

COMPARATIVE STUDIES ON RESPIRATION.

XXVII. THE MECHANISM OF OXIDATION IN RELATION TO CHLOROFORM ANESTHESIA.

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The preceding papers (1) in this series have shown that chloroform produces similar effects on the rate of production of CO_2 by living and killed tissue and by unsaturated organic acids. In the case of dead tissue and organic acids, the system consisted of oxidizable material, H_2O_2 and $\text{Fe}_2(\text{SO}_4)_3$. The experimental results indicate that similar processes are taking place in all cases. In this paper an attempt is made to verify this statement and to show that a mathematical interpretation of the curves indicates that the reactions are all of the same order.

There are certain considerations that must be taken into account in order to derive a formula which will approximate the conditions found experimentally. It is necessary to assume that there is present in the reaction mixture, before the addition of chloroform, a complex consisting of two, or possibly three, of the components of the reaction. It is plausible to assume that this condition exists in the reaction, for it is quite evident that here we have to deal with an "induced reaction." Mellor (2), in discussing this type of reaction, points out that it may be considered as taking place in two ways; either that the reaction is catalyzed by the presence of the third reacting substance, which may mean the formation of a complex, or that two (or possibly three) of the constituents of the reaction combine.

In the case which we are discussing—oxidation by means of H_2O_2 and $\text{Fe}_2(\text{SO}_4)_3$ —we consider that the complex is being broken down at a constant rate, with a production of CO_2 directly proportional to the rate of decomposition of the complex. We assume that the reaction starts from a substance, A , which in turn goes into the com-

plex (X), which is the factor concerned with the production of CO_2 ; and that X upon disintegration is transformed into B , which is non-reactive.

The addition of chloroform introduces a fourth factor into the reaction with the result that the changes taking place in the complex are definitely affected. Such a condition may exist if the chloroform acts as a catalyst. At the same time chloroform need not be considered to be entirely indifferent chemically for it is possible that it may unite with certain substances to form addition compounds. This is indicated by the work of Moore and Roaf (3) who worked with serum, and by that of Krüger (4) in the case of hemoglobin. If the action of the chloroform is catalytic in nature it does not preclude the conception that there is a loose combination with some constituent of the system.

The curve of the rate of production of CO_2 after the addition of chloroform is very much like the curve found by Rutherford and Soddy (5) for the decay of thorium compounds. These authors found that when the activity of a thorium compound is plotted against time, the resulting curve rises and falls just as in the case of the action of chloroform on the production of CO_2 . If we assume that the reactions $A \rightarrow X$ and $X \rightarrow B$ are monomolecular (and irreversible, or practically so) we may apply the formula developed by Rutherford and Soddy. This formula is

$$X_1 = \frac{A_0 k_1}{k_2 - k_1} (e^{-k_1 T} - e^{-k_2 T})$$

where X_1 is the concentration of the complex, X , produced from A during the time, T , after the addition of the chloroform; k_1 is the velocity constant of the reaction, $A \rightarrow X$, and k_2 of the reaction, $X \rightarrow B$; and e the base of natural logarithms. This formula gives the quantity of X without taking into consideration the amount present at the instant the chloroform was added. The amount of X present at this time diminishes (by the reaction $X \rightarrow B$) after the chloroform is added; if no formation of X occurred the amount remaining at the time, T , would be

$$X_0 = e^{-k_2 T}$$

where X_0 is the amount of X at the instant the chloroform was added, and T is the time which has elapsed since the addition of the chloroform.

To represent the whole process it is necessary to combine the formulæ given above. In this case let X_T be the total amount of X present in the solution at the time, T . Since we have given the experimental data in terms of per cent it will be necessary to multiply the value of X_T by a constant and to add a constant when the curve does not fall to zero. These constants will vary with the type of material used in the experiments.

The final formula will be

$$X_T = \left[X_0 e^{-k_2 T} + \frac{A_0 k_1}{k_2 - k_1} (e^{-k_1 T} - e^{-k_2 T}) \right] K + K'$$

This formula is practically the same as the one developed by Osterhout (6) for the resistance of living tissue.

In the calculations which follow it is assumed that at equilibrium (before the addition of chloroform) the ratio of X_0 to A_0 is as 1 to 30, and that the value of A_0 is 9. Therefore, the value of X_0 will be 0.3. The values of the constants K and K' are determined as follows: The base line of the curve is taken as the point at which the rate of production of CO_2 becomes constant. The difference between this and the normal rate of production of CO_2 (which is taken as 100 per cent) is divided by 0.3, thus resulting in the value of K . K' is the difference between the base line of the curve and the time axis.

