controlling life process is lowest in the case of the imaginal stage, and its critical increment determines that of the total life span.
It would be quite a mistake to suppose that all processes varying regularly with temperature should yield to this method of direct analysis. Two important exceptions may be cited. When an end-result is the expression of an equilibrium condition, the outcome of a balance between opposed or consecutive reactions differently influenced by temperature, then the relation between logarithm of effect and reciprocal of absolute temperature must produce not a straight line but a curve. In some instances these curves may be analyzable, but this need not be gone into here. Very pretty illustrations of the effect are provided by measurements of the activation of sea urchin eggs by acid at different temperatures, and by the relation between temperature and number of eye-facets in Drosophila.

R. S. Lillie (1917) in two series of experiments measured the “optimum” time for exposure of Arbacia ova to acid, at different temperatures, the optimum being defined as that duration of exposure providing the greatest proportion of larvae. The reciprocal of this
exposure time measures the velocity of the underlying parthenogenetic process, at the point where "activating" and "destructive" actions of the acid are (statistically) in a maximum equilibrium. The data (Fig. 3) obviously fall upon intersecting curves. The intersections occur at temperatures known from other studies to be "critical" temperatures for these eggs.

The numerous quantitative observations upon the relation of temperature to eye-facet number in various genetic races of *Drosophila* are equally illuminating. It is obvious that in such cases, since one does not deal with an unhampered velocity, the "temperature coefficients" are quite incapable of providing more than a crude indication that chemical forces are at work, a conclusion self-evident in the first place and which therefore is not especially instructive. The *bar* gene acts as an inhibitor of eye-facet development, the amount of eye reduction being greater at higher temperatures. One must therefore deal, not with the *number* of eye-facets, but with the efficiency of the reduction; this may be measured either as percentage reduction, or better by the reciprocal of the *number of facets*. I have carried out this procedure with the available facet counts in several bar and ultrabar stocks, and in the various F₁ heterozygotes. The figures for the

---

3 Seyster (1919), Krafka (1919–20), Hersh, R. K. (1924), and Hersh, A. H. (1924).
various series fall upon parallel curves, so that the multiplication of
each of the sets of measurements by a suitable factor brings them all
into coincidence (Fig. 4). The points fall upon two intersecting
curves, which cross at near 27°C. An analysis of the latitudes of
variation shows that on either side of 27° these follow a different course,
and confirms the result given in Fig. 4. The fact that temperature
influences so slightly the number of facets in the full-eye races (Hersh,
R. K., 1924) is further proof, if such were needed, that the facet
number is determined by an equilibrium. We may conceive that the

![Graph] Fig. 4. The logarithm of the reciprocal of the number of eye-facets in mutant
races of *Drosophila* containing the bar gene, plotted against reciprocals of absolute
temperatures. The different series of measurements have been brought together
for comparison by multiplying the determinations in each set by a factor. The
different series are:

Nos. 1, 2, Red bar-eye ♀ and ♂ (Seyster, 1919).
" 3, 4, White bar-eye, ♀ and ♂, unselected (Krafka, 1919–20).
" 5, 6, " ♀ and ♂, low-selected (Krafka, 1919–20)
No. 7, Bar, ♀ (Hersh, 1924).
" 8, Ultra-bar, ♀ (Hersh, 1924).
" 9, F₁ ♀ Bar × Ultra-bar (Hersh, 1924).
" 10, F₁ ♀ Full-eye × Ultra-bar (Hersh, 1924).
number of facets is determined by the amount of a formative catalyst, $B$, normally existing at a definite concentration which results in a system of the type

$$A \rightarrow B \rightarrow C,$$

in which $k_1$ and $k_2$ have not very different temperature characteristics. But in the bar-eyed race the amount of this "formative substance" is reduced, owing to the alteration of the reaction $A \rightarrow B$. The most convenient assumption would be that the bar mutant gene produces a negative catalyst for the process $A \rightarrow B$; this would automatically reduce the equilibrium concentration of $B$ and at the same time increase the value of $\mu$ for the reaction $A \rightarrow B$. Change of temperature would thus have a much greater effect than in the wild stock. (It must also be assumed that $A$ is differently affected, by subsidiary processes, on either side of 27°; cf. Fig. 4.) I wish merely to indicate the nature of an explanation for such cases. The relation of ultra-bar to bar (Morgan, Sturtevant, and Bridges, 1924), and the dominance effects on either side of 27°, are apparently susceptible to analysis in this way; but the matter need not be gone into here, beyond stating that the nature of the ultra-bar race as one in which two bar genes are present in a single chromosome is beautifully consistent with the observed similar effects of temperature on the two races. It may be pointed out that the type of equilibrium result seen in the case of the sea urchin eggs treated with acid, where competitive effects of the acid are involved, yields an instructively different sort of picture from that derived in the Drosophila instance, where the balance of consecutive processes is thought to be involved; the curves are, in the first case, convex to the temperature axis; in the second case, concave thereto. Where parallel reactions are involved log $K$ will show positive acceleration with increasing temperature; when the equilibrium concentration of an intermediate compound is measured, it may show negative acceleration as the temperature rises.\(^4\)

