

THE EFFECTS OF CERTAIN HEAVY METALS ON RESPIRATION.

By S. F. COOK.

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

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I.

The experiments here described were performed to determine the effects of copper, silver, and mercury on respiration. For purposes of comparison it was found advisable to include hydrogen (as hydrochloric acid). The organism used was *Aspergillus niger*, a fungus which, because of the rapidity of its growth and the ease with which it is manipulated, lends itself readily to such investigation. The effect of the above mentioned four elements is to check the respiration of *Aspergillus* in a definite and characteristic manner. An exceptional result was found with copper, in the form of a latent period which lasted a considerable length of time before the toxic effect became evident, and which will be discussed in detail in another paper (see Fig. 1). In this paper the general toxic effects of copper, mercury, silver, and hydrogen are described, together with the special effects of temperature and concentration; and an hypothesis is advanced by means of which the toxic effects may be placed on a mathematical and predictable basis.

II.

Respiration was measured by the indicator method, with an Osterhout respiration machine, the principles of which have been often described (*cf.* articles by Osterhout, Haas, Inman, Ray, Gustafson, *et al.* in *The Journal of General Physiology*). There is a closed system of tubes containing the organism and a tube with a solution of the indicator phenolsulphonphthalein. The pumping system, by means of a bulb, sends a continuous current of air through the apparatus, carrying the carbon dioxide from the respiring organism to the indicator, which changes from light pink to yellow. The limits of the color change are fixed by two tubes of buffer solutions made up as known pH standards, in this case pH 7.36

and 7.09. When the indicator solution has been changed to match the lower standard the current is switched into a U-tube containing sodium hydroxide which removes the carbon dioxide. The air free from carbon dioxide is then run through the indicator which sweeps out what carbon dioxide is held in it, thereby raising the pH and restoring the color to that of the upper standard. The shifting of the direction of the current was done with a three-way stop-cock.

The organism was placed in a test-tube $1\frac{1}{2}$ inches in diameter, fitted with a rubber stopper. When a solution was to be introduced after the beginning of the experiment it was done by means of a separatory funnel fused to the intake tube. The intake tube ran through the stopper to the bottom of the test-tube

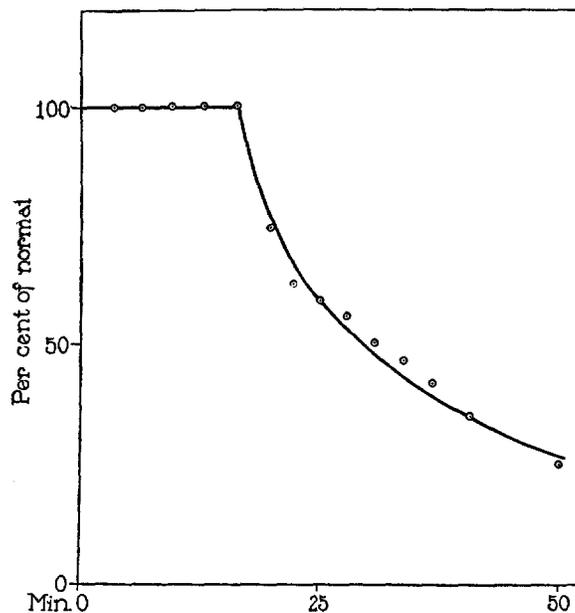


FIG. 1. Curve with copper chloride 0.25 M; K_p (monomolecular) = 0.022.

servng as respiratory chamber. In this way the liquid could be run directly into the chamber without opening the apparatus or allowing any outside air to get in. In some experiments it was necessary to replace the liquid in the chamber by an entirely fresh solution. For this purpose a tube was led off the closed system near the outlet from the chamber and connected with a U-tube filled with sodium hydroxide. A rubber tube was connected with the far side of the U-tube and a stop-cock closed the outlet securely when not in use. In this way carbon dioxide could be removed from air passing in or out of the apparatus. Another tube was run from the bottom of the respiratory chamber through the stopper to the outside and was likewise closed with a pinch-cock when not in use.

This system involved no significant leakage from the outside, as was determined by frequent tests. When it was desired to change the solution in the chamber, both exit tubes were opened and the liquid present was blown out, all carbon dioxide being removed from the incoming air by the sodium hydroxide in the U-tube. Then the pinch-cocks were closed and the new solution run in, in the usual manner, through the separatory funnel. This process was repeated as often as was necessary, in a very short time, and the organism could be washed quite free from the original fluid.

It was usually necessary to maintain a constant temperature. A thermometer was sealed into the respiratory chamber through the stopper. The whole chamber was set in a water bath with a mercury thermoregulator. The temperature was read from the thermometer inside and could be regulated to about 0.5°C., which was sufficiently close.

The normal respiration rate was obtained by determining the length of time to change the indicator from pH 7.36 to 7.09. When the toxic agent is introduced there is a change in the time of decolorization and hence a change in the rate of respiration. The indicator solution was made slightly alkaline (0.0001 M) with sodium bicarbonate. Ordinary tap water has been used, but only because there was about the right amount of alkali in it. Since the nature and quantity of this is unknown it is preferable to use distilled water and a known amount of the bicarbonate.

The results are expressed with reference to the rate of the carbon dioxide production and not with reference to absolute amounts. It is immaterial how much carbon dioxide is normally produced by the organism (within limits of convenience), because all subsequent changes are expressed as per cent of the normal. Thus it makes no difference if the total quantity of the fungus varies from experiment to experiment. The rate curves will always be comparable.

The organism, *Aspergillus niger*, was taken from a culture kindly supplied by Dr. W. H. Weston and has been kept in subcultures on potato agar. For use in these experiments it was grown on a liquid medium in Erlenmeyer flasks. The medium, adapted from that used by Gustafson in this laboratory (1918-19) was made up as follows: KNO₃, 2 gm.; KH₂PO₄, 0.75 gm.; K₂HPO₄, 0.25 gm.; MgSO₄, 0.5 gm.; sucrose, 40 gm.; and water 1000 cc. After being sterilized, the flasks were inoculated with a platinum or nichrome needle, special precautions being taken to avoid bacterial or fungus contamination. The mould was then placed in an incubator at about 30° and used after 48 to 60 hours. The growth was uniformly a solid mat which could be removed and handled easily. Pieces of appropriate size were cut out and washed free of the nutrient solution in distilled water. They were then placed in the respiration chamber in a 1 to 2 per cent glucose solution. The glucose was perhaps unnecessary, and no difference was ever detected in the toxic effect when the glucose was not used; but it seemed advisable not to force the mould to undergo so great a change in osmotic pressure as from the nutrient fluid to distilled water.

