COMPARATIVE STUDIES ON RESPIRATION.

XXV. THE ACTION OF CHLOROFORM ON THE OXIDATION OF SOME ORGANIC ACIDS.

BY GEORGE B. RAY.

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

(Received for publication, March 19, 1923.)

In a previous paper (1) it has been shown that *Ulva* which has been killed by drying and subsequently treated with H_2O_2 and $Fe_2(SO_4)_3$ may be made to produce CO_2 after the manner of the living organism, and that the rate of production of CO_2 may be affected by the presence of chloroform.

In order to throw some light on the mechanism of these reactions experiments similar to those described in the preceding paper of this series were undertaken, in which various organic acids were used in place of killed *Ulva*.

The first substance studied was oleic acid. In this experiment, the results of which are shown in Fig. 1, 50 cc. of the acid were placed in the reaction flask of the apparatus, and the rate of production of CO_2 was determined. Section AB of the curve shows that some CO_2 is produced. The time necessary to change the indicator tube from pH 7.78 to 7.36 was about 10 minutes. The addition of 25 cc. of approximately molar H_2O_2 caused the rate to increase. This is shown in Section BC. When $Fe_2(SO_4)_3$ is added to the mixture of acid and peroxide, one finds another increase, as is shown in Section CD.

Since colloidal metals are also known to act as oxygen catalysts, a solution of colloidal silver was also tested. This was prepared in the following manner. A direct current of 110 volts was allowed to pass between two silver electrodes placed in distilled water. The resulting dark brown solution was used as the catalyst. Its action is shown in Section CE of Fig. 1. As may be seen the effect is similar to that of iron. Since such solutions are difficult to standardize it was decided to use iron in further experiments.

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Tannic acid was the next substance tested; it was found to act in the same manner as oleic acid. In order to show the relation of the components of the mixture to the production of CO_2, varying concentrations of the constituents were placed in the reaction flask and the rate determined after 1 hour.

The composition of the mixtures is represented according to the method of Roozeboom (2) by means of an equilateral triangle (Fig. 2),

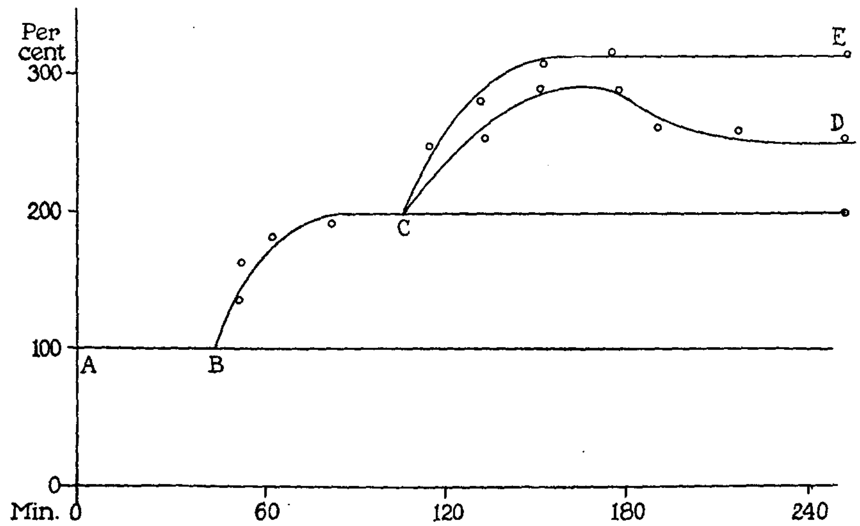

FIG. 1. The effect of the addition of H_2O_2 and the subsequent addition of either $Fe_2(SO_4)_3$ or colloidal silver to oleic acid. Section AB represents the rate of production of CO_2 by oleic acid alone (arbitrarily called 100 per cent); Section BC is the effect of the addition of 3 per cent H_2O_2; Section CD the effect of 25 cc. 0.0004 M $Fe_2(SO_4)_3$; and Section CE the addition of 25 cc. of a colloidal silver solution. The points represent the average of five experiments. The probable error of the mean is less than 5 per cent of the mean.