\(^4\) The use of this equation will only lead to unnecessary confusion unless its physical meaning is carefully kept in mind. Professor E. N. Harvey has called my attention to the fact that in Feldman’s treatment of the subject (Biomathematics, 1923, p. 236) there occurs a devastating error whereby the van’t Hoff equation for shift of equilibrium constant with change in temperature is identified with the
The present paper is intended to bring together, from the standpoint of critical increments, data on several oxidative processes in animals and plants, and to give a tentative explanation for the values of the critical increments found.

II.

It is desirable for an inquiry of this sort to be in possession of data obtained at short intervals of temperature, and to have the measurements of reaction velocity so numerous that the variation in velocity at constant temperature may itself be investigated. Few available sets of measurements satisfy these requirements, and interpretations may thus be, at times, in error. But even so it is remarkable to what extent the temperature characteristics of diverse respiratory phenomena, and of other processes demonstrably dependent upon oxygen tension (and thus governed by respiration) do show empirical agreement. The method of analysis developed in this connection (cf. Crozier, 1924–25) is strikingly justified by the manner in which the data supplied by independent experimenters, having themselves no thought of this theory, are reducible to systematic and rational order.

Figs. 5, 6, 7, and 8 show the relation to temperature as obtained from published observations upon CO₂ production and O₂ utilization. The resulting values of the critical thermal increments are summarized in Table I. For comparison, data on fermentation by yeasts (Fig. 9), and on photosynthesis, have been included.

equation for change in velocity constant. The Q in the van't Hoff equation, signifying heat of reaction, has nothing directly to do with the quantity μ; one mode of explanation which makes this clear is given by Lewis (1917). This error leads Feldman in a later paragraph (p. 237) into an absurd calculation of the "temperature coefficient" for the hydrolysis of ethyl butyrate on the basis of the heats of combustion of the reactant and resultants; so that not only is heat of reaction confused with heat of activation, but equilibrium constant is regarded as a velocity coefficient!

In the light of the discussion in the text it might be suggested that in some instances where two intersecting lines are drawn in the figures the data might better be fitted by a curve. If this were so one would not expect exact agreement with cases where the whole temperature range is adequately fitted by a single straight line. It will be noticed, however, that the agreements are quantitative.
Fig. 5. Oxygen consumption of *Arbacia eggs* (data of Loeb and Wasteneys, 1911). Measurements from seven sets of experiments (1 to 7) have been brought into juxtaposition by the use of a factor for each set. "Critical points" occur at 14°C. and about 25°C. The somewhat fragmentary data provided by Warburg (1908, 1914) for ova of other echinoids are not inconsistent with the assignment of the increment illustrated in this figure for the normal range of temperatures. The lower "critical point" coincides with the lowest temperature for normal development, but there is evidence sufficient to demonstrate that oxidative phenomena do not directly control the rate of cleavage.

Fig. 6. Upper graphs, O₂ utilization (crosses) and CO₂ production (circles) of starved crayfish (data of Brunow, 1911); lower, O₂ utilization of *Tenebrio pupae* (Krogh, 1914).
Vernon (1897) published numerous determinations of respiratory activity in various animals at different temperatures. The critical increments obtainable from these figures illustrate the diversity of processes which may control oxygen utilization. Considering only those series of measurements not obviously too irregular, the following critical increments are found:

*Helix*, 11,080; *Anguis*, 15,350; *Amblystoma*, 11,080 (below 15°C., 8,000 ±); *Molge*, 11,080 (below 15°C., 8,000 ±); *Rana temporaria*, 24,200 (below 15°C., 8,160); *Rana esculenta*, 8,160; *Bufo vulgaris*, 24,200 (below 15°C., 8,160).

The figures actually do exhibit greater consistency than Krogh (1916) seems to have believed. I call attention merely to the occurrence of increments of the orders 11,100, 16,000, and 24,000, these having been demonstrated in cases already presented; and to the discovery of 15° as again a "critical temperature."
8,000 ± may perhaps be understood as reflecting the velocity of central nervous activity concerned in breathing movements (cf. Crozier and Federighi, 1924–25, b).

