Absorption of Carbon Dioxide by the Application of Carbonic Anhydrase. II : Effect of Enzyme on the Rate of Absorption of Carbon Dioxide by Magnesium Oxide

Absorption of Carbon Dioxide by the Application of Carbonic Anhydrase. II Effect of Enzyme on the Rate of Absorption of Carbon Dioxide by Magnesium Oxide*

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Synopsis

In order to accelerate the rate of absorption of carbon dioxide by magnesium oxide, carbonic anhydrase or red blood corpuscles were added to magnesium oxide suspended in water as an accelerator. The rate of absorption was increased with the increase of the concentration of carbonic anhydrase up to 24 mg per litre. But by adding more amount of the enzyme, little further acceleration of the absorption rate was observed.

The rate of absorption was also increased with the concentration of magnesium oxide up to 20 g per litre, whether the enzyme was present or not. The enzyme action was almost independent of the particle size of the oxide. The optimum temperature of the absorption was 35°C in the absence of the enzyme and 45°C in its presence.

The enzyme lost its activity at 80°C. Red blood corpuscles, used instead of the enzyme, increased also the absorption velocity.

I. Introduction

One of the authors and his co-worker(1) have reported that carbonic anhydrase accelerated the absorption velocity in the absorption of carbon dioxide by sodium carbonate. In the present study, the effect of adding an enzyme, carbonic anhydrase, or red blood corpuscles on the absorption velocity of carbon dioxide by a suspended solution of magnesium oxide were measured.

II. Materials and experimental apparatus

(1) Carbonic anhydrase.

There are many preparative methods of the enzyme. Roughton's method(2) was adopted, because it was comparatively simple and gave relatively pure, stable and preservable enzyme. Fresh ox blood was mixed with a quarter volume of 3.8 per cent sodium citrate solution for preventing its coagulation. The blood corpuscles were separated centrifugally from the serum and then washed three times with the same volume of physiological salt solution. Then the washed blood corpuscles were agitated with the same volume of 40 per cent alcohol and a half volume of chloroform, and divided into three layers by the centrifugal machine:

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(1) K. Uchigasaki and R. Hara, Soc. Chem. Ind. Japan, **50** (1947), 106.

(2) N.O. Meldrum and F.J.W. Roughton, J. Physiol., **80** (1933), 120.

water-alcohol extraction layer, coagulated layer of blood corpuscles, and chloroform layer. The upper water-alcohol layer was dialyzed using a cellophane paper and evaporated to a thin film of light yellow luster at room temperature. The product was ground with agate mortar to a homogeneous powder and kept in a desiccator having calcium chloride at room temperature. The activity of the enzyme was measured by Brinkman's manometric method.(3) The activity of the enzyme, prepared on November 21, 1944 and used for the experiment was measured on May 13, 1945 and July 17, 1947, obtaining the values of 147 and 142, respectively, which showed the constancy of the activity for two years and eight months.

(2) Magnesium oxide.

The solubility and the dissolving velocity of magnesium oxide in water differ according to the original material, the ignition temperature and the manufacturing method, the oxide was, therefore, prepared by the following method under special attention. An aqueous solution of $MgCl_2 \cdot 6H_2O$ was heated at 70°C and mixed with concentrated ammonium hydroxide under vigorous agitation, producing a milky precipitate. After keeping the precepitate for 2 days with the mother liquor, it was heated at 70°C and washed until no chlorine ion was observed using a centrifugal machine. It was dried at 200°C and then heated at 810°C in an electric furnace for 30 minutes, obtaining α-MgO. It was crushed and sifted with a standard sieve. The particle size of magnesium oxide used in this experiment was 65~100 mesh (Tyler), if not specially stated.

