A LATENT PERIOD IN THE ACTION OF COPPER ON RESPIRATION.

By S. F. COOK.

(From the Laboratory of Plant Physiology, Harvard University, Cambridge.)

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I.

A preceding paper (Cook, 1925-26) has discussed the general effect of salts of copper and of other metals on the respiration of Aspergillus niger. It was demonstrated that the toxic action followed certain definite laws and could be formulated in a mathematical system. Mention was made of a peculiarity in the case of copper, wherein this element differed from the others studied. When copper chloride acts on Aspergillus niger there is at first no change in the rate of respiration, but later the production of carbon dioxide decreases in the manner characteristic of the action of all the heavy metals studied. The time which elapses between the introduction of the copper and the beginning of the drop in respiration rate has been termed the latent period.

Save where otherwise stated, the methods used in investigating this latent period were the same as those used in studying the other toxic effects of copper, mercury, and silver. All work on respiration was done by the indicator method with the Osterhout respiration machine as previously described. Fig. 1 shows a typical curve for the effect of copper chloride, including latent period and subsequent drop.

It might appear that the figure does not consist of a straight line followed by a distinct and clearly differentiated drop, but that it is really a continuous curve starting with the introduction of the copper, falling imperceptibly for a while and then with increasing velocity. In this case the curve should show a sharp bend toward the end of
the latent period, and not a cusp as here represented. That this is not true is maintained for two reasons:

1. Several hundred experiments were performed with different concentrations of copper and particular attention was paid to the readings at or near the end of the latent period. But in no instance was there any indication of a rounded curve at that point. The last reading of the latent period was always the same as the preceding readings, of which there were several, and there was no indication of a gradual falling off in respiration during that time. The first reading after the latent period, particularly with high concentrations, was usually a considerable distance below the normal level, so far indeed as to leave no doubt that the curve should be drawn as in Fig. 1.

2. If the curve is continuous from the zero abscissa then it must all represent the effect of the copper on the respiratory system. This system has been represented as a series of consecutive reactions $A \rightarrow X \rightarrow Y \rightarrow Z$ which is altered by the copper through its effect upon the velocity constants. If the entire curve represents the effect of
the copper on respiration then the entire curve should be reproducible by means of the formula for two consecutive reactions. A curve of this sort cannot be so duplicated (see Figs. 2 and 3). It seems reasonable to suppose, then, that the latent period is due to some delay whereby the copper does not directly affect the respiratory system until after a definite interval of time.

![Diagram](https://example.com/diagram.png)

**Fig. 2.** Solid line is curve with copper chloride 0.02 \( K_e = 0.021 \). Broken lines are curves calculated by the formula for consecutive reactions:

\[
M = A e^{-K_d} + B \left( \frac{K_1}{K_3 + K_1} \right) \left( e^{-K_d} - e^{-K_d} \right)
\]

where \( A = 1, B = 1, K_1 = 0.03, \) and in

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The question arises whether the mechanism responsible for the delay is of a chemical or physical nature, and whether it takes place outside or inside the cell. If the temperature is raised, say from 25° to 35°, it is found that the latent period is shortened, and on plotting the temperature against the latent period Fig. 4 is ob-
Fig. 3. Solid line same as Fig. 2. Broken lines are curves calculated as in Fig. 2 where $A = 1, K_1 = 0.01, K_2 = 0.1$, and in

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<td>5.454</td>
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Fig. 4. The temperature plotted against the length of the latent period.
tained. This shows that the temperature coefficient is nearly 2, a number characteristic of chemical rather than of physical processes. Of course the criterion is not absolute, for some physical processes have a coefficient as high as 2; but it may be considered fairly strong evidence in favor of the intervention of a chemical reaction rather than of a physical hold-up. Only three points on the temperature scale were used and further analysis using the recently developed method of Crozier (1924–25) might yield more information, but the general character of the system may be ascertained from only a few points.