Krogh's (1916) observations upon vertebrates, utilized by him for the construction of a "curve of standard metabolism," present a number of difficulties. The individual sets of measurements, pertaining to different species, were "averaged" by Krogh. It can be shown that the "critical temperature" at which a shift occurs from the dominance of one thermal increment to that of another, does not remain exactly constant for the different species; when series of determinations are "averaged," this results in a distortion of the fitted curve. This is most simply illustrated by reference to the case of Tenebrio larvae. Krogh (1916) says that the curve of $O_2$ consumption of these pupae as function of temperature differs fundamentally from the "curve of standard metabolism." Fig. 8 demonstrates that the
TABLE I.

<table>
<thead>
<tr>
<th>Object.</th>
<th>Observer.</th>
<th>Temperature range</th>
<th>Critical temperature (when evident)</th>
<th>Lower temperatures (°C)</th>
<th>Higher temperatures (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Arbacia</em> eggs (O₂ utilization).</td>
<td>Loeb and Wasteneys, 1911.</td>
<td>3 to 31</td>
<td>14, and 25</td>
<td>11,800</td>
<td>16,140</td>
</tr>
<tr>
<td><em>Mytilus</em> gill epithelium (O₂ utilization).</td>
<td>Gray, 1923-24.</td>
<td>1 to 34.5</td>
<td>15</td>
<td>16,700</td>
<td>11,500</td>
</tr>
<tr>
<td>Crayfish, starving (O₂ utilization and CO₂ production).</td>
<td>Brunow, 1911.</td>
<td>7.6 to 21.5</td>
<td>15.2</td>
<td>22,000</td>
<td>16,800</td>
</tr>
<tr>
<td><em>Tenebrio</em> pupae (O₂ utilization).</td>
<td>Krogh, 1914.</td>
<td>10 to 32.5</td>
<td>15.5</td>
<td>(28,000)</td>
<td>16,850</td>
</tr>
<tr>
<td><em>Dicyopus</em> adults (O₂ consumption and CO₂ production).</td>
<td>von Buddenbrock and von Rohr, 1922-23 (3 series).</td>
<td>2.5 to 20.9</td>
<td>16,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Closotricha</em> larva, <em>Anthera</em> larva (O₂ consumption).</td>
<td>von Buddenbrock and von Rohr* 1922.</td>
<td>4.5 to 33.5</td>
<td>17</td>
<td>16,000±</td>
<td></td>
</tr>
<tr>
<td><em>Limulus</em> heart ganglion (CO₂ production).</td>
<td>Garrey, 1920-21.</td>
<td>9.5 to 31</td>
<td>17±</td>
<td>16,300</td>
<td>11,000</td>
</tr>
<tr>
<td>Guinea pig uterus (O₂ utilization).</td>
<td>Evans, 1923.</td>
<td>1.25 to 25.1</td>
<td>16,500</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carp (O₂ utilization).</td>
<td>Lindsted, 1914 (in Krogh, 1916).</td>
<td>9.1 to 29.7</td>
<td>16,000±</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goldfish (O₂ consumption).</td>
<td>Edge and Krogh, 1914.</td>
<td>12.5 to 40</td>
<td>16,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toad (O₂ utilization).</td>
<td>Krogh, 1914.</td>
<td>16,100</td>
<td>16,800</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Winter frog (O₂ utilization).</td>
<td>&quot; 1914.</td>
<td>21,000</td>
<td>21,000</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pisum</em> (CO₂ production).</td>
<td>Kuiljper, 1910.</td>
<td>0 to 26</td>
<td>16,200</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast (CO₂ production).</td>
<td>Slator, 1906.</td>
<td>5 to 40</td>
<td>22.5</td>
<td>22,200</td>
<td>12,250</td>
</tr>
<tr>
<td><em>Utra</em> (CO₂ production).</td>
<td>Osterhout and Haas, 1918-19.</td>
<td>17 to 27</td>
<td>12,400</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pisum</em> (CO₂ assimilation).</td>
<td>Matthaei, 1904.</td>
<td>6 to 37</td>
<td>16.0</td>
<td>12,920</td>
<td>10,290</td>
</tr>
<tr>
<td><em>Utra</em> (CO₂ assimilation).</td>
<td>Osterhout and Haas, 1918-19.</td>
<td>17 to 27</td>
<td>10,300</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Determinations upon a number of other insect larvae and pupae, plotted by von Buddenbrock and von Rohr (1922) are somewhat erratic, but yield results probably not inconsistent with these.
only difference of any moment has to do with the portion of the curve above the critical temperature (cf. with Fig. 6).