III. Experimental apparatus and manipulation

When carbon dioxide is introduced into a solution containing magnesium oxide in suspension, the oxide gradually dissolves as carbon dioxide is absorbed and magnesium carbonate precipitates as the reaction proceeds. For measuring the absorbed amount of carbon dioxide, to take up a testing sample homogeneously containing magnesium hydroxide or carbonate is not easy. The authors have, therefore, adopted a method to determine the absorbed amount of carbon dioxide by measuring the decreased pressure of a carbon dioxide of a constant volume reaction vessel, instead of to determine the amount of carbon dioxide in the reacting solution.

The apparatus used was shown in Fig. 1, in which A shows the reaction vessel; B, an agitator; C, a reservor of carbon dioxide; M, a mercury pressure gauge; D, an inlet of carbon dioxide; and E, an outlet. Although the volume of the reaction vessel

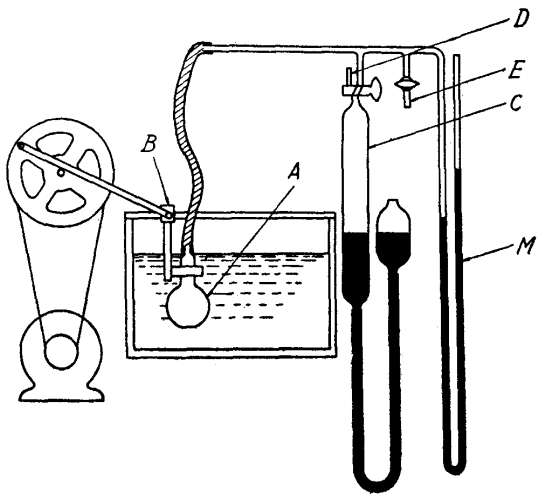

Fig. 1. Absorption apparatus.

(3) R. Brinkman, R. Margaria and F. J. W. Roughton, J. Physiol., **80** (1933), 116.

decreased, as the reaction proceded, by the volume due to movement of mercury in the mercury pressure gauge, the change was small comparing with the volume of the reaction vessel and almost equal volume decreased throughout all reactions, no correction on the volume was, therefore, not made. The experiment was conducted as follows: A definite amount of magnesium oxide and 50 cc of distilled water free from carbon dioxide were added in the reaction vessel, A, with or without the enzyme and air in it was exhausted with a water pump. A definite amount of carbon dioxide was introduced into C from a bomb and the whole of which was transfered into A and the connection between A and C was closed by D. After reading the pressure gauge, A was shaked by the velocity of 200 reciprocal movement per minute. Carbon dioxide was, therefore, always absorbed by a new surface of the liquid, not by a definite contacting surface. But as the shaking velocity was constant, the mean area of the contacting surface was presumed to be constant.

IV. Experimental results

Some of the experimental results are shown in Fig. 2, which show that the enzyme has clearly an accelerative action on the absorption of carbon dioxide by magnesium oxide. In considering the effect of the enzyme from the experimental results, it is difficult to derive a theoretical velocity formula, because the reaction mechanism is complex as the reaction system contains three phases of gas, liquid and solid. It was also not easy to derive a convenient experimental formula to express accurately the experimental results. Therefore, the following constants were calculated, by which the effects of many factors, such as the concentration of the enzyme, the reaction temperature, the particle size of magnesinm oxide, etc. were discussed.

