THE TOXIC ACTION OF COPPER ON NITELLA.

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(Accepted for publication, March 16, 1926.)

I.

A previous investigation (Cook, 1925–26) has brought to light certain facts regarding the effect of copper on Aspergillus niger. In the course of that investigation, where the criterion used was the respiration of the organism, a few subsidiary experiments were performed on the respiration of Nitella. This plant behaved in a manner very similar to Aspergillus. In connection with findings obtained with the respiration method, where the product of a chemical reaction is measured, the question arises whether there is a selective action exercised by the toxic agent on the individual cells. Expressing the situation differently, do the variations in resistance among the cells affect the form of the curve of the toxic effect? There has been considerable controversy among investigators concerning this matter; the reader is referred to the discussion of the question by Brooks (1918). For this particular case it seemed desirable to secure data concerning the effect of copper, using some organism and method which would bring out clearly variations in individual resistance. For this purpose recourse was had to measurements of turgidity with Nitella.

The Nitella used in these experiments was an undetermined species found growing in a brook near Sharon, Massachusetts. (To determine the species it is necessary to obtain the plant in a fruiting condition.) The cells averaged from 1 to 3 inches in length and were uniformly about 1 mm. in diameter. They could be handled easily without injury. The material was kept in tanks of running tap water, and no evidence of deterioration was observed during several months.

In preparation for an experiment three lots of fifty cells each were placed in about 200 cc. of water until needed. Three glass jars were placed on a wire frame in a 10 gallon glass tank filled with water, in such a way that the level of the water in the tank was about 1 inch above the level of the fluid in the jars. By means of tubing run from a hot and a cold water reservoir, and a stirring device,
the temperature of the water in the tank could be varied at will or be kept constant. Thermometers were placed in the jars and in the tank and the temperature could be controlled to within about 0.1°. The reagent, copper chloride, was made up to the desired concentration and placed in the small jars. After the proper temperature had been secured the three lots of fifty cells were placed in the toxic solutions. At regular intervals the cells were lifted out, one by one, and tested. The criterion of injury was the ability of a cell to support its own weight when held by one end. This is a purely arbitrary criterion because the process by which a cell becomes less and less turgid is continuous and not sudden. The stage where a cell will no longer bear its own weight, but will collapse, is only one point on a curve which, if it could be plotted, would represent the entire process. But it is a very distinct and convenient point and may be used with the understanding that it has significance merely as an index of a definite extent of injury.

Experience with many thousand cells has brought to light no correspondence between the size and shape of a cell and readiness with which it loses its rigidity.

In order to analyze the toxic effect of a substance on an organism it is desirable to secure data on three essential aspects of the problem: (1) the course of the toxic action with time, (2) the variation of the toxic effect with the concentration of the reagent, and (3) the variation with change of temperature. These three phases will be considered separately.

II.

There are three methods of plotting a curve of toxic action. The first is the so called survivor curve, where the number or the per cent of cells surviving is plotted against time. The second is the death curve, in which the number or the per cent of cells which have died (i.e. lost their turgidity) is plotted against time. The third is the mortality curve where the rate of dying is the ordinate. In Fig. 1 one set of data is plotted in these three ways. If $y$ represents the per cent surviving, the curve of the second type will be the reverse of that of the first type ($100 - y$ serving as ordinate instead of $y$), while the ordinate of the third type will be the first derivative of the equation of type 1 or 2. Any of these curves may be used according to circumstances.

A typical survivor curve is shown in Fig. 1, Curve A. It is evident by inspection that this is a curve of the sigmoid class; that is, of the sort expected from the assumption that the individual cells vary at random in their resistance to the action of the lethal agent.
this is true remains in the realm of conjecture unless, as in cases of hemolysis or similar phenomena, the cells may be observed individually. In the present instance there is no doubt that there is a wide variation in resistance. For example, in the experiment shown in Fig. 1, Curve A the first cell collapsed let us say at 10 minutes while the last remained turgid for 4 hours. The curve then does not represent the course of a reaction but is a statistical expression. It may be used as such, and two or more such statistical curves may be compared. For although each single curve expresses the individual

![Figure 1](image-url)

**Fig. 1.** Effect of copper chloride, 0.001 M, on *Nitella*. In Curve A the ordinate is the per cent surviving, in Curve B it is the per cent dead, and in Curve C it is the rate of mortality.