the apices of which represent (*A*) 100 per cent tannic acid (molar), (*B*) pure H_2O_2 (molar), (*C*) pure $Fe_2(SO_4)_3$ (0.0002 molar). Points on the sides of the triangle represent mixtures of the two solutions indicated by the adjacent apices. Therefore point G would represent 75 per cent H_2O_2 and 25 per cent $Fe_2(SO_4)_3$; H represents 50 per cent of each; I, 25 per cent of the peroxide and 75 per cent of the iron salt. In the same way P indicates 75 per cent H_2O_2 and 25 per cent tannic acid; E, 50 per cent of each; F, 25 per cent of the peroxide and 75 per cent of the acid. Points in the interior of the triangle represent mixtures of all three of the constituents. Thus O would indicate a mixture consisting of 50 per cent iron since it is on line HK; it is also on line PI which represents 25 per cent H_2O_2, and on line F which shows it to have 25 per cent tannic acid. In this manner the relation of the constituents of the system to each other may be defined. The ordinates, which represent the reciprocal of the time of production of a unit amount of CO_2, are plotted perpendicularly to the plane of the triangle. This gives a solid, as shown in Fig. 3.

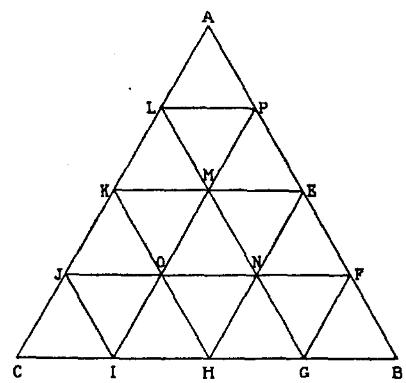

FIG. 2. The composition of the mixtures of tannic acid, H_2O_2, and $Fe_2(SO_4)_3$ according to Roozeboom's method. For explanation see text.

From these data it will be seen that tannic acid alone produced no CO_2, but on the addition of H_2O_2 a small amount is given off. $Fe_2(SO_4)_3$ alone does not oxidize the tannic acid, but when added to a mixture of H_2O_2 and tannic acid considerable CO_2 is produced. The rate of production of CO_2 is proportional to the amount of iron. The temperature at which these experiments were conducted was $20° \pm 2°C$. The probable error of the mean is less than 5 per cent of the mean.

Succinic acid and its unsaturated homologues, fumaric and maleic acids, present an interesting group for experimentation. First of all, they differ from the preceding compounds inasmuch as they give true solutions. They also form a series in themselves since they comprise a saturated acid and the cis- and trans- forms of the corresponding unsaturated acids. They are especially interesting from the biological point of view since fumaric and succinic are found in muscle tissue, while maleic is not. Verkade and Söhngen (3) found

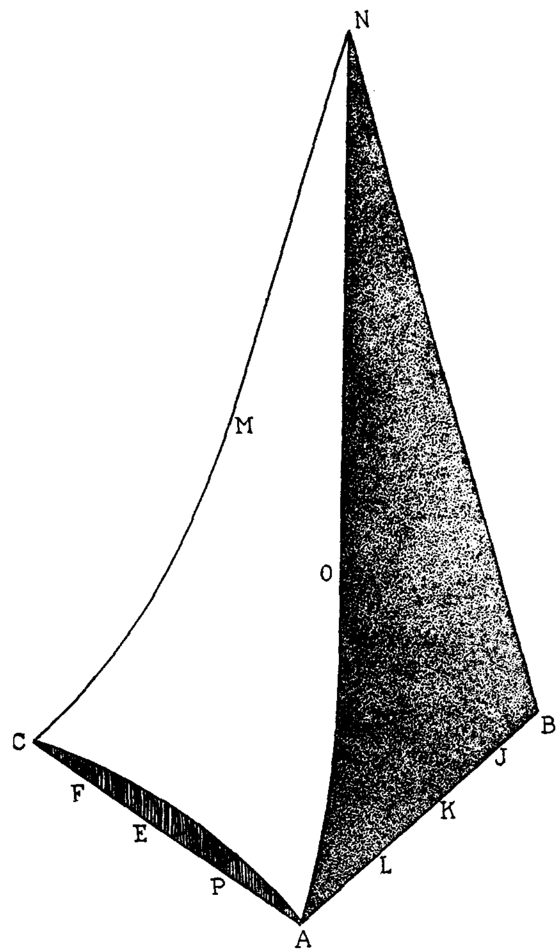

FIG. 3. The rate of production of CO_2 by the mixtures represented in Fig. 2.

that fumaric acid is attacked by *Aspergillus* and *Penicillium* in the presence of an excess of $CaCO_3$, while maleic is not. This was also found to be true of other cis- and trans- isomers.