Further evidence is found in the fact that if the toxic solution is removed from the respiration chamber (for the method see the preceding paper) before the end of the latent period, the drop in respiration will take place as usual and the rate of production of carbon dioxide will fall to a new, lower level and there remain (see Fig. 6). This phenomenon will be discussed again, but it may be pointed out here that it is very difficult to conceive of a trapping mechanism, such as an impermeable cell wall or membrane, or the adsorption of a substance on a surface, which would give this effect. If the copper is prevented from reaching the oxidative mechanism of the cell by any physical obstruction or barrier, which must first be saturated or broken down, it is hard to see how the copper can eventually get through, after it has been removed from the outside, and injure the cell. In the case of an ordinary physical process dependent on the presence of the external solution, that process could scarcely continue after the removal of the external solution. It is possible, however, if there is no physical barrier, and the copper gets into the cell immediately, that there might be a chemical system whereby the copper which had already penetrated might remain and ultimately damage if not kill the cell.

If the latent period is nothing more than the time required for a chemical reaction to take place somewhere inside the cell wall, then it ought to be possible to demonstrate the presence of copper inside the cell before the end of the latent period. For this purpose there are two methods of procedure, (a) the direct method, to test chemically for the presence of copper in the cell, and (b) an indirect method, to see if copper can injure the cell within the requisite time using criteria other than that of respiration.
A. *Aspergillus* does not lend itself readily to microchemical analysis because of the very small size of the cells. Therefore, an undetermined species of the stonewort *Nitella* was used. The material came from Woods Hole, Massachusetts, and was characterized by very large cells (1 to 4 inches long) and watery sap. *Nitella* gave a good respiration curve with copper. It was a little more sensitive than *Aspergillus* but the latent period was longer, being about 20 minutes with 0.01 M copper chloride. After determining the respiration curve, cells were put in the same concentration of copper for varying lengths of time and the sap then squeezed out (Osterhout, 1921-22; Irwin, 1922-23). 1 or 2 cc. of sap were usually obtained and tested with potassium ethyl xanthate. This reagent gives a yellow to brown color with copper, depending on the concentration, and is sensitive to about 0.00001 molar. In several experiments no color was obtained till 20 to 25 minutes exposure to 0.01 M copper chloride. Thereafter the color steadily increased. The result might be taken to signify that copper was not in the cell till after 20 minutes, that is till after the latent period. It is more probable that it was there but in such minute quantities as to be below the threshold for determination with xanthate.

Analogous experiments were performed with *Valonia* sp., a marine green alga growing in shoal waters at Bermuda. Unfortunately this organism did not produce sufficient carbon dioxide to measure in the respiration machine and consequently it is not known whether it has a latent period or not. In any case copper penetrates it rapidly. In 0.01 M copper sulfate in sea water a good xanthate test could be obtained after 15 minutes exposure. By evaporating 50 cc. of the sap nearly to dryness a good test was obtained after 5 minutes exposure, indicating the presence of copper in the cell at that time. If there is a latent period of respiration it is probably longer than 5 minutes, at this concentration of copper. Thus the direct method is suggestive but not conclusive. If the latent period with *Valonia* were certainly known to be more than 5 minutes it would be conclusive.

B. An indirect method was applied to the same organisms, *Nitella* and *Valonia*. With *Nitella* separate lots of twenty-five cells each were exposed for periods of varying duration to 0.01 M copper chlo-
ride. They were then placed in water and allowed to stand several hours before testing. A control lot of the same number of cells, which were not exposed to copper, was placed in water with the injured. The criterion of injury was the turgidity of the cells. If the cell was able to support its own weight when held by one end it was considered uninjured. If it broke and fell it was considered injured if not dead. This criterion makes use of a purely arbitrary point in the process of injury, for the loss of turgidity is progressive, but it is very convenient and useful. The results showed that even a very short exposure to copper (2 minutes) caused some of the cells to lose their stiffness. Of those which had been exposed for longer periods more were injured, until in the lots exposed for an hour or longer all were injured (or killed). In the control lot none, whatever, had lost its normal turgidity. This shows quite clearly that the copper injured the cell in many cases before 2 minutes had elapsed. In order to injure it must have penetrated at least as far as the plasma membrane and must have been exerting a toxic effect on the protoplasm even while it was not affecting the respiration.

