COMPARATIVE STUDIES ON RESPIRATION.

XXVI. THE PRODUCTION OF CO₂ FROM ORGANIC ACIDS IN RELATION TO THEIR IODINE ABSORPTION.

BY GEORGE B. RAY.

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

(Received for publication, March 20, 1923.)

In the preceding paper of this series the reaction of various organic acids with H₂O₂ and Fe₃(SO₄)₃ has been described. It was demonstrated that the behavior of these compounds towards certain anesthetics, as regards the production of CO₂, was similar to the response of the organism. It was found that only those acids which contain an unsaturated group are affected by chloroform.

In the light of this fact it was felt that a study of the unsaturated groups might prove to be of interest. This paper is a report of the changes in the iodine absorption of the compounds affected by the action of chloroform, as compared with the rate of production of CO₂.

The technique used in these experiments was as follows: A mixture of the acid to be studied was made up with H₂O₂ and Fe₃(SO₄)₃ with the same concentrations as in the experiments on the production of CO₂. At definite intervals measured portions of the reaction mixture were taken out and run into a definite quantity of Hanus' reagent. This was allowed to stand in a cool place for 1 hour. The amount of free iodine was then determined by titrating with 0.1 N Na₂S₂O₃. Starch paste was used as an indicator. The results are represented in terms of the thiosulfate used in titrating the iodine.

2 This reagent consists of a solution of iodine monobromide and is made in the following manner. 13 gm. of iodine are dissolved in 1 liter of glacial acetic acid by means of a gentle heat, and to this solution 3 cc. of bromine are added. In these experiments the bromine is calculated as iodine.
The first experiments were made with oleic acid. Preliminary trials were made to determine the changes in iodine absorption due to the addition of H$_2$O$_2$ and Fe$_2$(SO$_4$)$_3$. In this case the test sample was dissolved in chloroform before the addition of Hanus' reagent. The results are shown in Fig. 1. (For convenience in this and in all the subsequent figures the curve of the rate of production of CO$_2$ is given for comparison.) Section AB shows the amount of iodine absorption of the oleic acid alone; for convenience this is called 100 per cent. The shaded part represents the rate of production of CO$_2$. Portion BC is the iodine absorption when H$_2$O$_2$ has been added in the proportion of 25 cc. of 3 per cent solution to 50 cc. of oleic acid. It will be noted that there is an increase in the amount of iodine absorption.
absorbed and in the rate of production of CO₂. Section CD shows the effect of adding Fe₂(SO₄)₃ (25 cc. of 0.0004 solution to the mixture given above). In this case there is an increase in both curves. Control experiments were carried out to ascertain the influence of the peroxide and the iron on the iodine. They were found to be without effect.

![Graph showing the effect of chloroform on the absorption of iodine of oleic acid that has been treated with H₂O₂ and Fe₂(SO₄)₃. Curve A is the effect on the rate of production of CO₂, Curve B the effect of 1 per cent chloroform on the absorption of iodine, and Curve C the effect of 0.5 per cent chloroform on the absorption of iodine.]

**Fig. 2.** The effect of chloroform on the absorption of iodine of oleic acid that has been treated with H₂O₂ and Fe₂(SO₄)₃. Curve A is the effect on the rate of production of CO₂, Curve B the effect of 1 per cent chloroform on the absorption of iodine, and Curve C the effect of 0.5 per cent chloroform on the absorption of iodine.
When chloroform was added to a mixture of oleic acid, $\text{H}_2\text{O}_2$, and $\text{Fe}_2(\text{SO}_4)_3$, in the proportions mentioned above, the iodine absorption underwent a peculiar change. If the results of these titrations are plotted against time it is found that the curve approximates the curve for the rate of production of $\text{CO}_2$. In Fig. 2 Curve A shows the effect of the addition of 1 per cent chloroform on the rate of production of $\text{CO}_2$ by 50 cc. of oleic acid in the presence of 25 cc. of the peroxyde solution and 25 cc. of the iron, and Curve B illustrates the changes.
in the iodine absorption of a similar mixture under similar conditions. The iodine absorption of the system in the absence of chloroform is called 100 per cent. That the chloroform is the factor that determines the reaction is shown by the action of 0.5 per cent chloroform (Curve C). Here we find that the reaction is less than when 1 per cent is used.

The addition of 1 per cent chloroform to a mixture consisting of 50 cc. of a suspension of cinnamic acid equivalent to a 0.2 m solution, 25 cc. of H₂O₂, and 25 cc. of the Fe₂(SO₄)₃ solution is shown in Fig. 3. Although the agreement between the curve of the rate of production of CO₂ and the curve for the amount of iodine absorbed is not as good as in the case of oleic acid, there is a definite parallelism. Fumaric acid and tannic acid when examined in the same way gave similar results.

All of the curves shown above are the average of three or more experiments. The probable error of the mean is less than 10 per cent of the mean.

From the above experiments one would say that there is a definite relation between the rate of production of CO₂ and the ability of the compound to absorb iodine. A discussion of this, and of the results of the experiments previously reported, will be taken up in a future paper.