Fig. 4 shows the results of these experiments in which the same technique was used as in the case of the previous experiments. It is interesting to note that the acids which occur in the organism

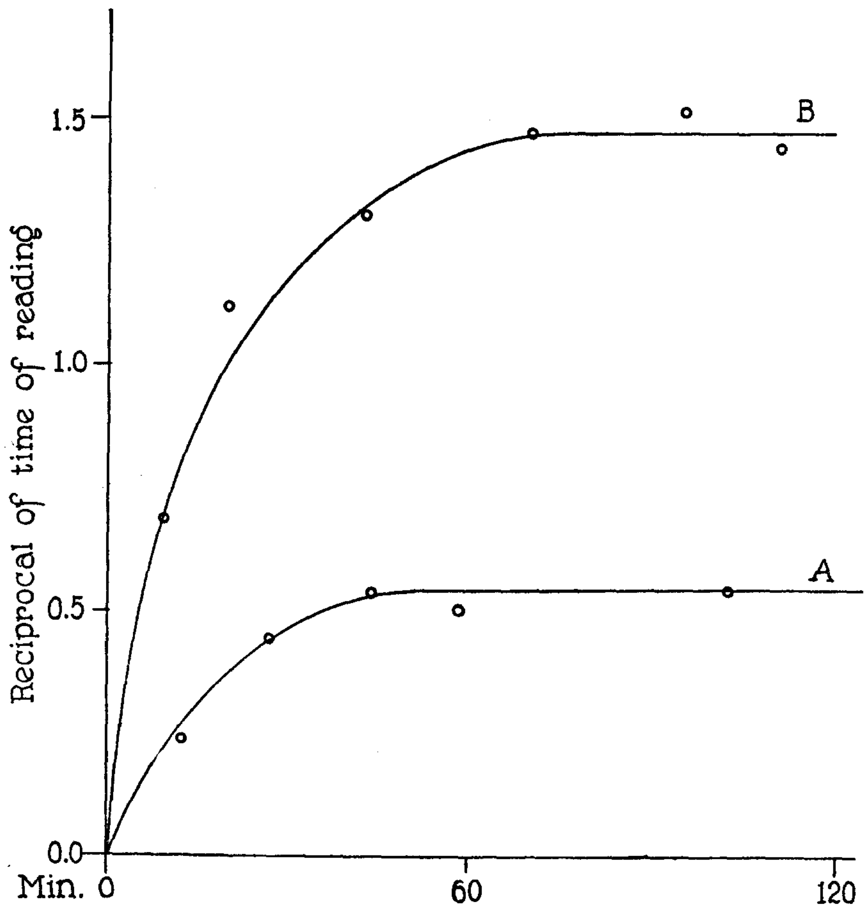

FIG. 4. The production of CO_2 by succinic (Curve A) and fumaric (Curve B) acids when treated with H_2O_2 and $Fe_2(SO_4)_3$. The curves are the average of five experiments. The probable error of the mean is less than 5 per cent of the mean.

are oxidized by H_2O_2 and $Fe_2(SO_4)_3$, while maleic acid did not produce sufficient CO_2 to be measured. These compounds were used in 0.2 M solutions or suspensions.

The following compounds were also tested and found to produce CO_2 in measurable quantities: pyrogallol, lecithin, cinnamic acid, hydrocinnamic acid, and glycocoll.

In all cases the hydrogen ion concentration of the mixture was between pH 3 and 4. This was determined by the use of indicators, and in case the compounds were turbid or dark colored the following procedure was used. The mixture was placed in a diffusion thimble and this was placed in a beaker of distilled water. After 1 hour the concentration of the hydrogen ions in the water was determined. This gave the pH of the reaction mixture since the buffer action was great enough to permit dilution without interference with the actual H ion concentration of the mixture.