The application of this formula to the experiments published in the previous papers shows some very interesting results.

Fig. 1 shows a curve obtained by averaging the curves which show the effect of chloroform on the rate of production of CO_2 by oleic, fumaric, and cinnamic acid. The calculated curve is shown as a broken line. The constants used in this case are $k_1 = 0.05$, $k_2 = 0.5$, $K = 133$, and $K' = 60$.

Fig. 2 gives the curves for the rate of production of CO_2 from killed *Ulva* in the presence of H_2O_2 and $\text{Fe}_2(\text{SO}_4)_3$, as compared with the calculated curves. The broken lines are the calculated curves and the solid lines the experimental. Curve A represents the effect of 1 per cent chloroform when 10 cc. of 0.002 M $\text{Fe}_2(\text{SO}_4)_3$ are present.

The constants for the calculation are $k_1 = 0.256$ and $k_2 = 2.5$. Curve B represents the effect of chloroform when 20 cc. of the iron solution are used. The constants in this case are $k_1 = 0.02$ and $k_2 = 1.00$. In both instances the values of K and K' are 200 and 50, respectively.

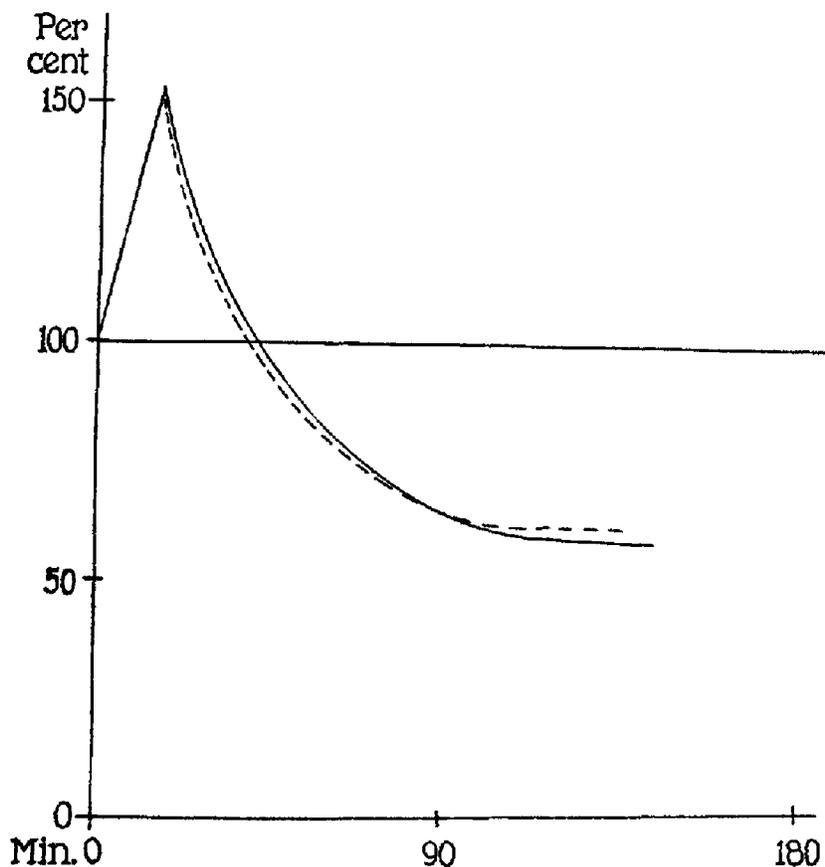


FIG. 1. The effect of chloroform on the rate of production of CO_2 by cinnamic, oleic, and fumaric acids. The solid line is the average curve of the experiments with these acids. The broken line is the value of X_T at various times (T) when the velocity constants are $k_1 = 0.05$ and $k_2 = 0.5$. K and K' are 133 and 60, respectively.