Similar results were obtained with Valonia. In this instance the criterion of injury was the presence of sulfate ion in the sap. Valonia normally does not give a test for SO$_4$ (Wodehouse, 1917), but if injured will allow this ion to enter. The same rough correspondence was observed between the duration of exposure to copper and the percentage of cells injured; i.e., giving the sulfate test. In fact it was here quite clearly logarithmic. The shortest exposure to copper showing injury to any of the cells was 10 seconds, in which experiment several of the cells were injured. This indicates that with Valonia at least copper penetrates from the start, regardless of any effect it may have on the respiration. Taken in conjunction with very similar results on Nitella where the latent period is known, it furnishes good evidence that copper is not held up on the outside by a physical barrier but that the latent period corresponds to a process which occurs inside the cell. We may say then, for present purposes at least, that the latent period is a chemical trapping mechanism situated inside the cell.

It should be noted here that when, with Aspergillus, the concentration is varied the duration of the latent period varies likewise
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(see Fig. 10). The relation between concentration and latent period is interesting, for it is the inverse of that observed in the case of velocity of toxic action; i.e., the length of the latent period varies inversely as a constant power of the concentration. Thus log latent period = \(\frac{1}{B}\) log C + log A, or latent period = \(A C^{-B}\). This fact also points to a chemical rather than to a physical explanation of the latent period and will be further discussed.

III.

It should now be possible to construct a hypothetical system, on a chemical basis, whereby the process underlying the latent period may be illustrated if not fully explained. It would be impossible at the present stage to state exactly what are the reactions involved. But a system which duplicates the experimental results should at least serve to bring out the characteristics of these reactions, even though it could not tell us the precise nature of the substances concerned. Any explanation must account for the following four experimental results.

1. It has been suggested previously that the normal oxidations of the cell take place in a series of catenary reactions each one of which is controlled by a catalyst and is characterized by its own velocity constant. The toxic effect of any metal is held to be due to the alteration of these velocity constants. But the metal in its raw state, so to speak, cannot do this. It must previously be activated, or undergo some chemical change (e.g. combination, change of valence, etc.). This activation has been assumed to take place through a reversible reaction involving a combination with a cell constituent called T. Thus if \(M\) represents the metal we can write
$M + T \rightleftharpoons MT$. To account for the latent period we must extend this hypothesis. Before combining with $T$ let us say that $M$ must diffuse from the external solution into the cell and that when it gets inside it exists in the form $MR$. This does not imply that $MR$ is a compound between $M$ and some cell constituent $R$, but is simply used for convenience to distinguish between the metal outside and inside the cell. The metal may undergo some change of state as from the ionic to the molecular form, from a hydrated to a non-hydrated condition or *vice versa*. Its exact nature is immaterial. We may treat it as if the metal simply diffused into the cell, and call the external metal $M$ and the internal metal $MR$. We then have this system:

$$MR + T \xrightleftharpoons[K_2]{K_1} MRT \quad \text{or} \quad CuR + T \xrightleftharpoons[K_4]{K_3} CuRT$$