Aspergillus is a very resistant fungus and will tolerate extreme conditions, but

the respiration curves were found to be affected by two principal factors: (1) The rate of normal respiration varies with the temperature, as was to be expected. It was found that the rate of respiration increased with temperature to a maximum at about 40°C. and then fell off rapidly and ceased at about 50°C. It was impossible, therefore, to use temperatures above 40°C. (2) The fungus changes in its resistance to toxic solutions with age. The culture should be at approximately the same age throughout a series of experiments. The best age is from 2 to 3 days, although a little older material will give good results. Over 4 days it should not be used.

III.

When a solution of copper chloride is run into the respiration chamber containing the organism there is no change in the rate of respiration for a definite length of time which depends on the temperature and on the concentration of CuCl_2 . Then suddenly the rate falls off and the carbon dioxide production gradually ceases (see Fig. 1). The drop is similar to that produced by many other substances, but the preliminary period during which there is no change is peculiar and has been found only with copper, iron, and tin. The interval observed with the latter two elements is much shorter than with copper. There are two names which might be applied to it: latent period or induction period. Of these the former seems more appropriate. The latter is a chemical rather than a biological term and is often taken to mean a period of slight activity followed by one of greater activity (Mellor, 1904), while here there is no detectable activity at all previous to the sudden change. This entire lack of activity is better described by the term latent period. Since the period occurs only with the three elements mentioned and has peculiar characteristics it will be discussed separately, and we will consider here only the drop in rate of respiration disregarding the latent period.

On analyzing the drop following the latent period it is found to follow approximately the course of a monomolecular reaction curve. In a monomolecular reaction there is a single substance undergoing decomposition and the amount decomposed in unit time is proportional to the amount present. Thus if a is the amount originally present, and x is the amount present after time t , then the rate of transformation $\frac{dx}{dt}$ is $K(a - x)$, or in the integral form, $K = \frac{1}{t} \log$

$\left(\frac{a}{a-x}\right)$. K is the velocity constant of the reaction. In the case of decreasing rate of respiration it might be termed the velocity constant of the toxic action.

In applying this formula to the curves obtained with copper and the other heavy metals it becomes evident that there is always a tendency for the value of K to diminish as greater and greater values

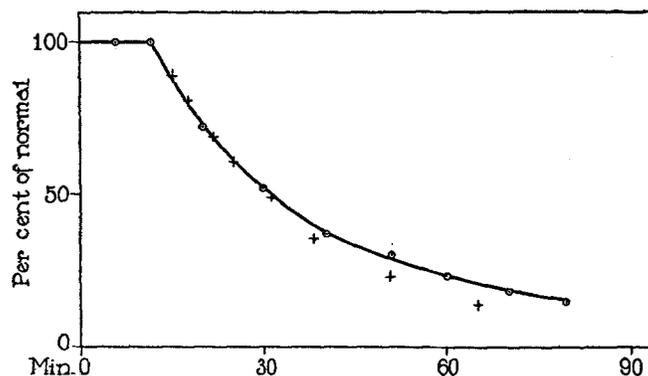


FIG. 2. Curve with copper chloride 0.0075 M. The average K_v is 0.0168. The crosses indicate the course of a curve using $K_v = 0.0168$ for all points. The values of K_v at various points of the experimental curve are:

t	K_v
2	0.0228
5	0.0194
9	0.0172
13	0.0170
19	0.0158
26	0.0153
38	0.0140
53	0.0137

of t are taken, indicating a reaction accelerated at the start. However, the differences in K are not sufficient to prevent the use of an average value as an index of the speed of the reaction. By using this average any two curves may be compared with sufficient accuracy and the monomolecular formula may be thus used on an empirical basis, for convenience only and without implication that in the measurement of respiration we have to do with an actual single mono-

molecular reaction. Indeed, the fact that there is a uniform diminution in the value of K throughout an experiment is evidence that the reaction is not a simple monomolecular one (see Fig. 2).

If we assume two consecutive reactions, each with its own constant, it is possible to duplicate the experimental curves much more closely

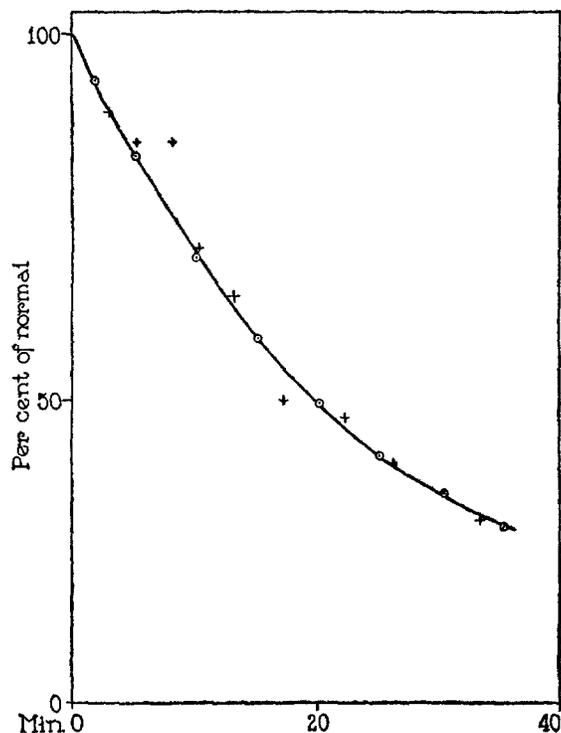


FIG. 3. Curve with copper chloride 0.02 M. $K_0 = 0.021$. The circles are points on a curve derived by the formula for consecutive reactions (see text), where $A = 1$, $B = 0.2714$, $K_1 = 0.0525$, and $K_2 = 0.06$. The crosses are the points obtained from the experimental data.

than by means of the formula for the single reaction. Osterhout (1922) has made this assumption with reference to similar curves obtained by the conductivity method. The mechanism of consecutive reactions will be discussed in another connection and here it will be sufficient to point out that a typical experimental curve ob-

tained with copper chloride can be duplicated (see Fig. 3), within the limits of experimental error, by using the formula for consecutive reactions. Similarly a composite curve obtained by averaging six experiments can be duplicated (see Fig. 4). Nevertheless, the simpler formula affords a good basis for comparison of curves under the influence of different conditions.

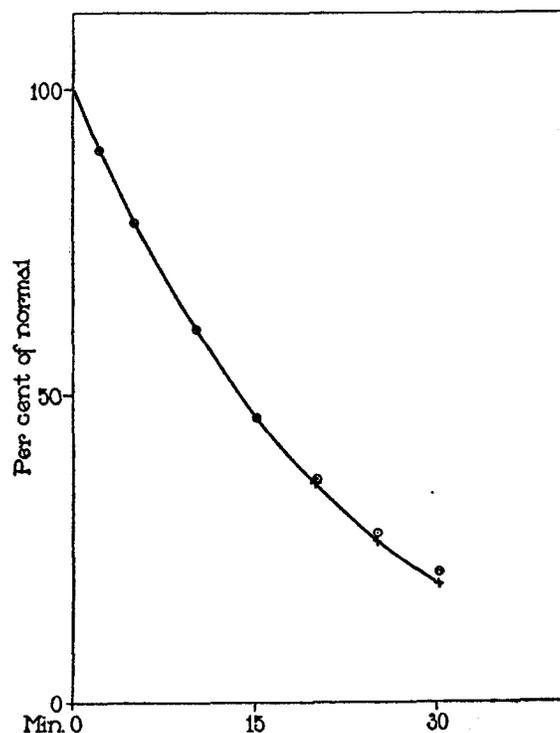


FIG. 4. The crosses are obtained by averaging six experiments with copper chloride 0.02 M. The circles are points on a curve derived by the formula for consecutive reactions (see text) where $A = 1$, $B = 0.2$, $K_1 = 0.0525$, $K_2 = 0.06$. The latent period is omitted.