Another serious difficulty with these measurements concerns the possible effect of anesthetics and narcotics, used to eliminate muscular movements during the experiments. For toad, and frogs under urethane, Krogh's figures yield \( \mu = 17,000 \); under curare the critical increment seems unquestionably lower (for frogs, \( \mu = 15,000 \); for dogs, 13,780). Knauthe (in Krogh, 1916) gives irregular data on carp under urethane which provide \( \mu = 9,400 \). According to Hill (1909) the velocity of the action of nicotine and of curare on frog muscle have high temperature coefficients; assuming no "break" in the curve of temperature relations, for the former \( \mu = 17,300 \); for the latter, 6,500. Veley and Waller (1910) give similar data for the toxic effect of quinine. It is not a new thought that alkaloids may act as catalysts of oxidative and other processes. In such event we might expect that the "temperature coefficient" for the action of the drug would be incapable of furnishing proof that the drug is "combining chemically" with the protoplasm. The critical increment for the effect of nicotine in producing mantle spasm in squid, from figures published by Moore (1918-19) is about 17,800 (assuming no "break" in the temperature range 14–24°, for which no data are given). If an introduced alkaloid should act as a catalyst of oxidations, we should expect to find the critical increment for these processes lowered; or if, as certain evidence suggests, the drug behaves as a negative catalyst, the temperature characteristic must become higher.

A point possibly significant involves the high increments found in some experiments with frogs (Vernon, 1897) and in Krogh's data in a winter frog (21,000 to 24,000); also, in the higher part of the temperature range, in the case of Brunow's tests with a starving crayfish (Table I). According to Morgulis (1923) laboratory confinement of Panulirus results in the rapid disappearance of glycogen from the blood. It is not difficult to conceive that under these and similar circumstances involving inanition, the limiting conditions for oxidative processes may be profoundly modified. It is tempting to regard these high values of \( \mu \) as due to the controlling influence of hydrolytic reactions which prepare the substrate of oxidations.

Not very much weight can be put, then, upon the "curve of standard metabolism." The examples of respiratory velocity most suitable for
analysis show unmistakably the presence of controlling reactions with critical increments \( \mu = 11,500 \) and 16,800; possibly an increment 16,200 is also to be distinguished.

The fermentative production of CO\(_2\) by yeasts (Slator, 1906) gives \( \mu = 12,250 \) (22.5-40\(^\circ\)) and 22,200 (5-22.5\(^\circ\)); the data are plotted in Fig. 9. It may not be an accident that 12,300 is the critical incre-
Examination of measurements which have been published upon a number of organic activities shows that they provide critical increments very similar to those given for oxidative phenomena. Since it is possible to show in certain instances that an activity in question is a function of oxygen tension, or in other cases may safely be presumed to involve cell respiration, the agreement of the values of \( \mu \) is scarcely to be conceived as accidental.\(^6\)

![Diagram](image)

**Fig. 10.** Martin's (1904) data on heart beat frequency; white circles, heart perfused with Ringer solution; black circles, perfused with Ringer solution plus 0.08 per cent KCl. The process having \( \mu = 16,800 \) is alone materially affected by the KCl.

1. The critical increment for frequency of contraction in the isolated auricle of the rabbit (Clark, 1920-21) is 16,000. Clark and others stress the extreme sensitivity of the auricle to \( O_2 \) deficiency.

2. For a good number of instances of heart rhythm \( \mu = 16,800 \pm \). These I shall consider in a later paper. The spontaneous rhythm of strips from septal and nodal regions of the heart gives \( \mu = 16,410 \) (Moorhouse, 1912-13). One case of

---

\(^6\) Arrhenius (1907, 1912) has commented in a general way upon the similarity of the values of \( \mu \) calculated for very diverse processes, but he offered no explanation.
particular interest is given in a paper by Martin (1904); in his data the value of $\mu$
for heart beat frequency is 16,800, above 22°±; Martin shows that if the potassium
in the perfusing fluid be increased, the heart frequency is proportionately lowered.
The value of $\mu$ is the same for a heart whose frequency is lowered by excess K
(Fig. 10); I have already pointed out (Crozier, 1924-25) that NaCl and KCl may
alter the velocity of toxic action, without disturbing the value of the critical incre-
ment; in the case studied (data of Koltzoff, 1913) the critical increment was also
that associated with respiration (16,800). One other case of toxic action in the
presence of salts (Dernoscheck, 1911) shows that the concentration of NaCl
determines the velocity of death of Daphnia at constant temperature, but the
value of $\mu$ is here 11,000.

3. The frequency of rhythmic movements in strips of rabbit intestine, de-
pendent on oxygen, provides $\mu = 16,150$ between 30° and 40°; from 20°-30°, it is
lower (8,000±); the curves are parallel for different levels of the intestine (data
of Taylor and Alvarez, 1917).