It may be seen, therefore, that it is possible to oxidize certain organic compounds in the presence of H_2O_2 and $Fe_2(SO_4)_3$ and obtain sufficient CO_2 to measure by the indicator method. What is the significance of this process in relation to biological oxidations? Apparently the best way to attack this problem is to subject the system to various conditions that affect the respiration of the cell, and to observe whether their response (in the production of CO_2) resembles that of the organism under similar conditions.

It has been shown that the production of CO_2 by *Ulva* is decidedly affected by chloroform, and that tissue which has been killed and then treated with H_2O_2 and $Fe_2(SO_4)_3$ reacts in the same general way (1). It is also well known that narcotics can affect chemical oxidations. Bigelow (4) found that the oxidation of Na_2SO_3 by air was inhibited by narcotics. He used copper as a catalyst. Titoff (5) extended this work with similar results. Attention may be called, incidentally, to the fact that Bredig and von Berneck (6) found that ether, ethyl alcohol, and amyl alcohol inhibited the destruction of H_2O_2 by colloidal platinum, although this is not an oxidation. Centnerszwer (7) worked on the oxidation of phosphorus to phosphorus trioxide under the influence of steam and found this reaction to be affected by narcotics. Warburg (8) showed that the oxidation of oxalic acid by blood charcoal was arrested by the presence of members of the urethane series. Vernon (9) and Baer and Meyerstein (10) have shown that narcotics inhibit the action of tissue oxidases on organic compounds. Battelli and Stern (11) found that tissue oxidases (they worked with muscle extract) oxidized pyrotartaric acid to maleic; this action was arrested by the action of an anesthetic.

If it is possible to vary the rate of production of CO_2 by means of the addition of an anesthetic, we may be able to gain an insight into the action of the narcotic on the respiratory system of the organism.

Fig. 5 shows the effect of various anesthetics on the rate of production of CO_2 by tannic acid when it is treated with H_2O_2 and $Fe_2(SO_4)_3$. The general technique of these experiments was as follows: A mixture of 50 cc. of 10 per cent tannic acid, 25 cc. of 3 per cent H_2O_2, and 25 cc. of 0.0004 M $Fe_2(SO_4)_3$ was placed in the reaction flask of the apparatus for measuring the rate of production of CO_2, and a current of air free from CO_2 swept through for 1 hour. Then a series of readings was made to determine the "normal" rate. For convenience this is called 100 per cent. Then the reagent was added and the rate was determined as often as possible until the reaction appeared to be over. The curves are the result of five experiments. The probable error of the mean is less than 10 per cent of the mean.

Curve A shows the effect of ether on the rate of production of CO_2. The amount of the anesthetic added was 10 per cent by volume. It will be seen that the effect of ether is to cause an increase in the rate of production of CO_2. In this connection it may be mentioned that Acton (12) found that ether would catalyze the oxidation of arsenious acid by air. Curve B shows the effect of the addition of 1 per cent chloroform (by volume). The effect in this case is to cause a decrease in the rate of production of CO_2. This is followed by an increase and then by a decrease. The effect of chloretone is shown in Curves C and D. Curve C is the effect of the addition of 5 per cent of a saturated solution of chloretone; Curve D is the effect of 10 per cent. As may be seen, both concentrations cause an increase in the rate of production of CO_2. Curve E shows the effect of adding 5 per cent of a 20 per cent solution of chloral hydrate. This also causes an increase in the rate.

It might be thought that the increases that have been observed in the preceding experiments were due to the oxidation of the anesthetic. That this is not the case is shown by the fact that in control experiments (without tannic acid) there was no production of CO_2.