The second metal used was mercury, as mercuric chloride. This element affects the respiration very much as does copper save that there is no latent period. The characteristic curve shows a steady drop (see Fig. 5). The monomolecular formula may be applied and

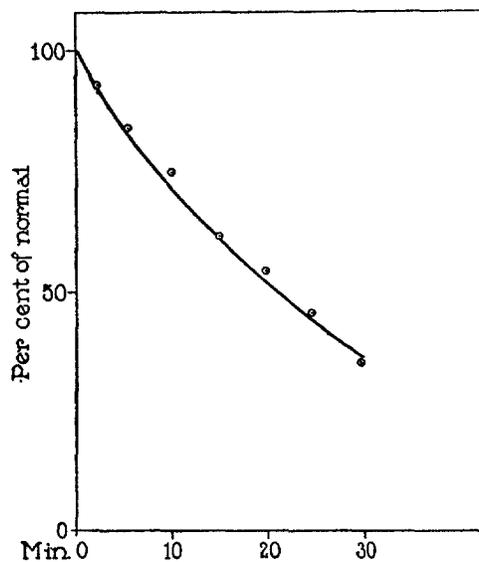


FIG. 5. Curve with mercuric chloride 0.0002 M $K_v = 0.012$.

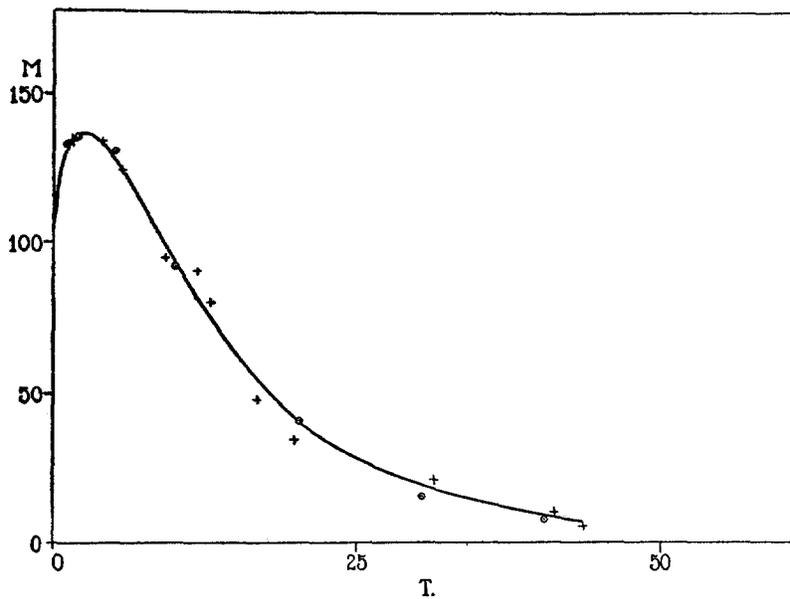


FIG. 6. Crosses indicate an experimental curve with silver nitrate 0.0001 M. $K_v = 0.03$. The circles are points on a curve derived by the formula for consecutive reactions (see text) where $A = 1.3$, $B = 2.333$, $K_1 = 0.12$, and $K_2 = 0.16$.

an average constant obtained. The formula for consecutive reactions may also be applied and the experimental curves duplicated.

Silver was used as silver nitrate. The curve showed an increase in the production of carbon dioxide followed by a decrease. Although the formula for a monomolecular reaction cannot be strictly applied to such a curve (see Fig. 6), yet a constant can be obtained by letting a equal either the maximum attained or the point where the descending limb crosses the 100 per cent line. Either method is purely arbitrary yet if used consistently will give comparable results, and as in the previous case will be a useful index of the speed of the toxic action. In this connection it should be noted that the formula for consecutive reactions will duplicate the curve of silver action, as in the case of copper and mercury (see Fig. 6). Although the curve with silver cannot by any means express a simple monomolecular reaction (for the latter cannot rise above 100 per cent), it can express the result of two consecutive reactions with different velocity constants.

In connection with the heavy metals experiments were performed with certain acids. The whole molecules seem to have considerable influence in the case of some of the aromatic organic acids such as salicylic, which proved to be highly toxic in a concentration of 0.001 M and which is practically undissociated. But the activity of acids of the mineral and aliphatic categories seemed to depend on ionization. Hydrochloric and nitric acids had the greatest effect while sulfuric was less active in the same molar concentration. In the case of HCl , H_2SO_4 , H_3PO_4 , trichloroacetic, and tartaric acids the effect of each was approximately proportional to the hydrogen ion concentration and not to the molecular concentration. Solutions of each at about the same pH value gave about the same velocity constant (see Fig. 7). The pH values were calculated from the molecular concentrations and the dissociation constants as obtained from Clark (1922).¹ With low concentrations of hydrochloric acid, which are known to be almost completely dissociated, the experimental curves were almost identical with those for copper and for mercury, and both the simple and consecutive reaction formulas will apply as in the other cases (see Fig. 8). Furthermore, the relation to concentra-

¹ Clark (1922), p. 462.

tion is the same. These results render it probable that hydrogen in the ionic form exerts a toxic action similar in nature to that of the heavy metals. If this assumption be made, then hydrogen may be classed with the metals and its action compared with theirs in the present investigation. If it is assumed that hydrogen acts in combina-