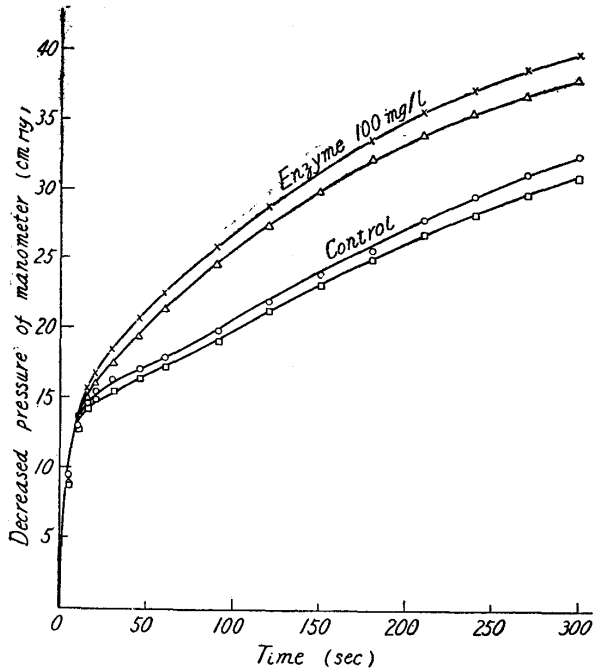

Fig. 2. Rate of absorption.
○ Run No. 1 □ Run No. 2
× Run No. 3 △ Run No. 4

As shown in Fig. 2, the relation between the pressure of the system and time roughly resembles to the exponential curve. So the reaction is assumed to be expressed as follows:

$$x = \alpha(1-e^{-kt}), \quad \text{or} \quad k = \frac{1}{t} 2.3025 \log \frac{\alpha}{\alpha - x} \qquad (1)$$

where, x: decreased pressure (cmHg) during t sec,

α: maximum decreased pressure (cmHg),

k: a constant.

The values of k obtained by putting the experimental value in (1) show that the reaction volocity may be expressed with (1) after about 90 seconds from the initial absorption, though the reaction velocity at initial 90 seconds is not expressed with (1). Let $k_{av.}$, mean value of k during 90~300 sec., express the absorption velocity of carbon dioxide by magnesium oxide. On considering the effects of many factors on the velocity, dp/dt obtained from the absorption curve at initial period, almost the same conclusion with $k_{av.}$ is obtained.

(1) The effect of the concentration of the enzyme.

When the reaction temperature was 15°C and the concentration of magnesium oxide 2 g/l, the absorption velocities were compared, changing the concentration of the enzyme, obtaining the result that the absorption velocity increased with the increase of the concentration of the enzyme up to 24 mg/l, but the increase of the velocity was small with further increase of the enzyme. The results were shown in Fig. 3.

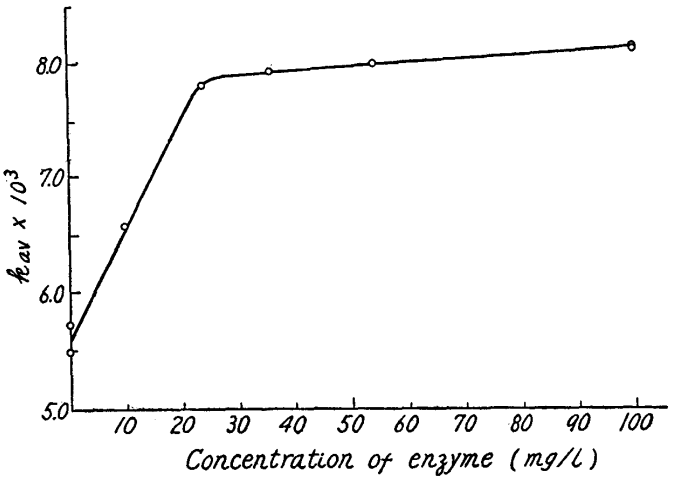

Fig. 3. Effect of concentration of enzyme.

(2) The effect of the concentration of magnesium oxide.

The measurement was conducted at 15°C at the concentration of magnesium oxide 2, 10, 20 and 35 g/l. The concentration of enzyme was 100 mg/l. In each case, as magnesium oxide was present above its solubility, undissolved oxide was suspended in the solution by vigorous agitation during the reaction, but $k_{av.}$ increased with the increase of the amount of magnesium oxide up to 20 g/l, whether the enzyme was pressent or not. At more concentrated solution of magnesium oxide, $k_{av.}$ was almost independent of the amount of the oxide. At the concentration of 35.4g/l of magnesium oxide, $k_{av.}$ seemed to decrease slightly in the presence of the enzyme. The results were shown in Fig. 4.