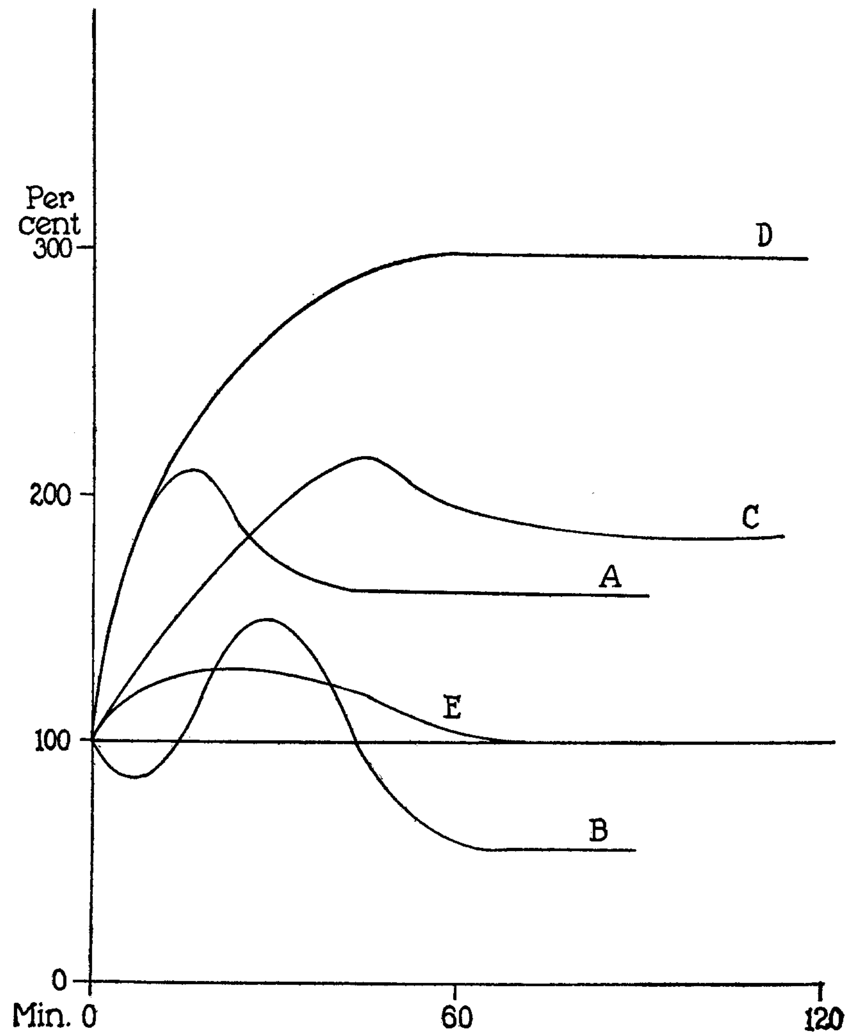

FIG. 5. The effect of various anesthetics on the rate of production of CO_2 by tannic acid in the presence of H_2O_2 and $Fe_2(SO_4)_3$. Curve A shows the effect of the addition of 10 cc. ether; Curve B of the addition of 1 cc. chloroform; Curve C of 5 cc. saturated chloretone; Curve D of 10 cc. of the chloretone solution; and Curve E the effect of 5 cc. of a 20 per cent solution of chloral hydrate. Each curve is the average of three experiments. The probable error of the mean is less than 10 per cent of the mean.

In planning further experiments it was thought desirable to concentrate effort on the study of one anesthetic. For this purpose chloroform was chosen since it is believed to be the most inert chemically.

Fig. 6 shows the effect of 1 per cent chloroform (by volume) on oleic and cinnamic acids. Curve A is the effect of the narcotic on oleic acid, and Curve B the effect on cinnamic acid. In both cases it is practically the same; there is a rise in the rate, which is followed by a fall. In the case of oleic acid, the mixture consisted of 50 cc. of the acid, 25 cc. of 3 per cent H_2O_2, and 25 cc. of 0.0004 M $Fe_2(SO_4)_3$. The mixture in the case of cinnamic acid was the same with the exception that 50 cc. of a 1 per cent suspension of cinnamic acid were used in place of the oleic acid.

Fig. 7 shows the effect of anesthetics on the rate of production of CO_2 by a mixture of fumaric acid, H_2O_2, and $Fe_2(SO_4)_3$. The amount of the acid was 50 cc. of 0.2 M. The concentrations of the other substances are the same, as in the preceding experiments. Curve A shows the effect of 1 per cent chloroform (by volume) and Curve B is the effect of 0.5 per cent. Both concentrations cause an increase in the rate, but only in the case of the greater concentration is there a drop below the normal rate. Curve C shows the effect of 10 per cent ether (by volume). The effect is almost the same as in the case of tannic acid; there is a continued rise in the rate for over an hour.

Experiments were also tried with hydrocinnamic acid, succinic acid, and glycocoll. In this case there was no change in the rate of production of CO_2. Apparently only those compounds that contain a double bond are affected.