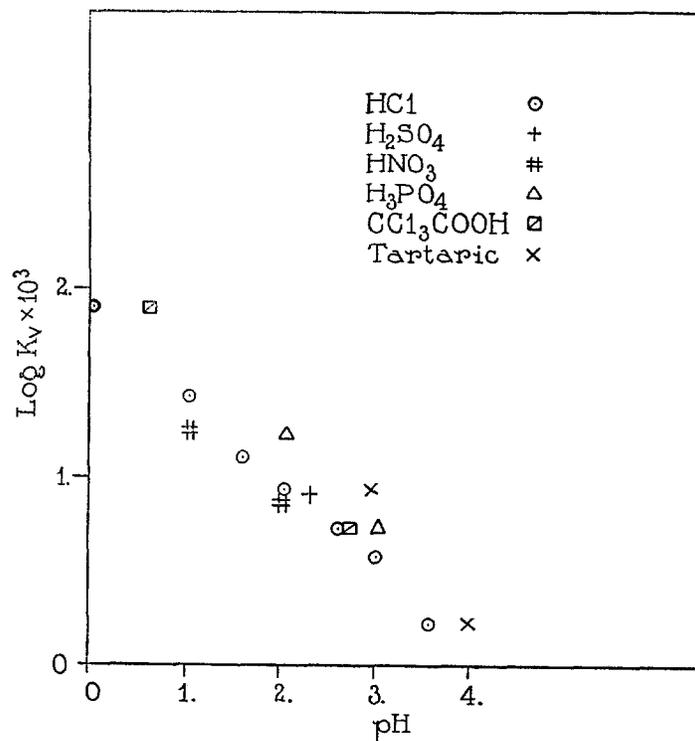


FIG. 7. The pH values of various acids plotted against the velocity constant.

tion then the comparison may still be made, for we may regard hydrogen chloride as comparable with cupric and mercuric chlorides and with silver nitrate.

IV.

We have next to inquire what is the effect on these curves of changes in temperature and concentration. Variations due to temperature were studied in the case of copper alone, but there is no

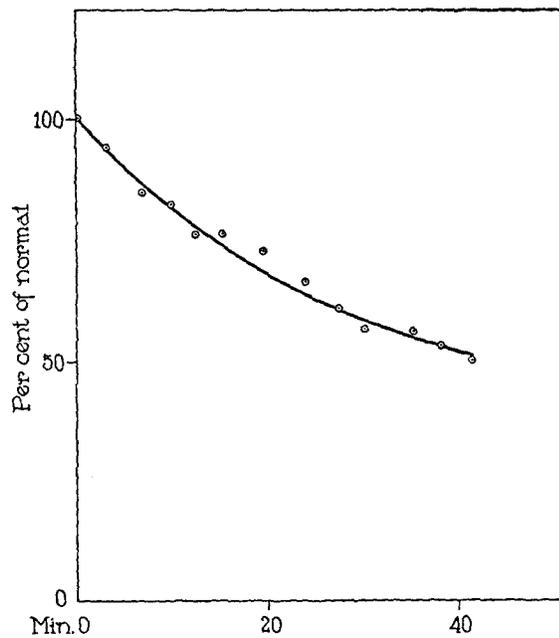


FIG. 8. Curve with hydrochloric acid 0.01 M.

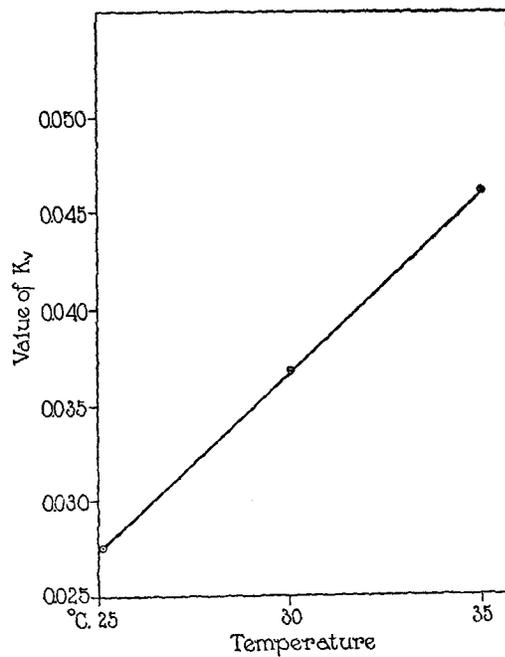


FIG. 9. The relation of temperature to the velocity constant of the action copper chloride.

reason to suppose that it affects the action of the other elements differently. The concentration effect was determined for each of the four elements.

The temperature of the respiration chamber was varied by means of the thermostat and several experiments were performed with copper chloride, keeping the concentration constant. The temperatures were then plotted as ordinates against the value of the velocity constants as abscissæ. The result (see Fig. 9) showed that the temperature coefficient of the toxic action was about 1.8 for this range of

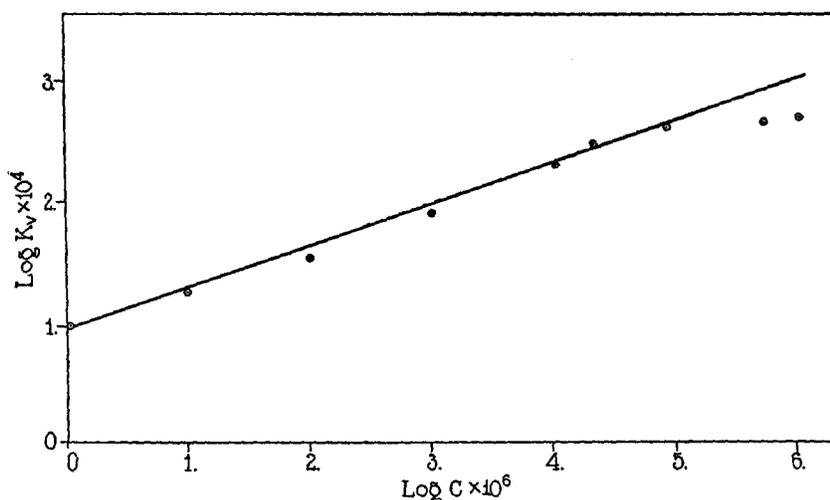


FIG. 10. The relation of the logarithm of the concentration to the logarithm of the velocity constant with copper chloride.

temperature (25–35°) at least. This is of the order commonly met with in chemical reactions and seems to exclude physical phenomena which usually have much lower coefficients but the work on temperature has been carried only far enough to determine the general order of the Q_{10} coefficient. The recent results of Crozier (1924–25) indicate that much further investigation would be profitable in this connection.

In studying the effect of concentration a range of 0.1 M to 0.000,001 M was investigated. With the other elements the concentrations lay between these values, but did not necessarily reach the extremes.

If, with any one element, the velocity constants for the curves are plotted against the concentrations as abscissæ the resulting curve is an hyperbola. When the logarithms are plotted the points fall very nearly on a straight line. In the case of copper, when high concentrations are reached the experimental line bends and tends to approach the horizontal (see Fig. 10). With the exception of this deviation, which will be again referred to, the relation may be expressed as follows: if v is the velocity, c the concentration, and b a constant, then $\log v$ is proportional to $\log c$ and $v = cb$. This is the well known power relation which has been derived biologically a number of times (Chick, 1908; Paul, Birstein, and Reuss, 1910) in work on the effect of toxic substances.

v.

The generally accepted theory of cell respiration is that the carbon-containing substances are oxidized by the instrumentality of catalysts. Opinion differs as to the nature of these catalysts and their mode of action. For present purposes, and until toxic action shall be better understood, it is necessary to assume merely that respiration occurs in a series of consecutive reactions, each step being catalyzed by a different substance or by the same substance in different states. All the seriously considered hypotheses make some such assumption.