4. Gray (1923-24) shows that ciliary activity and O$_2$ consumption in the gill
epithelium of Mytilus are in constant proportion. I have pointed out that the
critical increments are practically identical (1-15°, 16,700; and 15-34.5°, 11,590;
Crozier, 1924-25).

5. The rate of regeneration of polyps in Tubularia (Moore, 1910) gives $\mu =
16,800$ (14-25°); oxygen is necessary for the regeneration (Loeb, 1891).

6. The velocity of the latent period process in strips of turtle ventricle (Snyder,
1911) yields $\mu = 16,170$ (14-30°) and $\mu = 24,750$ (0-14°).

7. The velocity of the latent period process in the contraction of strips of cat
bladder (Stewart, 1900) gives $\mu = 17,000$.

8. The velocity of conduction of the heart wave in Ascidia atra (Hecht, 1917-18)
gives $\mu = 16,400$.

9. The velocity of the process underlying first order periods in the discharge of
the electric organ of Malapterurus (Koike, 1910) shows $\mu = 16,500$.

10. The velocity with which the action current in frog spinal nerve rises to a
maximum (Gasser and Erlanger, 1922) gives $\mu = 16,180$.

11. The activities of the nerve net in the body of the colonial coelenterate
Renilla (Parker, 1920) and in the foot of the gasteropod Limax (Crozier and Pils,
1923-24) yield $\mu = 11,700$ and 16,100 (Renilla) and $\mu = 10,700$ (Limax).

12. The velocity of the process underlying the first oscillation in the electromyo-
gram of frog gastrocnemius (Judit, 1923) gives $\mu = 11,000$ (2.4-15°) and $\mu =
16,000$ (15-20°).

13. The frequencies of respiratory movements in the aquatic Libellula larva
(Babšk and Rócek, 1909) give $\mu = 11,000$ (above 15°) and 16,400 (below 15°);
and in Dixippus $\mu = 16,800$ (von Buddenbrock and von Rohr, 1922-23) and
in another series of measurements, 11,500; the frequency of the movements is
dependent on the oxygen tension; the rates of breathing movements in the fish
Barbus (Kuijper, 1907) give a value of $\mu$ agreeing fairly well with these (15,000 or
higher).
14. The velocity of the "presentation time" processes for geotropic and phototropic curvatures in *Avena* yield respectively $\mu = 16,300$ and $\mu = 16,500$ (Fig. 11).

15. The velocity of elongation of the radicle of *Pisum*, under certain conditions of experimentation, yields 16,450 (Leitch, 1916; Fig. 13).

It is difficult not to believe that these various processes yielding sensibly identical critical thermal increments involve some common fundamental process, of a (relatively) simple character. The evidence is strong that the basic reaction is a catalyzed respiratory oxidation. Organic activities which show quite different critical increments we may safely assume not to be controlled by the same reaction. The relations of cell division and growth to temperature are in this connection especially interesting. There is but scant indication that oxidative processes of the sort involved in measurements of respira-
Oxidations as Function of Temperature

...tion have anything to do with the control of the velocity of cell division, differentiation, or growth. Loeb (1913) recognized that by the indications afforded by temperature coefficients the velocity of respiratory oxidations was shown not to be the independent variable in determining the velocity of the initial cleavage in Arbacia eggs. For these ova (Loeb and Wasteneys, 1911; Loeb and Chamberlain, 1915) the critical increments for the speed of the first cleavage (Fig. 12) are quite different from those associated with respiration.

For velocity of segmentation in frog ova Krogh's (1914) data yield (below 13.5°) \( \mu = 22,600 \) and (above 13.5°) \( \mu = 10,200 \). This method of inquiry, it may be emphasized, is able to differentiate between "growth" due to different causes. Miss Leitch (1916) measured the early growth of the radicle of Pisum at different temperatures, in one series of experiments extending over periods of 22.5 hours and in a second series extending over periods of \( \frac{1}{2} \) hour. The first series thus included the hours of darkness, and the daily rhythm of cell division in plants would suggest that the limiting condition for the

![Diagram](attachment:image.png)

**Fig. 12.** Velocity of segmentation (first cleavage) in Arbacia ova; black circles, data of Loeb and Wasteneys (1911); white circles, data of Loeb and Chamberlain (1916).
rate of elongation of the radicle would thus be expected to differ in the two cases. One is therefore not surprised to find (Fig. 13) that while the increment 16,450 is associated with the rate of elongation during brief (daytime) periods, the increments 20,300 and 8,170, intersecting at 15°, are clearly demonstrated in three independent sets of measurements based upon elongations achieved during periods of 22.5 hours.

![Graph](image)

**Fig. 13.** The rate of elongation of the radicle of *Pisum*—lowest curve, period of 0.5 hour; upper curves, three series of data from measurements of elongation during period of 22.5 hours (Leitch, 1916).