The next step is to analyze this system with respect to the rate of formation of $CuRT$. In calculations concerning these reactions it is permissible to let the velocity constants be whatever seems desirable. We may also assume that the rate of penetration of $Cu$ into the cell is much more rapid than the rate of its diffusion out again. This might be due to the physical nature of the cell wall or membrane or to chemical reactions involving the copper as it entered or left the protoplasm. Indeed the temperature coefficient (approximately 2) indicates that a chemical reaction might possibly be connected with the entrance of the copper into the cell. Furthermore we may say that the rate of entrance of the copper is greater than the rate of combination of the copper with $T$. If the difference between these rates is great enough the penetration of the copper will be practically instantaneous (a few seconds) while the formation of $CuRT$ will take a considerably longer time (a matter of minutes). For practical purposes we may consider that the concentration of the copper inside the cell ($CuR$) will be at equilibrium with that of the copper outside ($Cu$) before the reaction $CuR + T \rightleftharpoons CuRT$ begins. The simplest assumption is that the concentrations are the same outside and inside or at least that the ratio between them is always the same.

On this basis the reaction $CuR + T \rightleftharpoons CuRT$ may be considered...
by itself. Starting with CuR at an arbitrary concentration of, say, 100, and considering the reaction as monomolecular on the ground that T is present in great excess, it is possible to plot the formation of CuRT.

If x is the amount of CuRT formed after time, t, a is the concentration of CuR at the start and $ is the amount of CuRT present at equilibrium, then

$$\frac{dx}{dt} = K_3 (a - \xi) - K_4 \xi.$$

But at equilibrium $\frac{dx}{dt}$ is zero, and $K_3 (a - \xi) - K_4 \xi = 0.$

Then

$$K_3 = \frac{K_3 (a - \xi)}{\xi}.$$

But at any moment during the reaction,

$$\frac{dx}{dt} = K_3 (a - x) - K_4 x;$$

or, substituting,

$$\frac{dx}{dt} = K_3 (a - x) - K_3 \frac{a - \xi}{\xi} x.$$

This equation when integrated takes the form

$$\frac{1}{\xi} \log \frac{\xi}{\xi - x} = \frac{K_3 a}{\xi}.$$

But, since

$$K_4 = \frac{K_3 (a - \xi)}{\xi}, \quad \xi = \frac{K_3 a}{K_3 + K_4},$$

Substituting and rearranging,

$$\log \frac{\xi}{\xi - x} = (K_3 + K_4) \xi.$$

Expressed in the exponential form:

$$x = \xi - \xi e^{-(K_3 + K_4)}.$$
and, finally, substituting for $\xi$, we get

$$x = \frac{K_1 a (1 - e^{-(K_1 + K_4) t})}{K_1 + K_4}$$

If we let $a$ have the value of 100, where $K_3 = 0.016$ and $K_4 = 0.001$, and plot the curve of the formation of CuRT we get Fig. 5, Curve A. It will be observed that the rate is rapid at first and progressively becomes slower and reaches equilibrium only at infinity. Since the toxic action of copper depends on its presence in the form CuRT

![Graph showing formation of CuRT over time](image)

**Fig. 5.** Curve A shows the amount of activated copper (CuRT), or in the case of any metal (MRT), plotted against time. For formula see text. Curves B and C show the effect of removing the toxic agent after 50 and 10 units of time respectively. For method see text.

It is obvious that there can be no toxic action without CuRT. The hypothesis here advanced to account for the existence of the latent period is this: a certain definite amount of CuRT must be formed before the action on the respiratory system can begin. Since a measurable time must elapse before such a quantity is formed there will be a period of inactivity previous to the observed fall in respiration, and this is called the latent period. There is involved the idea of a threshold, analogous to, although differing in many respects from, other thresholds known to biology and chemistry. The latent
period here observed may be compared on the one hand to the threshold of nerve stimulus and on the other to certain induction periods observed by chemists. It resembles the former in that a definite intensity must be attained before there is any visible response on the part of the organism, but here the stimulus is chemical whereas with nerve it is more likely physical (cf. Lillie, 1923). The resemblance to the chemical induction periods is based on the fact that in both cases there is a chemical reaction taking place for a considerable time before there are any apparent effects (cf. Forbes, Estill, and Walker, 1922). Hecht (1923-24 etc.) has made similar assumptions with regard to the effect of light on Mya. There seems to be no reason for not maintaining, in the case of copper, that there is a threshold which must be passed in order for the toxic activity to occur, and that the threshold is a function of the formation of activated copper, CuRT. In Fig. 5 we may place the threshold arbitrarily at 50 units of CuRT, or at a concentration of 50. On the scale here used about 30 units of time are consumed during the latent period. If we say 30 minutes we shall not be far from the experimental facts.