The time honored theory of Engler and Bach states that oxygen is activated and handed on, as it were, by means of oxidases. The molecular oxygen is first brought into combination with an autoxidizable substance and by means of the energy furnished by this reaction forms also a peroxide. The active oxygen of the latter is handed on from one substance to another until it forms carbon dioxide, as the final number of a series of compounds thus: $O_2, AO_2, BO_2, \dots, CO_2$. The precise nature of the intermediate compounds has never been discovered, nor indeed has much of the mechanism been made clear, save that it is controlled by the oxidizing enzymes and proceeds by a series of consecutive reactions.

Wieland (1912, 1913, 1914) has developed the idea that the essential process in oxidation is not the activation of oxygen but of hydrogen. Thus a substance is not oxidized by the addition of oxygen but by the removal of hydrogen, the latter if necessary being fur-

nished by water. The pertinent enzymes are called by him dehydrogenases, and by Thunberg (1920, 1921) hydrogen transportases. According to this theory a series of reactions involving hydrogen can be constructed thus: $AH_2 \rightarrow BH_2 \rightarrow CH_2 \rightarrow \dots \rightarrow D$.

According to Hopkins (1921, 1922, and Meyerhof (1924) the activator of cell oxidations is some substance containing the sulfhydryl group $HS-SH$ (glutathione of Hopkins, cysteine, thioglycollic acid, etc.). The essential feature of this system involves the alternate addition and removal of hydrogen. This in a way depends on dehydrogenation and is effective through a series of consecutive reactions.

Warburg (1921, 1925) has recently developed the idea of iron as the active catalyst. According to him the sulfhydryl group is present but plays a subordinate rôle, and oxygen, not hydrogen, is activated. His scheme is of this sort: Bivalent iron + oxygen \rightarrow trivalent iron + organic material \rightarrow bivalent iron + oxidation products. This involves the alternate oxidation and reduction of iron and proceeds likewise through a series of consecutive reactions.

We may then assume that respiration does take place in successive steps, each step under the influence of a catalyzer, and we may draw up a conventional representation of the process as follows:

$A \xrightarrow{K_1} X \xrightarrow{K_2} Y \rightarrow Z$ where A represents the initial substance, Z the end-products, and X and Y intermediate stages.

To explain the mechanism of the toxic action of the heavy metals and of hydrogen the hypothesis is advanced that these substances alter the velocity constants of these reactions, either increasing or decreasing them. Mellor (1904) and Osterhout (1922) have given analyses of such reaction systems. If we have the substance X which is being converted into Y at a constant rate K_1 , and Y is being converted into Z at a constant rate K_2 , then the quantity of Y present will always be constant. The substance X is being replaced as fast as it is decomposed, from a precursor A . If any factor enters the system which changes the rates of formation of X and Y and Z the quantities of each component will change. For instance let the reaction $A \rightarrow X$ stop and let K_1 be increased. Then Y will increase because it is being formed faster than it is broken down. At the

same time X is being rapidly decomposed because it is no longer being formed from A . After it sinks below a certain level it will be unable to form Y as fast as Y is decomposed and, therefore, Y will decrease from this point on. Instead of increasing K_1 , we may increase K_2 . Then Y will be decomposed faster than it is formed and will decrease from the start. If both the constants are multiplied by the same factor the speed will be greater but the shape of the time curve of Y will be the same except that all the abscissæ will be divided by this factor.

Stated as a differential equation, $\frac{dY}{dt} = \frac{dX}{dt} - \frac{dZ}{dt} = K_1X - K_2Y$.

This may be integrated so that the quantity Y present at any time t will be expressed thus: $Y = A (e^{-K_1t}) + B \left(\frac{K_1}{K_2 - K_1} \right) (e^{-K_1t} - e^{-K_2t})$.

In this form of the integrated equation K_1 and K_2 are the velocity constants as explained above, A represents the amount of Y present when the equilibrium is disturbed by the toxic agent, and B represents the amount of X at the same moment. This is the previously mentioned formula for consecutive reactions, by the use of which the experimental curves may be duplicated. In the case of the cell, Y would represent the catalyst which activates directly the formation of carbon dioxide and when we measure the rate of production of carbon dioxide we have a direct index of the quantity of Y present.

In other words, when a metal interferes with the system $X \xrightarrow{K_1} Y \xrightarrow{K_2} Z$ so as to alter K_1 and K_2 , the quantity of Y changes according to the formula and the observed rate of carbon dioxide production changes in an identical manner.

One point of fundamental importance in this theory is that the metals are thought of as either inhibiting or accelerating the reactions governing the formation of the catalysts. If the metal promotes the formation of the catalyst it has an effect which might be compared to the so called *promoter action*. In speaking of catalysis by iron W. C. McC. Lewis (1923)² says, "The presence of a mere trace of

² Lewis (1923,) vol. i, p. 479.

certain oxides and salts . . . greatly enhances the activity of the catalyst proper. These are usually spoken of as promoters." Referring to the oxidation of ammonia to nitric acid he also says,³ "Recently iron has been employed as a catalyst in this process. The activity of iron is greatly enhanced by promoters such as cerium, thorium, bismuth, tungsten, or copper." The latter statement is particularly suggestive in the light of Warburg's theory of iron as the respiratory catalyst. The promoter action might be brought about in various ways, but for our present purpose we may regard it as due to the change in the quantity of the catalyzer Y . This in turn is caused by the action of the toxic agent in altering the velocity constants K_1 and K_2 .

VI.

A further extension of this hypothesis is necessary to account for the concentration effect of the metals. In the system $A \xrightarrow{K_1} X \xrightarrow{K_2} Y \rightarrow Z$ at the same time that K_1 and K_2 are altered by the metal we have assumed that the reaction $A \rightarrow X$ ceases altogether. The other two stages then continue and the decrease of Y usually resembles a monomolecular reaction inhibited or accelerated at the start, having the apparent velocity constant K_z . It is this apparent velocity constant which appears in the experimental data. It will be directly proportional to the amount of heavy metal present in active form and this renders it necessary to establish the relation between the metal in active form and the metal in the toxic solution as first applied to the cell. It may be assumed that after the metal, for instance copper, enters the cell, regardless of whether it penetrates in the ionic or in the molecular form, it encounters a substance which we may call T . With this substance the copper must combine chemically to form CuT if it is to alter the velocity constants of the reactions $X \rightarrow Y \rightarrow Z$. In other words, the copper must itself be activated before it can exert its characteristic toxic effect. We may note that according to Warburg iron must be activated in order to catalyze oxidation. The suggestion might be made that the activation of copper consists in a change of valence instead of a chemical combina-

³ Lewis (1923), vol. i, p. 480.

tion, but the kinetics of the toxic action are more easily treated on the latter basis than on the former.