In conclusion one might say that organic acids are oxidized by H_2O_2 and $Fe_2(SO_4)_3$, producing CO_2 at a rate that can be measured by the indicator method. In the case of unsaturated acids the rate of production of CO_2 may be affected by the addition of an anesthetic. Compared with the experiments reported in the preceding paper of this series on the effect of chloroform on *Ulva*, these results show a striking resemblance to the response of the organism.

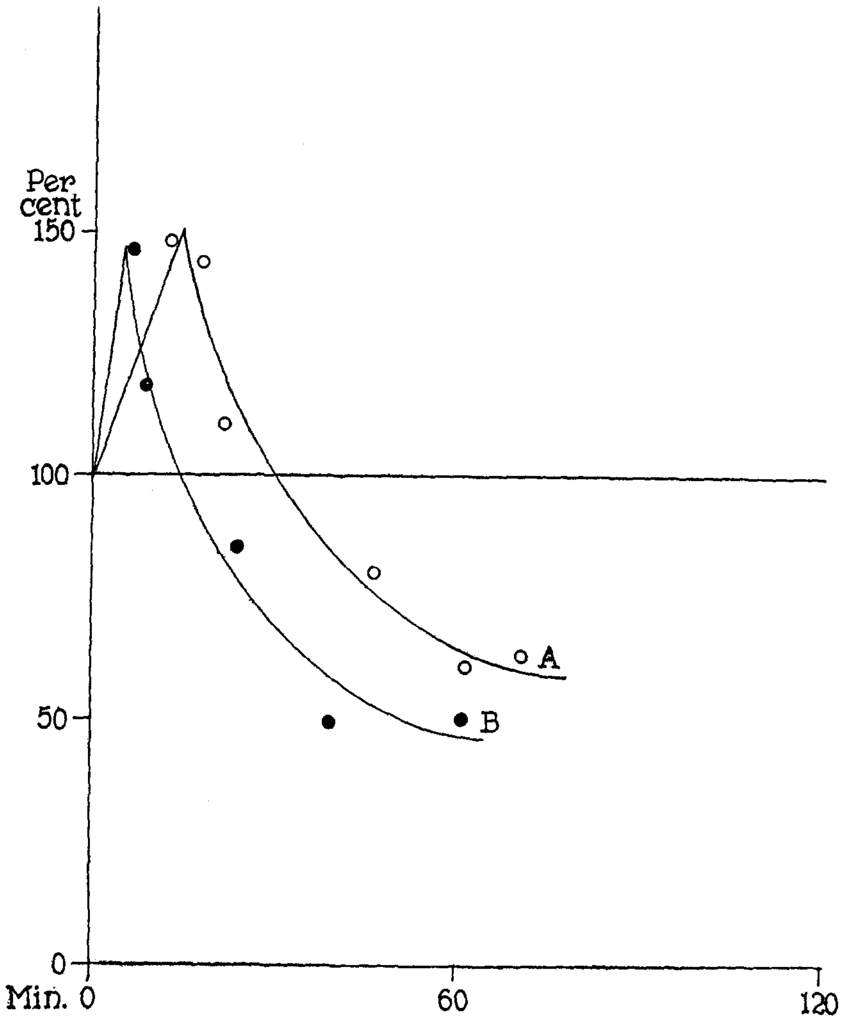

FIG. 6. The effect of the addition of 1 per cent chloroform on the rate of production of CO_2 by oleic acid (Curve A) and cinnamic acid (Curve B) in the presence of H_2O_2 and $Fe_2(SO_4)_3$. Each curve is the average of five experiments. The probable error of the mean is less than 10 per cent of the mean.