In order to analyze a curve or compare it with others we should know its equation. In the present case it is not feasible to try to deduce the equation. Consequently it is necessary to find some arbitrary and empirical equation which will fit the experimental data.

Many curves of the sigmoid type have been encountered in biological work, as for example those discussed by Brooks (1918), Fulmer and Buchanan (1923), Lotka (1923), Reed and Holland (1919), Ross
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(1911), Robertson (1907, 1908), etc. Equations of various sorts have been applied to these almost all of them involving an exponential function. Although the fit is not absolute, the survivor curves obtained with Nitella are best fitted by an equation in this form:

\[ y = ae^{-pt^n} \]

where \( y \) represents per cent surviving, \( t \) the time, \( a \) is a proportionality constant, and \( p \) and \( n \) are arbitrary constants. Fig. 2 shows several curves obtained with different concentrations of copper chloride and points representing equations of the above type. It will be observed

![Curves showing the effect of various concentrations of copper chloride on Nitella. The concentrations are as follows:](image)

<table>
<thead>
<tr>
<th>Curve</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.1 M</td>
</tr>
<tr>
<td>II</td>
<td>0.01 M</td>
</tr>
<tr>
<td>III</td>
<td>0.001 M</td>
</tr>
<tr>
<td>IV</td>
<td>0.0001 M</td>
</tr>
<tr>
<td>V</td>
<td>0.00001 M</td>
</tr>
</tbody>
</table>

For the equations of these curves see Table I. The roman numerals of the curves correspond to those in the table.
that although the correspondence is not absolute, it is sufficiently close to justify the use of the equation for the comparison of the curves.

The time curve of the effect of copper chloride on *Nitella*, then, is of the sigmoid survivor type and can be represented by an empirical equation with a fair degree of accuracy.

![Graph showing the relation between concentration and time to reach a given point on the survivor curves. Curve A represents time to reach 40 per cent surviving and Curve B, 60 per cent.](attachment:fig3.png)

**Fig. 3.** Relation between concentration and time to reach a given point on the survivor curves. Curve *A* represents time to reach 40 per cent surviving and Curve *B*, 60 per cent.

### III.

Fig. 2 shows the curves obtained with five concentrations of copper chloride from 0.00001 M to 0.1 M, at constant temperature. There is considerable change in toxic effect with change of concentration. In order to determine the quantitative relations several methods may be used.
1. The simplest procedure is to select some point on the ordinate and plot the logarithm of the length of time necessary to reach this point against the logarithm of the concentration. In Fig. 3 the points chosen are 60 per cent and 40 per cent. Although the points on the concentration curve are irregular, nevertheless they fall approximately on a straight line. There is, therefore, a relation between concentration and toxicity which may be expressed as:

$$\log t = -n \log C + \log a, \quad \text{or,} \quad t = aC^{-n}.$$  

This relation is one commonly encountered in studies of toxicity and has been discussed in a previous paper (Cook, 1925-26).

![Graph showing the relationship between concentration and the maximum tangent to the death curves.](image)

**Fig. 4.** Relation between concentration and the maximum tangent to the death curves.

2. Instead of considering the length of time necessary for a given percentage of the cells to collapse, it is possible to consider the rate of mortality at the moment of most rapid killing. The slope of the survivor curve being an expression of the rate, we may find the tangent to the curve at the steepest point. For this purpose it is better to use a curve of the type of Fig. 1, Curve $B$—the death curve—the reason being that while the value of the tangent will be the same in either type $A$ or $B$, in type $B$ it is positive whereas in type $A$ it is negative. If this value
of the tangent to the steepest point of the experimental curves is obtained by means of suitable instruments, and the logarithm of the tangent is plotted against the logarithm of the concentration, Fig. 4 is the result. Like the preceding curve, it is linear and indicates the following relation:

\[
\log \tan \gamma = n \log C + \log a, \quad \text{or} \quad \tan \gamma = aC^n,
\]

where \( \tan \gamma \) is the tangent to the curve at its steepest point, or in other words is the maximum tangent to the curve. But \( \tan \gamma \) is equivalent to \( \frac{dy}{dt} \) (at its maximum) in an equation relating \( y \) to \( t \), and simply states that the maximum rate of toxic action is proportional to some power of the concentration.