Thus the critical increments for oxygen utilization and for CO₂ production do not appear to be characteristic for measurements of growth (Figs. 1, 12, 13). Since development must depend upon synthetic processes, it would indeed be very surprising to discover any such relation. Yet some have not hesitated to argue, in the absence of suitable quantitative evidence, that the most rapidly "metabolizing"
(i.e., in practice, *respiring*) portion of an embryo must control and direct the differentiation of the whole (cf. Child, 1915). It is possible that these questions may be best examined by the method of critical increments suggested in the present papers, for the outcome of the inquiry seems to give a means of characterizing protoplasmic processes which involves no destructive interference with the living material.

IV.

According to the theory arising from these studies of critical thermal increments, it is conceived that the velocity of a process such as cell respiration is governed by that of a system of linked reactions which have each a characteristic catalyst. It is consistent with general ideas of protoplasm and its complexity that the velocities of such processes, in the natural state of the organism's adaptation, should be in delicate dynamic equilibrium. The possibility of adaptive response must depend upon this condition. One aspect of this

This conclusion cannot very well be tested by the investigation of enzyme preparations. The natural mechanism for the formation of active enzyme molecules is bound to be interfered with. The mode of preparation seems to be in itself important. It cannot be assumed that in pulped tissues or in tissue extracts the conditions of action are comparable with those in the organism (cf. Rahn, 1915-16). Harris and Creighton (1912, 1915) have published two series of measurements of the velocity of enzymatic reduction (1) of soluble Prussian blue and (2) of oxyhemoglobin (determined spectroscopically). The "reductase" was supplied by pulped liver. I find for these series \( \mu = (1) 9,580 \) and (2) 13,400. It is entirely probable that conditions (of acidity and of "heterogeneity," among others) are introduced in these experiments such as do not obtain in the living protoplasm. There are other similar instances.

The average of Amberson's (1921-22) figures for the oxidation (dehydrogenation) of luciferin by luciferase with production of light, gives \( \mu = \) approximately 25,000; the system is known to be very sensitive to OH ions, and is not affected by KCN (Harvey, 1920); the controlling reaction appears in this case to be an hydrolysis. On the other hand data from Heymans and Moore (1923-24) suggest that the exhaustion of luminescence in slime isolated from the medusa *Pelagia noctiluca* may be due to a process essentially similar to the respiratory oxidations. For two series of determinations in 0.6 M MgSO\(_4\), \( \mu \) is found approximately 10,500 and 16,600; for another series of measurements, in 0.9 M NH\(_4\)OH, \( \mu \) is about 15,500; the data are irregular. The further investigation of bioluminescence from the standpoint of critical increments might permit a decision as to the chemical similarity of the mechanism of light production in different animals.
dynamic balance is believed to be revealed by the occurrence of unmistakable shifts in the nature of the relationship between temperature and velocity, in the neighborhood of a certain (significantly "normal"?) temperature. This frequently occurs at about 15°.

To what extent and in what way this temperature is chemically an "inevitable" critical temperature for protoplasm cannot be decided until poikilothermous forms naturally adjusted to very warm and to very cold environments have been more fully investigated. The very general occurrence of this critical temperature, in tissues of invertebrates, frog, mammals (e.g. in connection with the spontaneous rhythm of the excised intestine of rabbit and cat (Magnus, 1904)) encourages the notion of its association with some general property of protoplasm—though why 15° should be a critical temperature for objects so far removed as the rabbit intestine and the egg of the clam Cummingia (cf. Heilbrunn, 1924), or the sweet pea, is hard to explain.

In connection with the occurrence of two critical increments for a single activity it should be pointed out that in a number of instances the lesser increment is associated with the higher zone of temperature. This fact cannot be used theoretically, because (as illustrated in the present paper) there are a good number of cases in which the reverse is true.

V.

It should be very desirable to obtain some hint as to the nature of the catalysts concerned. While admittedly having the status of a suggestion, the outcome of inquiry in this direction is distinctly attractive and leads to a consistent provisional interpretation of the velocity of respiration as affected by temperature.

Biological oxidations are now believed to involve quite frequently, if not generally, a chemical mechanism of dehydrogenation (Meyerhof, 1918, 1923, a and b; Hopkins, 1921; Wieland, 1922). The relation between temperature and the time of reduction of methylene blue by bacteria in the presence of Na succinate has recently been measured under controlled conditions by Quastel and Whetham (1924), although no analysis of the result is given by them. The reduction system in this case includes the reaction

\[
\text{Succinic acid} + \text{methylene blue} \rightleftharpoons \text{fumaric acid} + \text{leucomethylene blue}.
\]
This system is thought to have close analogy with the reduction system in fresh muscle and other tissues (Hopkins, 1921), which Hopkins has shown to be of very general occurrence.