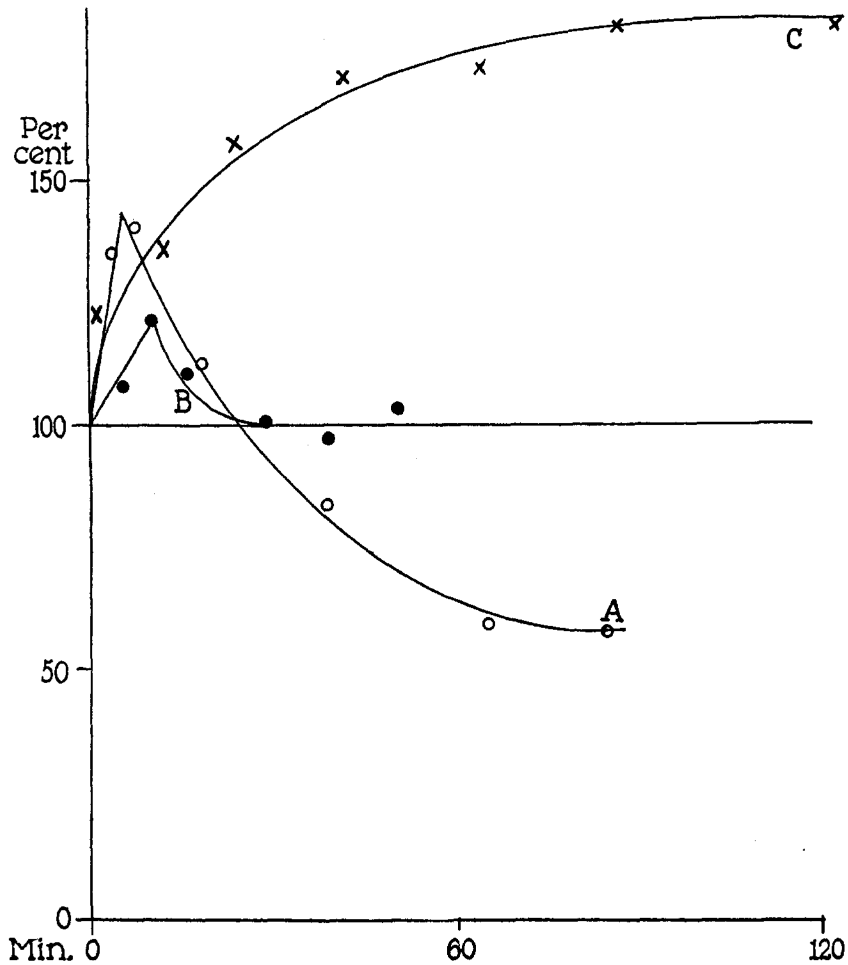

FIG. 7. The effect of anesthetics on the rate of production of CO_2 by fumaric acid in the presence of H_2O_2 and $Fe_2(SO_4)_3$. Curve A represents the addition of 1 cc. chloroform; Curve B the addition of 0.5 cc. Curve C is the effect of the addition of 10 cc. ether. Each curve is the average of five experiments. The probable error of the mean is less than 10 per cent of the mean.

SUMMARY.

1. Organic acids when treated with H_2O_2 and $Fe_2(SO_4)_3$ produce CO_2 at a rate that can be measured by the indicator method.

2. In the case of acids containing a double bond, the rate of production of CO_2 can be varied by the addition of an anesthetic. The changes in the rate of production of CO_2 under the influence of a typical anesthetic, such as chloroform, show a striking resemblance to the reaction of the organism.

BIBLIOGRAPHY.

1. Ray, G. B., *J. Gen. Physiol.*, 1922–23, v, 469.
2. For a description of the method see Findlay, A., The phase rule and its application, London, 1915, 246.
3. Verkade, P. E., and Söhngen, N. L., *Centr. Bakt.*, *2te Abt.*, 1920, l, 81.
4. Bigelow, S. L., *Z. physik. Chem.*, 1898, xxvi, 493.
5. Titoff, A., *Z. physik. Chem.*, 1903, xlv, 641.
6. Bredig, G., and von Berneck, R. M., *Z. physik. Chem.*, 1899, xxxi, 258.
7. Centnerszwer, M., *Z. physik. Chem.*, 1898, xxvi, 1.
8. Warburg, O., *Arch. ges. physiol.*, 1914, clv, 547.
9. Vernon, H. M., *Biochem. Z.*, 1912, xlvii, 374.
10. Baer, J., and Meyerstein, W., *Arch. exp. Path. u. Pharmakol.*, 1910, lxiii, 441.
11. Battelli, F., and Stern, L., *Compt. rend. Acad.*, 1905, cxli, 1044.
12. Acton, L. T., *Proc. and Trans. Roy. Soc. Canada*, 1913, vii, series 3, 209.