3. Instead of the actual experimental curves the equations of the curves may be used, and here again the concentration may be related to the time in which a given amount of activity occurs or to the maximum rate of the action. The most convenient point to select for the time value is that time necessary for the maximum rate to be reached, or the point on the curve at which the tangent has its maximum value. (It will be noted in passing that this point of greatest activity falls in all the curves at approximately the same distance along the ordinate from the intersection with the abscissa. Using the values of \( y \), it falls at about 50 per cent, or at the time when half the cells have been killed.)

Consider the equation \( y = ae^{-\theta t} \). This is based on the curve of type A, the survivor curve. In order to keep the sign positive it is better to use type B, where

\[
y = 100 - ae^{-\theta t}.
\]

When the rate is at a maximum \( \frac{dy}{dt} \) will be at a maximum and \( \frac{d^2y}{dt^2} \) will equal zero.

But

\[
\frac{d^2y}{dt^2} = \theta a e^{-(n-1)t} \theta e^{-(n-1)t}.
\]

Simplifying, we have

\[
\frac{d^2y}{dt^2} = \theta e^{(n-1)t} - (n-1)e^{(n-1)t}.
\]

When \( \frac{dy}{dt} \) is at a maximum and \( \frac{d^2y}{dt^2} = 0 \), then

\[
\theta e^{(n-1)t} = (n-1)e^{(n-1)t}.
\]

Solving, \( t = \sqrt{\frac{n-1}{\theta}} \).
For any equation the constants $n$ and $p$ are determined and we may solve for $t$. This gives the length of time necessary for the tangent to attain its greatest value, or for the lethal action to be at its maximum. To obtain an expression for the amount of action this value of $t$ may be substituted in (2) and the result will be the value of $\frac{dy}{dt}$ which is the equivalent of $\tan y$ (at its maximum) in the experimental curves. Table I summarizes these values.

Fig. 5 shows the logarithm of the concentration plotted against the logarithm of $t$ and $\frac{dy}{dt}$. In both instances the curves are linear. Therefore

$$\log t = -n \log C + \log a \quad \text{or} \quad t = a C^{-n}$$

and

$$\log \frac{dy}{dt} \text{ (max.)} = n \log C + \log a \quad \text{or} \quad \frac{dy}{dt} \text{ (max.)} = a C^n.$$  

<table>
<thead>
<tr>
<th>Concentration.</th>
<th>$y$</th>
<th>$\frac{dy}{dt}$</th>
<th>$\frac{dy}{dt}$ (max.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M</td>
<td>100</td>
<td>0.303</td>
<td>223.8</td>
</tr>
<tr>
<td>0.01 M</td>
<td>100 (1 - $e^{-2.32}$)</td>
<td>0.7051</td>
<td>117</td>
</tr>
<tr>
<td>0.001 M</td>
<td>100 (1 - $e^{-0.032}$)</td>
<td>1.699</td>
<td>83.3</td>
</tr>
<tr>
<td>0.0001 M</td>
<td>100 (1 - $e^{-0.0032}$)</td>
<td>1.889</td>
<td>58.3</td>
</tr>
<tr>
<td>0.00001 M</td>
<td>100 (1 - $e^{-0.00032}$)</td>
<td>5.655</td>
<td>30.2</td>
</tr>
</tbody>
</table>

The exponent $n$ or $-n$ has nearly the same value in all these curves.

In Fig. 3. Curve $A$ $-n = 0.336$

" 3. " $B$ $-n = 0.332$

" 4. $n = 0.340$

" 5. " $A$ $-n = 0.325$

" 5. " $B$ $n = 0.222$

The uniformity with which the value $0.325 - 0.340$ occurs is significant evidence that we are dealing with one and the same process in the cell whether we consider it from the point of view of time or of intensity of toxic action, by means of the experimental curves or by means of empirical equations.