Fig. 3 gives the experimental and the calculated curves for the action of chloroform on the rate of production of CO_2 by living *Ulva*. A is the curve for the action of 0.5 per cent chloroform, and B the

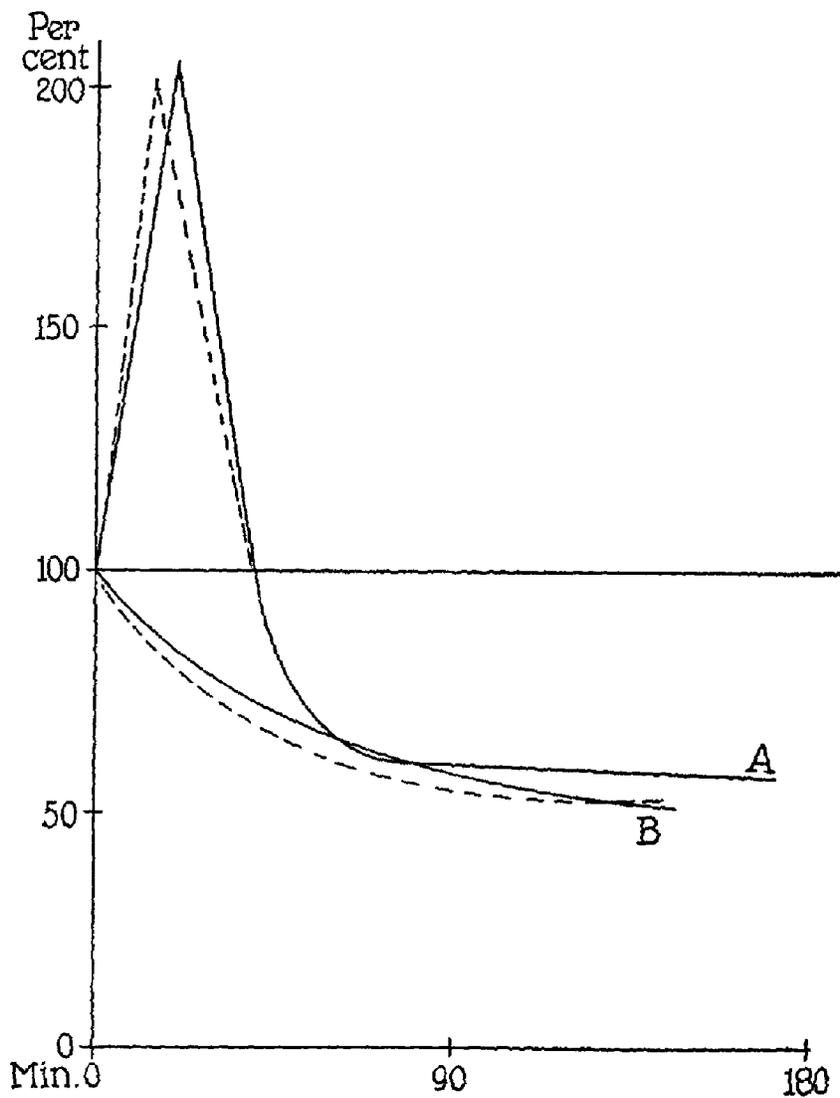


FIG. 2. The calculated (broken line) and the experimental (solid line) curves of the effect of chloroform on the rate of production of CO_2 by killed *Ulva* when treated with H_2O_2 and $\text{Fe}_2(\text{SO}_4)_3$.

- A. 10 cc. of iron. $k_1 = 0.256$, $k_2 = 2.5$, $K = 200$, and $K' = 50$.
 B. 20 " " " $k_1 = 0.02$, $k_2 = 1.00$, $K = 200$, " $K' = 50$.

curve for 0.25 per cent. The values for k_1 and k_2 in the case of A are 0.0186 and 0.54, respectively, and 0.018 and 0.4, in that of B. The values of K and K' are 266 and 20, respectively.