I have calculated the velocities of reduction from the determinations plotted by Quastel and Whetham, and the outcome is given in Fig. 14. The critical increment is \( \mu = 16,700 \). So striking an agreement with the values of \( \mu \) already given for tissue respiration is distinctly encouraging. Quastel and Whetham (1924) have further shown that the velocity of reduction at constant temperature is a function of the hydrogen ion concentration. It is of interest that the reduction does not occur at acidities greater than pH 5.0 (or less); according to Rice (1923) the catalytically neutral point for water is pH 5.6. Between pH 6 and about 7.5 I find from their figures that the logarithm of the velocity of reduction is directly proportional to the pH. These two facts are consistent with the view that the veloc-
ity of the reduction is a function both of the hydroxyl ion concentration and of the temperature, and make it possible to suggest why in biological oxidations the increments 11,500 and 16,800 are both found. The assumption is, that in a reduction system of the kind identifiable in many living tissues the velocity of oxidation reduction is determined by two catalytic processes, one of dominant speed throughout in some cases, the other in other cases, while in still different instances the relative velocities of the two processes "intersect" at a certain temperature; the value 11,500 ± is rather definitely associated with hydroxyl ion catalysis (Rice, 1923).#}

A further point concerns the possibility that the values \( \mu = 16,700 \pm \) and \( \mu = 16,100 \pm \) are really distinct. So small a difference (3.6 per cent) is scarcely outside the limit of error of determination, and the chemical environment may be sufficiently different to produce a real variation of this magnitude. Yet the fact must be given due emphasis that for the oxidation of Fe'' to Fe''' (by KClO₃, in acid solution) Noyes and Wason (1897) found \( \mu = 16,180 \). The earlier experiments of Hood (1885) on the oxidation of FeSO₄ yield a value of \( \mu \) concordant with this. The significance of this possibility lies in the rôle of iron as respiratory catalyst. Slator (1903) found for the reaction between chlorine and benzene \( \mu = 16,880 \), when catalyzed by FeCl₃; with other metal ions as catalysts \( \mu \) differed considerably from this. It is of course possible that in different cases two respiratory mechanisms may exist with critical increments respectively 16,100 ± and 16,700 ±. Note that in the respiration of echinoderm eggs (Fig. 5) with which (Warburg, 1914; Warburg and Meyerhof, 1913) the theory of catalysis by iron is intimately connected, \( \mu = 16,140 \); while for the deoxygenation of hemoglobin (by CO) I have calculated from the data of Hartridge and Roughton (1923, b) \( \mu = 16,525 \) (Crozier, 1924–25).# The velocity of oxygenation of hemoglobin (Hartridge and Roughton, 1923, a) I find to yield \( \mu = 25,650 \) (acid

# It is of interest that the oxidation of NaH₂PO₄ by palladium black (Sieverts and Peters, 1916) yields \( \mu = 11,000 \).
# These experiments are important as demonstrating that the method of critical increments is applicable to very rapid reactions.
# The reduction of KMnO₄ by gaseous CO (Just and Kauko, 1913) provides \( \mu = 11,060 \).
solution) and 20,880 (alkaline solution); these increments are not surprising in view of the role of the hydrogen ion (Hartridge and Roughton, 1923, b) in this process.

The fact is remarkable, and important for my argument, that a somewhat exhaustive search through published data has failed to show a single clearly understood reaction, other than those cited, in which \( \mu = 16,000 \) to 17,000. The purely chemical data are much more fragmentary than one could wish, but suffice to show that many oxidative reactions provide \( \mu = \) as low as 5,200. The exceptional case of castor lipase acting on triacetin \( (\mu = 16,700) \) is not well enough understood to disturb the general conclusion. The data of Drushel and Dean (1913) and of Dean (1914) lead to \( \mu = 16,500 \pm \) for the hydrolysis of ethereal salts by HCl, but lower values are obtained from experiments by other workers. The oxidation of iodine, in acid solution, in different processes yields 15,850 or 18,550 (in the dark); the reaction between CHI₃ and acid \( (\mu = 15,400 \pm) \) and the decomposition of trichloroacetic acid \( (\mu = 16,230, \) in the dark), like the first two reactions cited, may be catalyzed by iron (cf. Dushman, 1904; Plotnikow, 1907, 1910–11; Dhar, 1917; Banerji and Dhar,

\[ \text{Fig. 15. The velocity of reduction of methylene blue by bacteria is proportional (pH 6 to 7.25) to a fractional power (0.5 \pm) of the hydrogen ion concentration (from data of Quastel and Whetham, 1924).} \]
1924). (It is doubtful if the actual measurements, for any of these processes, are of more than moderate precision.)