Any one of these methods of comparison will lead to the same result. The conclusion is therefore justified that the toxic effect of the copper chloride varies as a constant, fractional, power of its concentration.
This relation is of importance because it is analogous to that which holds in the case of respiration (*Aspergillus niger*; Cook, 1925–26) and demonstrates that although the time curves obtained with the two criteria are very different, yet in some fundamental way the toxic action of the metal is the same with both organisms. Just how fundamental is the similarity is evidenced by the fact that the power relation is found very frequently in other work on toxicity.

**FIG. 5.** Relation between concentration and the equations representing the death curves. In Curve A, log C is plotted against the logarithm of \( \frac{dy}{dt} \) at its maximum and in Curve B, log C is plotted against the logarithm of the time for \( \frac{dy}{dt} \) to reach its maximum.
When the temperature instead of the concentration is varied, we find that with increase in temperature the time necessary to kill a constant percentage of the cells becomes shorter. In Fig. 6 the logarithm of the reciprocal of this time is plotted against the reciprocal of the absolute temperature. The result is a series of intersecting curves. The reciprocal of the time necessary to reach a definite point

![Graph showing the effect of varying the temperature.](image)

**Fig. 6.** The effect of varying the temperature. The reciprocal of the absolute temperature is plotted against $\log \frac{1000}{t}$ where, in the upper curve, $t$ is the time necessary to reach 60 per cent surviving and, in the lower curve, 40 per cent.

in the toxic action is equivalent to the activity of the toxic agent at a given temperature.

It will be observed that the slope of the curves falls off with rising temperature till about 32°, above which there is little change in toxicity with temperature (if any it is negative) up to 39°. Then the points rise rapidly, and in a linear fashion. This rise doubtless is due to the physical effects of the heat, such as coagulation of proteins etc. Evi-
idence in favor of this supposition is the high value of \( \mu \) (about 40,000). In the lower range of temperatures the points of intersection (or cusps) fall quite definitely at 16°, 27°, and 32°. In the vicinity of 23–24° there is another break, but owing to the irregularity of the data it appears to fall at 23° in one case and at 24° in the other. In general breaks are known to occur quite frequently at these temperatures. In a recent paper Crozier (1925–26) has assembled data which show that the points where breaks occur most often include 15°, 27°, and 30°. Cases are also known where a break occurs at 23°.

The formula for the effect of temperature on a chemical reaction as found by Arrhenius is:

\[
\frac{K_2}{K_1} = e^{\frac{\mu}{T_1} - \frac{\mu}{T_2}},
\]

where \( K_1 \) and \( K_2 \) are velocity constants, \( T_1 \) and \( T_2 \) the corresponding absolute temperatures, and \( \mu \) is a constant which is characteristic of the system and ideally represents the heat of activation. When the logarithm of \( K \) is plotted against \( 1/T \) abs. (and here \( \log \frac{1}{T} \) is equivalent to \( \log K \)) one or more straight lines are usually obtained. Crozier (1924–25) has recently plotted the data for a great many biological activities and has found that there are often two or more such lines which intersect at rather definite points on the temperature scale (e.g., 15–16°). For a fuller discussion of the situation, reference should be made to his papers. Usually straight lines are obtained. Lillie, however, has obtained data with sea urchin eggs in which the points for parthenogenesis with acid fall along curved lines similar to those here obtained with copper.

The question has been raised whether the so called straight lines are in fact geometrically straight or are curved so slightly that the curvature cannot be detected by ordinary methods. If this is true, and all temperature plots are really curves, then between the usual cases and the one here recorded there is a difference not in principle but only in degree. But for present purposes it is not necessary to decide this question. Since the curvature of the ordinary lines is

\[1 \text{ Quoted by Crozier (1924–25), p. 193. Crozier has plotted the data published by Lillie.}\]
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so slight as to defy detection they may be considered straight, and the
effect of copper on Nitella may be held an exception to the general
rule. On this basis the action of copper must involve some process
or state which is not present in the normal vital processes. It is
worth while to try to gain some conception of the situation underlying
these temperature effects by setting up a hypothetical normal system
and by assuming an appropriate mode of action of the copper. There
would be no proof that such a hypothetical system actually exists.
It would simply be shown that if such a system were present
the effect of temperature variation would be such as that observed
experimentally.