The effect of the other metals may be considered at this point. It was suggested previously that they are all activated in a manner similar to that assumed for copper. If so there should be a latent period with these elements likewise. It is the belief of the writer that there is one, but that it is often too short to be detected experimentally. The longest latent period observed with copper was 120 minutes. If that with mercury, for equivalent concentration, were one hundredth as long it would be about 1 minute, and a latent period of 1 minute would be impossible to observe when the subsequent drop is very slow. With higher concentrations there would be still less chance of detection. It is only necessary to assume, with mercury for instance, that the value of the product HgRK₃ is much larger than with copper. Thus HgRT is formed much more rapidly and the threshold is passed immediately. If we let the presence or absence of a latent period depend on the relative value of K₃ then it follows logically that one of the effects of all metals is a latent period of greater or less duration, only a few of which are long enough to permit experimental detection. Iron and tin, as well as copper, show noticeable latent periods but they are only about half
as long. We can say that FeRT and SnRT are formed about twice as fast as CuRT but very much slower than HgRT and AgRT. The metals, therefore, may be distinguished by the rates at which they are activated.

2. Reference has been made to phenomena which occur when the toxic substance is removed shortly after its introduction. Figs. 6 and 7 show the effect of the removal of copper after 3 and 17 minutes exposure. In the first instance the latent period continued a little longer than usual, then the respiration rate fell off and gradually attained a new level at about 70 per cent of the normal. In the second example the removal of the toxic agent occurred after the end of the latent period and the respiration continued to fall, gradually reaching equilibrium at about 25 per cent of the normal. Whether any secondary, permanent injury was sustained which ultimately killed the organism was not ascertained, but the equilibrium level was held for several hours at least and secondary effects do not enter into the present discussion.

Fig. 8 illustrates the result of the removal of mercury. After removal the equilibrium was attained before the next reading could
be taken, and was held during the time of the experiment. This is in direct contrast to copper where the equilibrium was only gradu-

![Graph 1: Effect of removing copper chloride 0.0075 M after 17 minutes exposure.](image1)

**Fig. 7.** The effect of removing copper chloride 0.0075 M after 17 minutes exposure.

![Graph 2: Effect of removing mercuric chloride 0.0002 M after 2 and 20 minutes exposure.](image2)

**Fig. 8.** The effect of removing mercuric chloride 0.0002 M after 2 and 20 minutes exposure.

ally attained. Fig. 9 shows the effect of the removal of silver, (A) at the peak of the curve and (B) on its descending limb. The same
situation obtains here also, for equilibrium is attained before readings are resumed and, curiously, bears no reference to the rise in respiration, characteristic of the action of silver. These results can be interpreted in the light of the previously outlined hypothesis.

During the formation of CuRT (to consider the case of copper) the concentration of CuR was held constant by being in equilibrium with the copper outside the cell, and in the formula

$$x = \frac{K_3 \cdot a \left(1 - e^{-\left(K_3 + K_4\right)}\right)}{K_3 + K_4}$$

![Figure 9](https://example.com/fig9.png)