It is justifiable to assume that the combination of copper with T is reversible. Indeed, most chemical reactions which are homogeneous, *i.e.* which do not involve a change of state, are reversible (Lewis, 1923).⁴ The reaction may be expressed in its most general form thus: $\text{Cu} + T \rightleftharpoons \text{CuT}$, but the concentration effect depends on the proportions of the components of the reaction.

Mention has been made previously of the fact that if, in the case of copper chloride, the concentration be plotted against the velocity constant (as derived from the monomolecular formula) the resulting curve is hyperbolic, and therefore if the logarithms be plotted the result will be a straight line, for all except the highest concentrations (see Fig. 10). We may ignore these highest concentrations for the present. Expressed mathematically $K_v = AC^B$ and $\log K_v = B \log C + \log A$. $\log A$ will be equal to the distance from the origin to the point where the line would cross the 0 ordinate if the line were so far extended. We shall see that on theoretical grounds we are not justified in so extending it, but for the present $\log A$ may be retained in the formula.

This equation has been derived by Chick (1908) and by Paul, Birstein, and Reuss (1910), and has been employed in various forms by investigators who have used other methods (Ikeda, 1897; Krönig and Paul, 1897; etc.). It seems, therefore, to have a general application to the biological effects of concentration. A numerical value can be obtained for both the constants A and B when two or more concentrations and their velocity constants are known.

$$\begin{array}{r} \log K_1 - \log A = B \log C_1 \\ - \log K_2 - \log A = B \log C_2 \\ \hline (\log K_1 - \log K_2) = B (\log C_1 - \log C_2) \end{array}$$

or

$$B = \frac{\log K_1 - \log K_2}{\log C_1 - \log C_2}$$

This value of B may be substituted and the value of A obtained. Using this method the value of B in the case of copper is 0.4.

⁴Lewis (1923), vol. i, p. 120 ff.

Referring now to Fig. 11 it will be seen that similar concentration curves can be obtained from the experimental data for mercury, silver, and hydrogen. This suggests that the action of all these elements may be subject to the same laws and that the general formula $K_v = AC^B$ may hold in all cases. A striking feature, furthermore, is that the slope of the logarithmic curves appears to be the same for each ion within the limits of experimental error. The exponent B expresses the slope of the curve, and if the slope is the same B is the same.

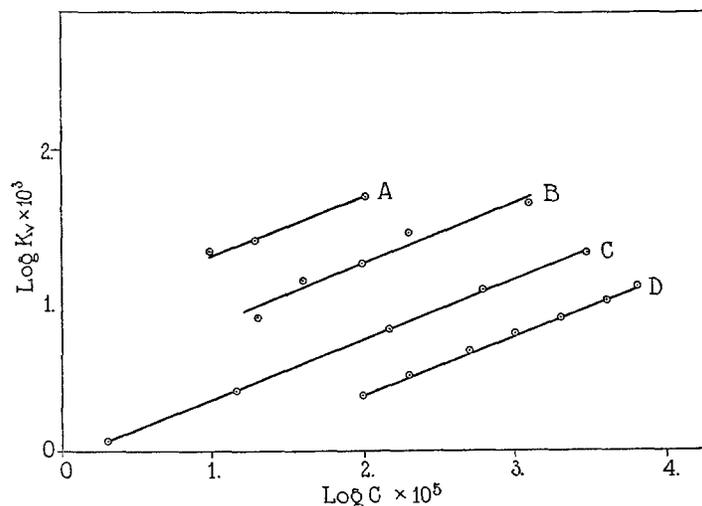
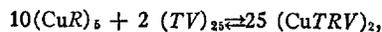


FIG. 11. The relation of the logarithm of the concentration to the logarithm of the velocity constant for: (A) AgNO_3 , (B) HgCl_2 , (C) CuCl_2 , (D) HCl .

Having reached this point in the analysis of the experimental data it is appropriate to return to the hypothetical reversible reaction and to see if by this method the experimental data can be explained or at least duplicated. Consider the case of copper and the reaction $\text{Cu} + T \rightleftharpoons \text{CuT}$. Since nothing is actually known of the substances entering into this reaction, it is permissible to assume any molecular proportions or any formulas for these substances that we wish. Certainly the substance T must be organic in nature and more or less complex. If we assume that the reaction runs as follows:



we can treat it as an ordinary reaction. R and V are used in combination with Cu and T because Cu_5 and T_{25} could scarcely exist alone as polyatomic ions but R and V are entirely neutral as far as the reaction is concerned. This point being understood we can write for simplicity: $10 \text{Cu}_5 + 2 T_{25} \rightleftharpoons 25 (\text{Cu}T)_2$. Now if the reaction proceeds to equilibrium, the equilibrium constant K_e will equal the ratio between the products of the reacting molecules on each side of the equation. In the simple reaction $\text{Cu} + T \rightleftharpoons \text{Cu}T$ the equilibrium constant K_e will equal $\frac{(\text{Cu})(T)}{(\text{Cu}T)}$, since there is one molecule each of Cu and T on the left side and one molecule of $\text{Cu}T$ on the right side. In the special case above we can divide the coefficients of both sides by the same number, and if we so divide by 5 we obtain the following equation: $2 \text{Cu}_5 + 0.4 T_{25} \rightleftharpoons 5 (\text{Cu}T)_2$. When this is in equilibrium $K_e = \frac{(\text{Cu}_5)^2 (T_{25})^{0.4}}{(\text{Cu}T)_2^5}$. Now if p be the number of molecules of Cu_5 at the beginning of the reaction, q the number of molecules of T_{25} , and at equilibrium r is the number of molecules of $(\text{Cu}T)_2$, then at equilibrium the number of molecules of Cu_5 is equal to $(p - r)$ and of T_{25} $(q - r)$. We may now write $K_e = \frac{(p-r)^2 (q-r)^{0.4}}{r^5}$. By rearranging and putting into the logarithmic form we obtain:

$$\log (p - r) \frac{\log K_e + 5 \log r - 0.4 \log (q - r)}{2}.$$

If we assume values for p , q , and K_e we can calculate the value of r . In this manner the following table is obtained ($K_e = 0.016$):

p	q	r
0.1001	1000	0.1
1.03	"	1
21	"	10
188	"	30
366	"	40
960	"	60
3546	"	100
19050	"	200
114000	"	400
742000	"	800
6313000	"	999.9

If the logarithm of p is plotted against the logarithm of r , Fig. 12, Curve B is obtained. This curve comes reasonably close to coinciding with the experimental curve for copper in the same range of concentration. If the proportions of the reacting substances were assumed with greater exactness a much more accurate duplication would be the result.