Let us begin by assuming that any vital activity (A) which may be
under consideration depends on a reaction or a series of reactions hav-
ing a velocity $M_1$. There is also a reaction in the reverse sense with
a velocity $M_2$. The exact nature of these reactions is immaterial.
The essential point is that the intensity or rate of the activity, A,
depends on the net velocity of the forward reaction. The reverse
reaction nullifies the effect of the forward reaction and therefore the
net intensity of A which is observed bears a definite ratio to the net
velocity of the forward reaction. The net velocity is the velocity
$M_1$ minus the velocity of the reverse reaction, $M_2$. If $M_1$ is equal
to, or less than, $M_2$ there will be no observed activity. Hence $M_1$
must always be the larger.

Now suppose that the value of $M_1$ depends on a catalyst B while
that of $M_2$ depends on another catalyst Y. Then if B and Y vary,
$M_1$ and $M_2$ will vary, and A will vary correspondingly. Since this
system represents a very general case and is not intended to fit any
particular instance it is made as simple as possible, but with the under-
standing that it might be extended considerably as occasion might
require.

In varying B and Y it is assumed that each is the intermediate
product of a series of consecutive reactions, thus:

$$P \xrightarrow{K_1} B \xrightarrow{K_2} C \quad \text{and} \quad P \xrightarrow{K_3} Y \xrightarrow{K_4} Z.$$ 

If $P$ is very great in quantity with respect to $B$, $C$, $Y$, and $Z$, then the
quantity of $B$ and $Y$ will depend on the values of the velocity con-
stants $K_1$, $K_2$, $K_3$, and $K_4$. Thus if $K_1$ is increased while $K_2$ remains constant, $B$ will be formed more rapidly until the concentrations come to equilibrium in accordance with the equation $PK_1 = BK_2$. The same holds true for $PK_3 = YK_4$. Similarly, if $K_2$ or $K_4$ is increased $B$ and $Y$ will decrease.

What, now, will be the effect of varying the temperature? In each step of these reactions the velocity constant will be determined by the critical thermal increment ($\mu$) of that step. For instance let us assume that at 0°C, $K_1$, $K_2$, $K_3$, and $K_4$ each equals unity. Then if $P$ is indefinitely large it will remain practically constant throughout the temperature range here considered (0° to 50°). It may therefore be disregarded numerically and the equations may be reduced to:

$$B = \frac{K_1}{K_1} \quad \text{and} \quad Y = \frac{K_3}{K_4}.$$ 

Then, since at 0° all the constants equal unity, $B = Y = 1$, $B - Y = 0$, and $M_1 - M_2 = 0$. This indicates that the observed vital activity, $A$, ceases at 0°. Certainly most activities cease at that temperature, and if they continue the rate must be exceedingly slow.

As the temperature rises the ratios $\frac{K_1}{K_2}$ and $\frac{K_3}{K_4}$ will no longer equal unity since the critical thermal increments are not the same for each. If, in any one instance, we take 10 degree intervals then the formula of Arrhenius may be written:

$$\frac{K_2}{K_1} = e^{\frac{\mu}{10}}$$

The left-hand term, $\frac{K_2}{K_1}$, is often expressed as $Q_{10}$, and represents the increase in velocity for a rise of 10 degrees. $Q_{10}$ will itself vary depending on the absolute temperature, and if at 0° the velocity constants equal unity, their values for other temperatures will be as shown in Table II.
Now assume the following two sets of values for the critical thermal increments:

<table>
<thead>
<tr>
<th></th>
<th>I.</th>
<th>II.</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>$\mu = 6,300$</td>
<td>$\mu = 14,000$</td>
</tr>
<tr>
<td>$K_2$</td>
<td>$\mu = 1,540$</td>
<td>$\mu = 6,300$</td>
</tr>
<tr>
<td>$K_3$</td>
<td>$\mu = 14,000$</td>
<td>$\mu = 22,000$</td>
</tr>
<tr>
<td>$K_4$</td>
<td>$\mu = 1,540$</td>
<td>$\mu = 6,300$</td>
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</table>

By means of the equations $B = \frac{K_1}{K_2}$ and $Y = \frac{K_2}{K_4}$ the values of $B$ and $Y$ may now be calculated for 10 degree intervals starting with 0°.