**Fig. 9.** The effect of removing silver nitrate 0.0002 M after 5 and 10 minutes exposure.

a was kept at the arbitrary concentration of 100. Now if the outside copper (Cu) is removed so that its concentration is practically zero then the situation is as follows: Any uncombined copper in the cell (CuR) escapes into the outside fluid and is for our purposes lost. There will be no further diffusion of the copper into the cell, but on the other hand there will be a slow diffusion outward, whereby CuR in the cell will become lost as Cu in the surrounding medium. At the same time the reaction CuR + T ⇔ CuRT is still going on but some
of the copper is continually being lost. This loss, or outward diffusion, may be assumed to go on at a rate always proportional to the amount of copper (CuR) left in the cell. It will thus follow the law of an irreversible, monomolecular reaction and will have a velocity constant \( K_2 \). Stated another way the amount of CuR remaining in the cell will equal \( ae^{-K_2t} \) where \( a \) is the amount of CuR present when the outside copper was removed. This situation may be represented

\[
\frac{K_3}{K_2} \text{ thus: } \downarrow \text{Cu} \rightleftharpoons \text{CuR} + T \rightleftharpoons \text{CuRT}.
\]

If, as we have supposed in the case of copper \( K_2 \) is smaller than \( K_3 \) more copper will for a while be converted into CuRT than will escape from the cell. If it is larger the reverse will be true, although CuRT will increase for a relatively short time, depending on \( K_4 \). For, suppose the product \( \text{CuRT}K_4 \) is larger than the product \( \text{CuR}K_3 \), then the reaction \( \text{CuR} \rightleftharpoons \text{CuRT} \) will run to the left and CuRT will diminish from the moment of the removal of the outside solution. If the reverse is true then CuRT will increase until CuR is nearly exhausted, and the rate of exhaustion will depend on the size of \( K_2 \). In the case of copper the constants assumed were \( K_2 = 0.003, K_3 = 0.016, \) and \( K_4 = 0.001 \).

In the formula \( a \) was constant at 100, but now the outward diffusion of CuR must be considered. If CuR is being lost at the rate \( K_2 \) then the value of \( ae^{-K_2t} \) can be substituted for \( a \) and the equation may be written:

\[
x = \frac{K_3ae^{-K_3t}}{K_2 + K_4}.
\]

Curves B and C in Fig. 5 were calculated by means of this formula. It is evident that the value of \( x \) (CuRT) rises for a while and then falls. If we take 50 as the threshold we see that after removing the reagent the CuRT increases to the threshold, but takes longer in the process, exerts a toxic action for a short time, and then falls again. During the period that CuRT is above the threshold the respiration falls, and when CuRT drops below the threshold the oxidation system regains its equilibrium but at a lower level. This lower level indicates that the respiratory catalysts have been permanently reduced by the removal of \( A \) and \( X \). The duration of the toxic activ-
ity is dependent on the exposure to the copper solution and consequently on the quantity of \( \text{CuRT} \) formed before removing the copper.

When mercury is removed something similar takes place. The velocity constants of this reaction have been assumed to be greater than in the reaction involving copper, and the further assumption may be made that here \( K_4 \) is greater than \( K_3 \). Then \( \text{HgRT} \) decreases from the moment the external mercury is removed \( \text{HgR} \) diffuses and since all the reaction velocities are much greater \( \text{HgRT} \) very rapidly sinks below the threshold. So rapid is the decomposition of \( \text{HgRT} \) that when the next readings are taken the toxic action has ceased and the respiratory system \( A \rightarrow X \rightarrow Y \rightarrow Z \) has had time to come to equilibrium in accordance with the now reduced quantities of \( A \) and \( X \).

The same reasoning applies to the case of silver. Here the striking feature is that if the silver is removed at the peak of the curve, the new level is far below the normal. The curve of silver has been explained by saying that in the reactions \( A \rightarrow X \rightarrow Y \rightarrow Z \) the velocity constant of \( X \rightarrow Y \) is increased more than that of \( Y \rightarrow Z \). The reaction \( A \rightarrow X \) ceases while the metal is present, and \( A \) is being continually reduced in quantity. This results in an increase in \( Y \) for a short time and consequent acceleration of the carbon dioxide production. Meanwhile \( X \) is being exhausted and ultimately the value of \( Y \) falls. If the silver is removed and the toxic action stopped while \( Y \) is at a maximum the normal velocity constants will be restored very soon. \( Y \) will fall to a new value in accordance with the now permanently reduced value of \( A \) and \( X \). This must all take place in the interval between the readings.