Regardless of the precise numerical value of the exponent, it is apparent that the relation is: $r = ap^b$ in which a is a constant. This is just the relation found where $K_s = AC^B$ as determined experimen-

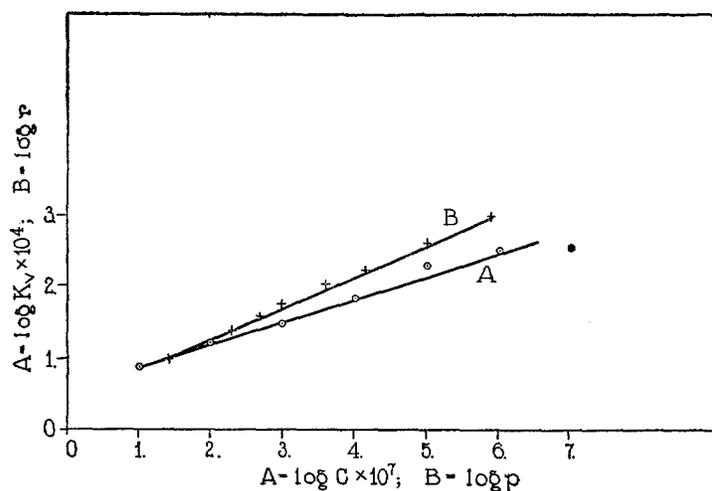


FIG. 12. Circles indicate the concentration effect with copper (*cf.* the middle portion of Fig. 10). The crosses are obtained by the use of the formula:

$$\log (p-r) = \frac{\log K_e + 5 \log r - 0.4 \log (q-r)}{2}.$$

tally. K_s corresponds to r , A to a , C to p , and B to b . The exponents B and b differ slightly, as explained above, because the assumed values in the calculation did not exactly fit the experimental case. In the experimental curve C represents the actual external concentration of the copper, in the calculated curve it represents the same thing. In the experimental curve K_s represents the speed of the toxic action; in the calculated curve r represents the amount of the compound CuT present at equilibrium. But it has been pointed out that the speed of the toxic action represented by K_s , or the change

in the rate of carbon dioxide production, is directly dependent on the amount of active copper (CuT) present. Thus although these considerations cannot be said to prove the hypothesis of a reversible reaction, nevertheless by that hypothesis the experimental concentration effect can be duplicated.

In the experimental curves of the metals and of hydrogen (see Fig. 11) it will be observed, as already noted, that the relation between $\log K_e$ and $\log C$ for each one appears to be linear, and that the lines are parallel. This indicates that the exponent B is the same in all cases but the coefficient A is different. That is to say, the lines if prolonged backward apparently would cross the 0 abscissa at various distances from the 0 ordinate. Furthermore, if prolonged forward they apparently would continue indefinitely without change of slope. There is no direct experimental evidence concerning the extensions of these curves and, this being the case, we are not justified in extrapolating the curves beyond the points actually obtained by experiment. If the lines should not cross the 0 abscissa in the manner we have assumed in evolving the formula $K_e = AC^B$, then we have no right to use the coefficient A except empirically, and then only in treating those portions of the curves which are furnished by the experimental data. In the calculated curve

$$K_e = \frac{(p-r)^2 (q-r)^{0.4}}{r^5},$$

or

$$\log (p-r) = \frac{\log K_e + 5 \log r - 0.4 \log (q-r)}{2}.$$

Now suppose we let r become smaller. Then r tends to approach the value of p as shown in the table (*e.g.* when $r = 0.1$, $p = 0.1001$) and in Fig. 13, Curve B where these points are plotted. The solid lines of the experimental region are extended as dotted lines backward past the intersection of the ordinates. If, on the other hand, r approaches q the curve will bend over, as indicated by the dotted line to the right in Fig. 13, Curve B , and p will become indefinitely large. Thus since the quantity of q is always the same, the quantity of r can never exceed it, no matter how great the concentration of p . That a similar situation exists experimentally is shown in Fig. 10,

where the points tend to approach a horizontal line at the right of the curve. It was pointed out previously that with the higher concentrations of copper the toxic effect ceased to increase rapidly with increase in concentration. We can here account for the phenomenon by saying that there is a definite amount of T (represented by $q = 1000$) in the cell which limits the amount of CuT which can be formed with increasing concentrations of copper. It is evident that we

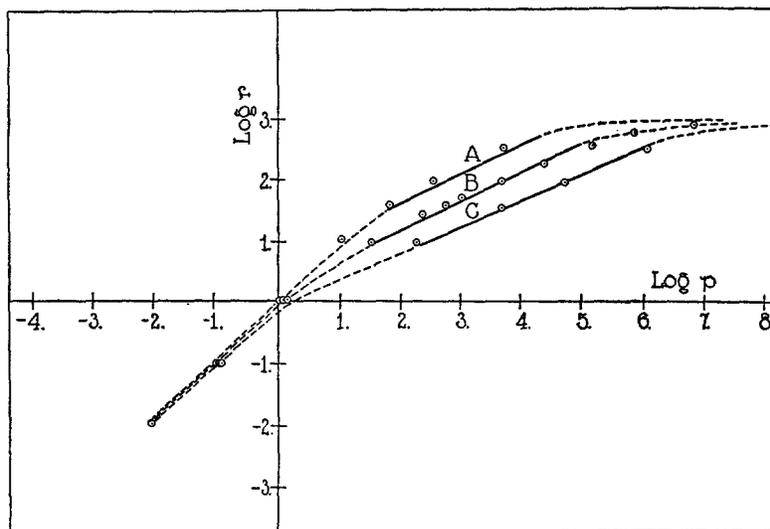


FIG. 13. Curves A , B , and C are calculated by means of the formula:

$$\log(p-r) = \frac{\log K_0 + 5 \log r - 0.4 \log(q-r)}{2}$$

where $K_0 = 0.00004$, 0.016 , and 2.5 respectively. Curve B is the same as in Fig. 12, Curve B . The solid lines represent the middle portions of the curves such as are obtained experimentally. The dotted portions represent the theoretical continuations of the curves when r approaches p and q .

should not be justified in extrapolating the graphs in Fig. 13 to the right as continuations of the straight lines.

Returning to the calculated curves in Fig. 13, the dotted portion to the left makes it clear that since $\log p$ and $\log r$ both approach minus infinity the curve will not cross the 0 ordinate at any such distance ($\log A$) from the 0 abscissa as a continuation of the experimental curve would lead us to suppose. It will actually cross below

the intersection of the ordinates. It is apparent from an inspection of the experimental and calculated curves that the experimental data give us the middle portion of the entire curve and that the middle portion approximates a straight line. For this portion, therefore, the relation $K_s = AC^B$ holds, and we may use it, as we use the formula for the monomolecular reaction, as sufficient working index to the concentration effect. But we cannot consider it to be the precise relation throughout the entire range of concentrations from zero to infinity. The exponent B will differ at very small and at very great concentrations, and since the value of A represents an extension of the straight line which does not really occur, the simple power relation can be of value only empirically and with a limited range of concentrations.