### Table II.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$\mu = 1,540$</th>
<th>$\mu = 6,300$</th>
<th>$\mu = 14,000$</th>
<th>$\mu = 22,000$</th>
<th>$\mu = 28,000$</th>
<th>$\mu = 34,000$</th>
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<tbody>
<tr>
<td>0-10</td>
<td>1.104</td>
<td>1.503</td>
<td>2.47</td>
<td>4.16</td>
<td>6.12</td>
<td>8.91</td>
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<td>10-20</td>
<td>1.097</td>
<td>1.462</td>
<td>2.32</td>
<td>3.76</td>
<td>5.41</td>
<td>7.76</td>
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<td>20-30</td>
<td>1.091</td>
<td>1.424</td>
<td>2.20</td>
<td>3.45</td>
<td>4.86</td>
<td>6.78</td>
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<td>30-40</td>
<td>1.085</td>
<td>1.397</td>
<td>2.10</td>
<td>3.22</td>
<td>4.43</td>
<td>6.11</td>
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<tr>
<td>40-50</td>
<td>1.078</td>
<td>1.350</td>
<td>1.98</td>
<td>2.96</td>
<td>3.95</td>
<td>5.31</td>
</tr>
</tbody>
</table>

### Table III.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>$\mu = 1,540$</th>
<th>$\mu = 6,300$</th>
<th>$\mu = 14,000$</th>
<th>$\mu = 22,000$</th>
<th>$\mu = 28,000$</th>
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<td>1.503</td>
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<tr>
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<td>26.5</td>
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<td>2563</td>
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<td>50</td>
<td>1.53</td>
<td>5.91</td>
<td>52.5</td>
<td>514.2</td>
<td>2812</td>
<td>15174</td>
</tr>
</tbody>
</table>

They are shown in Table III. (The values for 15°, 25°, and 35°, printed in italics, are obtained by plotting the curves of $\frac{K_1}{K_2}$ etc. and interpolating. They are of course less accurate than the calculated values.)

By subtracting $Y$ from $B$ the values of the net velocity $M_1$, or the rate of the observed activity, may be obtained. These values are
also given in Table III. If the logarithm of \((B - Y)\) is plotted against the reciprocal of the absolute temperature Fig. 7, Curves I and II, is the result. It will be seen that there are two intersecting lines. It cannot be maintained that the lines are geometrically straight, but they are as straight as those obtained from experimental data which is as far as it is necessary to carry the analogy. Since \(B - Y = 0\) at 0° it is obvious that at the extreme right the line must be curved, but it is also evident that if any observed vital process ceases at 0° the line representing it, also, must curve as it approaches 0°. Only the range from 10° to 50° need be considered here.

**TABLE III.**

<table>
<thead>
<tr>
<th>Temperature °C</th>
<th>I. B</th>
<th>Y</th>
<th>B - Y</th>
<th>II. B</th>
<th>Y</th>
<th>B - Y</th>
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</thead>
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<td>0.000</td>
<td>1.000</td>
<td>1.000</td>
<td>0.000</td>
</tr>
<tr>
<td>10</td>
<td>2.245</td>
<td>1.363</td>
<td>0.882</td>
<td>2.773</td>
<td>1.646</td>
<td>1.127</td>
</tr>
<tr>
<td>15</td>
<td>3.1</td>
<td>1.55</td>
<td>1.55</td>
<td>4.5</td>
<td>2.05</td>
<td>2.48</td>
</tr>
<tr>
<td>20</td>
<td>4.735</td>
<td>1.733</td>
<td>3.002</td>
<td>7.109</td>
<td>2.604</td>
<td>4.505</td>
</tr>
<tr>
<td>25</td>
<td>6.7</td>
<td>2.0</td>
<td>4.7</td>
<td>11.0</td>
<td>3.25</td>
<td>7.75</td>
</tr>
<tr>
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<td>9.618</td>
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<td>7.230</td>
<td>17.23</td>
<td>4.02</td>
<td>13.21</td>
</tr>
<tr>
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<td>13.3</td>
<td>2.7</td>
<td>10.6</td>
<td>26</td>
<td>5</td>
<td>21</td>
</tr>
<tr>
<td>40</td>
<td>18.66</td>
<td>3.084</td>
<td>15.58</td>
<td>39.65</td>
<td>6.05</td>
<td>33.6</td>
</tr>
<tr>
<td>50</td>
<td>34.31</td>
<td>3.863</td>
<td>30.45</td>
<td>87.1</td>
<td>9.5</td>
<td>76.6</td>
</tr>
</tbody>
</table>