To summarize it may be stated that the phenomena observed on removing the toxic agent may be accounted for by differences produced in the velocity constants governing the reactions \( M + R \rightleftharpoons MR + T \rightleftharpoons MRT \).

3. There remains the effect of the concentration on the length of the latent period. The experimental data (see Fig. 10) indicate that there is an inverse logarithmic relation between them; \( i.e., \) latent period \( = A \ C^{-B} \) in which \( C \) is concentration and \( A \) and \( B \) are constants. The complete reaction for the activation of copper implies
molecular proportions of a high order. In discussing the effect of the concentration on the toxic action in general, use was made in a previous paper of the equilibrium constant of the second half of the reaction and the experimental curve was duplicated. It was necessary to assume the molecular proportions mentioned in order to account for the fractional exponent in the equation \( K_s = A C^B \). For present purposes we will disregard the fractional exponent and assume that the reaction \( CuR + T \rightleftharpoons CuRT \) involves only the simple proportions of 1:1. If we were to attempt to duplicate the slope of the curve of latent period and concentration it would be necessary to assume the former complex proportions, but for simplicity of calculation the reaction \( 10(CuR)_5 + 2T \rightleftharpoons 25(CuRT)_2 \) may be replaced by: \( CuR + T \rightleftharpoons CuRT \).

For concentrations within the experimental range we may regard \( T \) as greatly in excess of \( CuR \) and treat it as a reversible monomolecular reaction, for a bimolecular reaction may be so treated when one of its components is in excess. In calculating Fig. 5 the external concentration, and therefore that of \( CuR \) (a in the formula) was called 100. If \( T \) is indefinitely large with respect to \( a \) then we may take different values for \( a \) and determine \( t \), the length of the latent period. This has been done in Fig. 11 for values of \( a \) between 60 and 10,000, when \( x \) is held constant at 40, and the logarithm of \( a \) plotted against the logarithm of \( t \). It is evident that \( \log t = \frac{1}{B} \).
\[ \log a, \text{ or } t = A C^{-B}. \]

\( B \) equals unity because of the molecular proportions assumed in the equation. If the proportions were correct \( B \) would be a fraction as in the experimental case. It is clear, however, that the relation between the length of the latent period and the concentration is logarithmic, both in the experiments and in the theoretical system.

As \( a \) approaches the value of \( T \), which is finite (however large), the relations change. When the values of \( a \) and \( T \) are nearly equal the system is clearly bimolecular and the monomolecular formula

\[ \text{FIG. 11. The logarithm of the time plotted against the logarithm of } A. \text{ For method see text.} \]

cannot be used. It is evident that as \( a \) becomes greater than \( T \) the similarity to a monomolecular reaction will again appear, but now with \( a \) as the component in excess. Variation in its concentration will have little effect because the value of \( CuRT \) is approaching the value of \( T \) beyond which it cannot go. The result will be, then, that the logarithmic relation will hold until high concentrations of the toxic reagent are reached, at which time the sloping line in the plot will become more nearly horizontal. That this is true experimentally is shown in Fig. 10.
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SUMMARY.

1. When copper chloride is allowed to act on Aspergillus niger there is at first a period during which there is no change in the rate of the production of carbon dioxide, following which the rate of respiration falls. The interval of no change is called the latent period.

2. When the copper is removed from the external solution before the end of the latent period this interval is prolonged. The rate of respiration then falls to a new level below the normal level.

3. Experiments on Nitella and on Valonia indicate that the copper penetrates the cell almost immediately.

4. The length of the latent period varies inversely as a constant power of the concentration of the copper.

5. These results are explained by assuming that the copper is made active in the respiration system by means of a reversible reaction. By using appropriate velocity constants the experimental curves can be duplicated by calculated curves.

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