The experimental curves with the other metals (Fig. 11) likewise appear to be straight lines. But we know from the case of copper that their continuations will not be straight. Probably the experimental data have given a portion of each which is approximately straight. However, the fact that they do not coincide in this range of concentrations shows that they differ to the extent that for a given concentration the toxic effect of some is greater than that of others. On the basis of the experimental curves alone this variation in toxicity would be referred to differences in $\log A$. This would provide a working basis for the comparison of the elements, but it would furnish no clue to the nature of the differences between them. And on theoretical grounds $\log A$ is an unsafe criterion since the use of it depends on an arbitrary extrapolation of the curves. A sounder criterion is the equilibrium constant of the reaction $M + T \rightleftharpoons MT$ in which M stands for any cation.

Since the curves show that for a given concentration the toxic effect of the elements varies, we can take this to mean that more MT is formed by some than by others. In order for more MT to be formed by the same concentration of M the ratio $\frac{(M)(T)}{(MT)}$ must be smaller. This means a difference in the equilibrium constants of the reaction. There is no method for determining the equilibrium constants of the experimental curves directly, but we may start with the calculated curve for copper (Fig. 12, Curve B) and try to duplicate the curves

of the other metals. In Fig. 13 there are three curves calculated by the usual formula using $K_e = 0.00004, 0.016,$ and 2.5 . It was previously pointed out that the middle portions of each (solid lines) are approximately straight lines and are parallel, whereas the extremes (dotted lines) are unquestionably curved and converge as p approaches zero and infinity. If we had only the middle portions we

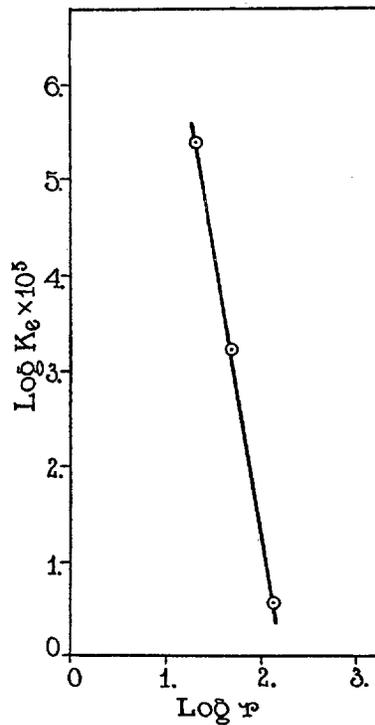


FIG. 14. Points obtained from the curves of Fig. 13 when $\log p = 3$. $\log K_e \times 10^5$ is plotted against $\log r$ when $\log p$ is thus fixed.

should be very close to the experimental curves for mercury, copper, and hydrogen, and should consider the calculated curves to be straight lines.

If we consider, in the calculated curves, a given concentration, say that represented by 1000 ($\log 1000 = 3$ on the curve), and plot the logarithm of r against the logarithm of K_e we obtain Fig. 14.

Apparently here also is a power function, since the relation of the logarithms appears to be inversely linear. Then

$$\log K_e = -n \log r, \quad \text{or} \quad K_e = r^{-n}.$$

If we take different values for r :

$$\frac{\log K_{e1} = -n \log r_1}{\log K_{e2} = -n \log r_2} \\ \frac{-}{(\log K_{e1} - \log K_{e2}) = -n (\log r_1 - \log r_2)}$$

or

$$\left(\frac{K_{e1}}{K_{e2}}\right) = \left(\frac{r_1}{r_2}\right)^{-n} \quad \text{or} \quad \left(\frac{K_{e2}}{K_{e1}}\right) = \left(\frac{r_2}{r_1}\right)^{-n}.$$

Now r in the calculation is equivalent to MT , or the amount of active metal, and this in turn is proportional to the speed of the toxic action K_v . Therefore we may substitute K_v for r and write:

$$\left(\frac{K_{e2}}{K_{e1}}\right) = \left(\frac{K_{v2}}{K_{v1}}\right)^{-n} \quad \text{or} \quad K_{e2} = \left(\frac{K_{v2}}{K_{v1}}\right)^{-n} \times K_{e1}.$$

In the original calculated curve of copper (Fig. 12, Curve *B*, and Fig. 13, Curve *B*) the constant $K_e = 0.016$ was assumed. We now wish to find K_e for mercury, using the experimental data. This we may do for any concentration, say 0.001 M. Making the appropriate substitutions for K_{v1} and K_{v2} , from actual experiments, we get $K_{e2} = \left(\frac{0.028}{0.0085}\right)^{-n} \times 0.016$. If we let $n = 5$ and solve, we get $K_{e2} = 0.00005$ where the calculated value was 0.00004 (Fig. 13, Curve *A*). Similarly, using the experimental data for hydrogen, $K_{e2} = 1.39$ where the value used in calculating Fig. 13, Curve *C*, was 2.5. These values are fairly close though not as close as might have been obtained had the experimental curves been exactly duplicated by the calculated ones. They are, however, of the same order of magnitude. Furthermore the relation $K_e = r^{-n}$, as with the relation $K_v = AC^B$, cannot hold with very small or with very great values of r . It holds empirically, however, for the middle ranges of concentrations which are subject to experimental analysis. This fact being granted, we may use this formula, as well as the other, in analyzing experimental data.

VII.

Any hypothesis must account for as many as possible of the observed facts. It is believed that in this instance the facts may be explained by assuming a reversible reaction which activates the toxic elements (Cu, Ag, Hg, H) and enables them to inhibit or destroy in a characteristic manner the normal oxidation catalysts. The various phenomena observed in connection with this action are explicable on the assumption of appropriate reaction velocities. This hypothesis is advanced solely as a working model and no claim is made for its ultimate truth. However, it provides a convenient and rational way in which to correlate a large number of otherwise isolated and unrelated facts. An attempt has been made to put all relations on a purely chemical basis. This has been done deliberately and in the light of the knowledge that alternative theories might well be advanced. For instance, many of the phenomena here recorded could be treated from the point of view of surface action or adsorption and some of the curves duplicated; notably, the curve of concentration effect. But it is the opinion of the writer that an explanation from a purely chemical point of view accounts for more of the results and is in general more satisfactory.

SUMMARY.

1. The effect of the heavy metals on the respiration of *Aspergillus niger* is to cause the rate of carbon dioxide production to decrease from the first or to increase and subsequently diminish.
2. The speed of the toxic action varies as a constant power of the concentration.
3. The temperature coefficient of the toxic action is between 1.5 and 2.
4. An hypothesis is advanced to account for the action of the heavy metals, by means of which the experimental results may be accounted for. It is assumed that the metal is activated by a chemical combination with a cell constituent. This active compound alters the velocity constants of the normal respiratory reactions, and thus causes the observed changes in the rate of carbon dioxide production.

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