A system has thus been set up which may be diagramed as follows:

```
  B ----> C
     |    K2
    K1 ----> P
       |    M1
      P -----> M1
         |    M1
       Y ----> Z
         |    K4
```

We have next to enquire how, by the introduction of a toxic agent, the straight lines may be converted into curves. For the sake of convenience we will use copper, although many other substances might have the same effect. The suggestion has been made in a previous
paper (Cook, 1925-26) that copper is activated in the cell by entering into a reversible combination with a cell constituent such as $T$. This reaction has the form $\text{Cu} + T \rightleftharpoons \text{CuT}$. In considering the effect of temperature we may assume that at constant temperature the concentration of $T$ is constant at, say, 1, while that of the copper is so much greater that it remains practically unchanged when $T$ varies.

The reaction may then be treated as an equilibrium between $T$ and $\text{CuT}$. If there is another substance, $V$, with which the copper combines, and which is present in the same concentration as $T$, there will

---

**Fig. 7.** Theoretical temperature curves using the data for $(B - V)$ as given in Table III. The roman numerals correspond with those in the table. The bracketed points are those obtained by interpolation in the calculated curves $K_1$, etc.
be two forms of activated copper, $\text{CuT}$ and $\text{CuV}$. The amount present of each will depend on the velocity constants of the respective forward and reverse reactions:

$$\frac{K_5}{K_6} \text{CuT} \rightleftharpoons \text{CuT} \quad \text{and} \quad \frac{K_7}{K_8} \text{CuV} \rightleftharpoons \text{CuV}$$

But $T$ and $V$ are limited. The amount of each, whether by itself or in combination, cannot, let us say, exceed 1.

### Table IV.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
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<td>1.000</td>
<td>0.500</td>
<td>0.001</td>
<td>0.250</td>
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<td>0.314</td>
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<td>0.017</td>
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<td>2.00</td>
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<td>0.130</td>
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<td>0.261</td>
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<td>0.712</td>
<td>0.562</td>
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<tr>
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<td>3.084</td>
<td>0.196</td>
<td>0.669</td>
<td>0.772</td>
<td>0.924</td>
<td>0.752</td>
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<tr>
<td>50</td>
<td>5.47</td>
<td>3.86</td>
<td>0.154</td>
<td>0.908</td>
<td>1.23</td>
<td>1.40</td>
<td>1.21</td>
</tr>
</tbody>
</table>

Column 1: values of $B$ when $K_1 = 1$ at $0^\circ$ with $\mu = 28,000$ and $K_2 = 1$ at $0^\circ$ with $\mu = 22,000$.

Column 2: values of $Y$ when $K_3 = 1$ at $0^\circ$ with $\mu = 6,300$ and $K_4 = 1$ at $0^\circ$ with $\mu = 1,540$.

Column 3: values of $\text{CuT}$ when $K_5 = 1$ at $0^\circ$ with $\mu = 22,000$ and $K_6 = 1$ at $0^\circ$ with $\mu = 28,000$.

Column 4: values of $\text{CuV}$ when $K_7 = 0.001$ at $0^\circ$ with $\mu = 34,000$ and $K_8 = 1$ at $0^\circ$ with $\mu = 1,540$.

Column 5: values of $B - Y + 0.5 \text{CuT} - 0.5 \text{CuV}$.