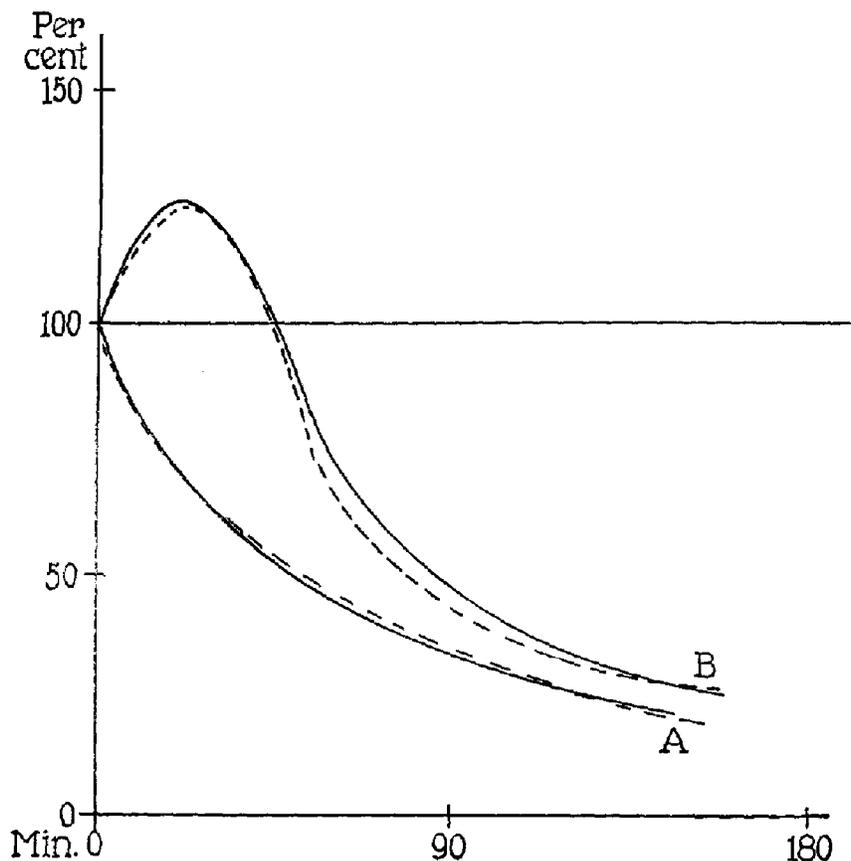


FIG. 3. The effect of chloroform on the rate of production of living *Uta*. A is the action of 0.5 per cent chloroform, and B is the effect of 0.25 per cent. The solid lines are the experimental curves and the broken lines the calculated values of X_T . In A, $k_1 = 0.0186$ and $k_2 = 0.54$. In the case of B, $k_1 = 0.018$, and $k_2 = 0.4$. In both A and B, $K = 266$ and $K' = 20$.

As may be seen from the figures, there is a close agreement between the calculated and the experimental curves. This would seem to indicate that the same process is taking place in each case. At the

same time it must not be considered that the fact that we have a formula based on consecutive monomolecular reactions means that these are the exact processes taking place. It is only feasible to conclude that there are two processes going on simultaneously in the reaction.

There may be two interpretations of these facts. We may assume that the action of chloroform is catalytic or that a new compound has been formed.

There is some evidence to show that the action of chloroform may be catalytic. The fact that chloroform accelerates production of CO_2 in low concentration and hinders at higher concentration has many analogies. Thus, Hoyer (7) found that certain acids in low concentrations hastened the action of the enzyme. In higher concentrations the effect of the acid was to retard the action. This may be true in the experiments considered here. It is possible that the chloroform as it combines with the complex tends to accelerate the production of CO_2 , and that as the amount of chloroform in the complex increases, decomposition is inhibited. It has been found that when a small concentration (0.5 per cent) is used there is no drop below the normal rate of production of CO_2 , although one still finds the increase.

In this connection attention may be called to the experiments of Acton (8), who found that ether catalyzed the oxidation of arsenious acid by the air. The results of the experiments reported in the preceding papers show that ether can increase the rate of production of CO_2 from tannic and fumaric acids. Chloretone and chloral hydrate have a similar effect.

It would seem, therefore, from a consideration of the calculations together with the evidence cited from the literature, that the effect of chloroform in these experiments may be chemical in nature. Whether this conception will apply to other narcotics, especially those known as indifferent narcotics, is a matter for further experimentation.

It is evident that certain widely accepted theories of anesthesia fail to explain these results. It does not seem probable that lipid solubility could play a part in this reaction since it has been shown that the process will take place in solutions where lipoids are absent. Such is also the condition in regard to the possible effect on the

colloids. It has also been considered that narcotics owe their action to the changes produced in the membranes of the cell. Without doubt this may play a part in certain life processes, but the effect of chloroform on the oxidative system of the cell seems to be concerned directly with that system and to be distinct from the effects on permeability.

SUMMARY.

A mathematical analysis of the effects of chloroform on the production of carbon dioxide by living *Ulva*, killed *Ulva*, and unsaturated organic acids indicates that the same process is taking place in all three cases. On the basis of this interpretation it is reasonable to conclude that the action of chloroform on the oxidative mechanism of the cell is chemical in nature and that it acts either by catalysis or by the formation of a loose compound with some portion of the system.

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