Conceivably, iron is associated as promotor catalyst with the functioning of the sulf-hydryl reduction-oxidation system (Meyerhof, 1918, 1923, a, and b; Hopkins, 1921); perhaps its place may be to some extent taken by other metals (Cu, Mn, Va). The implication of iron in such processes seems definitely established, but the erratic manner in which these oxidations may or may not be inhibited by cyanide is in itself sufficient to suggest that the mechanism is not altogether a simple one (cf. also Baudisch and Welo, 1924). For purposes of orientation in planning further experiments one may be required to choose between two alternative interpretations: either the different critical increments associated with respiration are connected with different processes involved in the formation of active molecules of a respiratory catalyst, or else with different steps in the history of the respiratory substrate (glycogen). Each of these alternatives has certain advantages, and if it is recalled that temperature characteristics akin to those of hydrolytic processes are also encountered (under inanition) it can be assumed that in the general analysis of respiration and of processes dependent thereon both hypotheses may be useful and not mutually exclusive.

SUMMARY.

1. The critical thermal increments are calculated for respiratory processes (O₂ consumption, CO₂ production) in various plants and animals. They are characteristically found to be of two, possibly three, types: \( \mu = 11,500 \), and 16,100 or 16,700. The first is commonly encountered above 15°, the second below that temperature, but these relations may be reversed. (The value of \( \mu \) may be significantly changed in inanition.)

2. For reduction of methylene blue by bacteria, through removal of H from succinic acid, \( \mu = 16,700 \). This process (Quastel and Whetham, 1924) at constant temperature is a function of the hydroxyl ion concentration. The suggestive identity is pointed out of the critical increment for this reduction phenomenon with that deduced for biological respirations in which a dehydrogenation mechanism is supposed to be of widespread occurrence, and in connection with which
Fe very likely has a catalytic rôle. The action of OH' is believed to be revealed in the value \( \mu = 11,500 \), frequently obtained in connection with respiration.

3. A somewhat lower \( \mu (16,140) \) is associated with the oxidation of Fe', and may be compared with (1) that of respiration in sea urchin eggs, for which (Warburg) iron is catalyst, and (2) that for some simple reactions in which Fe is known to serve as catalyst; it is not found for oxidative reactions in which Fe is not involved.

4. The bearing of these findings is discussed in relation to the theory of functional analysis of concurrent catalyzed reactions in protoplasm. It is shown that for a number of activities in which the effects of respiration may safely be assumed, the values of the critical increments are consistent with those determined for processes of respiration.

5. The further development of these views may lead to an extremely important method of identifying controlling reactions in undisturbed living matter.

CITATIONS.

Papers referred to which do not appear in this list are cited in the monograph by Kanitz (1915) or in that by Krogh (1916).

Evans, C. L., 1923, *J. Physiol.*, iviii, 22.
ixxxv, 486; 1915, J. Biol. Chem., xx, 179.
Harvey, E. N., 1920, The nature of animal light, Monographs on experimental
biology, Philadelphia and London.
Hersh, A. H., 1924, J. Exp. Zool., xxxix, 55.
Hersh, R. K., 1924, J. Exp. Zool., xxxix, 43.
Hood, J. J., 1885, Phil. Mag., xx, 323.
Koltzoff, N. K., 1913, Arch. ges. Physiol., clix, 327.
Krogh, A., 1916, The respiratory exchange of animals and man, Monographs on
biochemistry, London.
Loeb, J., 1891, Untersuchungen zur physiologischen Morphologie der Thiere. I,
Wurzburg: 1913, Artificial parthenogenesis and fertilization, Chicago. Loeb,
Northrop, J. H., 1917, J. Biol. Chem., xxxii, 103. Loeb, J., and Wasteneys,
H., 1911, Biochem. Z., xxxvi, 345.
Magnus, R., 1904, Arch. ges. Physiol., cii, 150.
Meyer, J., 1909, a, Z. physik. Chem., lxvi, 81; 1909, b, Z. physik. Chem., lxvii,
257.
Meyerhof, O., 1918, Arch. ges. Physiol., cxxxi, 432; 1923, a, Arch. ges. Physiol.,
cxcix, 531; 1923, b, Arch. ges. Physiol., cc, 1.
Morgan, T. H., Sturtevant, A. H., and Bridges, C. B., 1924, Carnegie Institution of
Murgulis, S., 1923, Carnegie Institution of Washington, Year Book No. 21, 173.
Palladin, W., 1913, Ber. bot. Ges., xxxi, 80.
Rahn, O., 1915–16, Biochem. Z., lxxii, 351.