Column 6: values of $B - Y + 0.5 \text{CuT} - 0.5 \text{CuV}$ when $K_5$ and $K_8$ are equal and have the same thermal increment.

Column 7: values of $B - Y + 0.4 \text{CuT} - 0.5 \text{CuV}$.

Then if

$$T = \frac{\text{CuTK}_4}{K_4} \quad \text{and} \quad \text{CuT} + T = 1,$$

$$1 - \text{CuT} = \frac{\text{CuTK}_4}{K_4},$$
TOXIC ACTION OF COPPER ON NITELLA

Rearranging,

\[ \text{CuT} = \frac{K_4}{K_4 + K_4'} \]

and, similarly,

\[ \text{CuV} = \frac{K_5}{K_5 + K_5'} \]

If to \( K_4, K_5, K_7, \) and \( K_8 \) are assigned appropriate values at \( 0^\circ \), and each has its corresponding critical thermal increment, we can calculate the amount of \( \text{CuT} \) and of \( \text{CuV} \) present at any temperature. Table IV gives some of these values.

![Graph](https://via.placeholder.com/150)

**Fig. 8.** Theoretical temperature curves using the data in Table IV. Curve A is based on Column 5 in Table IV and the ordinate is the logarithm of \( B - Y + 0.5 \text{CuT} - 0.5 \text{CuV} \). Curve B is based in a similar way on Column 6 and Curve C on Column 7.

The mode of action of the activated copper is assumed to be the following: The activated copper acts as a catalyst, not altering or inhibiting the normal reactions which form \( B \) and \( Y \), but acting as a secondary catalyst to further catalyze the reactions designated by \( M_1 \) and \( M_2 \). \( \text{CuT} \) acts in conjunction with \( B \) to alter the value of \( M_1 \) and \( \text{CuV} \) acts with \( Y \) to affect \( M_2 \). Then in general the expression for the ordinate is: \( \text{CuT} + B - Y - \text{CuV} \). It is not necessary that \( \text{CuT} \) and \( \text{CuV} \) should have the same catalytic power as \( B \) and \( Y \). It
is possible for them to be more or less powerful when present in the same quantity. Thus CuT might be one-half as effective as B. Then instead of adding CuT and B we would add 0.5 CuT and B. Similarly CuV might be one-half as powerful a catalyst as Y and we would subtract 0.5 CuV. Using these figures the above expression becomes:

\[0.5 \text{CuT} + \text{B} - \text{Y} - 0.5 \text{CuV}\]

Using these figures we obtain Fig. 8, Curve A. Table IV gives data for three such curves (plotted in Fig. 8). It will be observed that these lines are unquestionably curved, and resemble in principle the results obtained with copper chloride and Nitella. No attempt is made to precisely duplicate the experimental case.

The system outlined above is rather complex, but there is no reason to believe that what actually occurs is any less so. In fact it is likely to be much more complicated. A hypothetical system of this sort does not pretend to give a picture of what goes on in the cell in all its details. It merely indicates that if some such system were present it would give curves similar to those actually secured. At the same time it furnishes a tangible representation of the vital processes, a sort of working foundation on which we may build a more substantial edifice of fact. This is its main justification.

SUMMARY.

1. Using the loss of turgidity of the cells as a criterion it is found that the toxicity curve of copper chloride with Nitella is sigmoid. An empirical equation can be constructed which will approximately fit the curve.

2. When the concentration of the copper chloride is varied the toxic effect varies as a constant, fractional, power of the concentration. This relation holds when the concentration is plotted against either (1) the time necessary to reach a given point on the ordinate of the survivor curve, (2) the maximum speed of toxic action as shown by the tangent to the survivor curve or (3) the first derivative of the equation which fits the survivor curve.

3. When the temperature is varied and the logarithm of the reciprocal of the time necessary to reach a given point on the survivor
curves is plotted against the reciprocal of the absolute temperature
the resulting figure consists of several intersecting curves. A hypo-
thesis is described which will give straight lines under normal
conditions and curves when acted upon by a toxic agent.

CITATIONS.

Robertson, T. B., 1907-08, *Arch. Entwicklungsmechn. Organ.*, xxv, 4; 1908, *Arch.