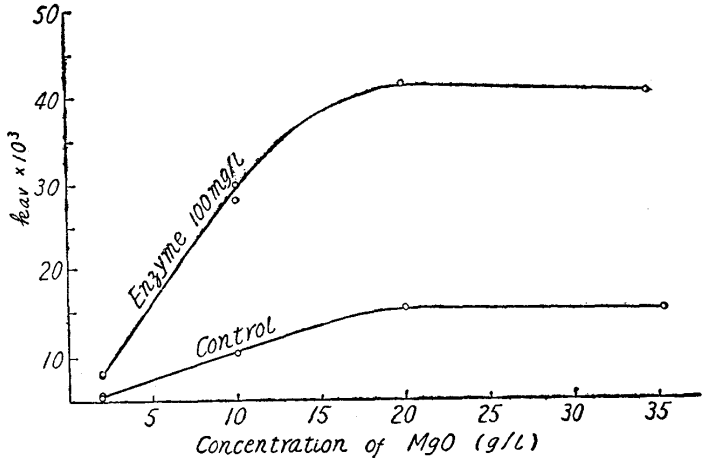

Fig. 4. Effect of concentration of MgO.

(3) The effect of the particle size of magnesium oxide

As the solubility of magnesium oxide in water is very small, the oxide is suspended in water in case of absorption of carbon dioxide by magnesium oxide, the particle size of magnesium oxide would, therefore, give an effect on the absorption velocity.

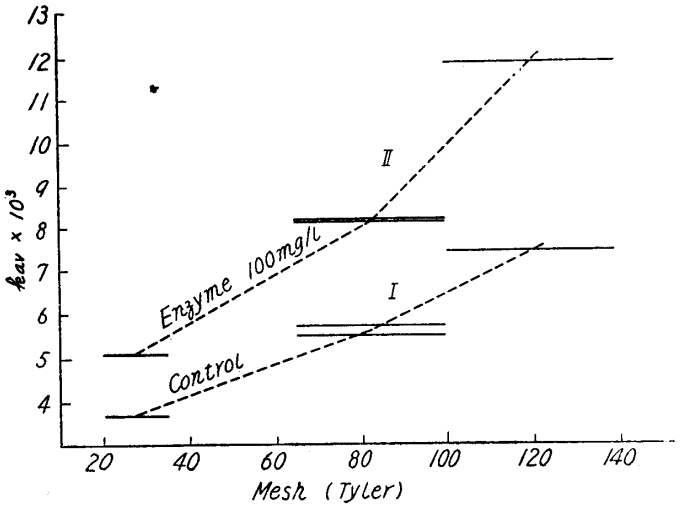

Fig. 5. Effect of particle size of MgO.

The effect of the particle size on the enzyme effect was examined at 15°C, using magnesium oxide of 20~35 mesh (Tyler), 65~100 mesh and less than 100 mesh at the concentration of 2 g/l. The concentration of enzyme was 100 mg/l. The results were shown in Fig. 5, which was drawn by the following method. When the particle size was 20~35 mesh, $k_{av.}$ was 3.7, which was taken on the ordinate and lines parallel to the abscissa were drawn between 20~35 mesh. Other two cases were drawn by the same method. Two curves I and II were drawn by connecting the center of these horizontal lines.

The effect of the enzyme had almost little relation with the particle size.

(4) The effect of temperature.

The experimental solution contained 2 g/l magnesium oxide, to which 100 mg/l of the enzyme were added. The absorption rate had a maximum at about 35°C in the absence of the enzyme and at about 45°C in the presence of the enzyme. Namely, when the enzyme was added to the solution, the absorption velocity increased with the increase of temperature until about 45°C, but decreased at higher temperature. No effect of the enzyme was observed at 80°C. In the first report, it was observed that the catalytic action of carbonic anhydrase on the absorption of carbon dioxide by sodium carbonate was lost at 50°C. The temperature difference between the two cases was assumed to be due to the effect of pH of the solutions,

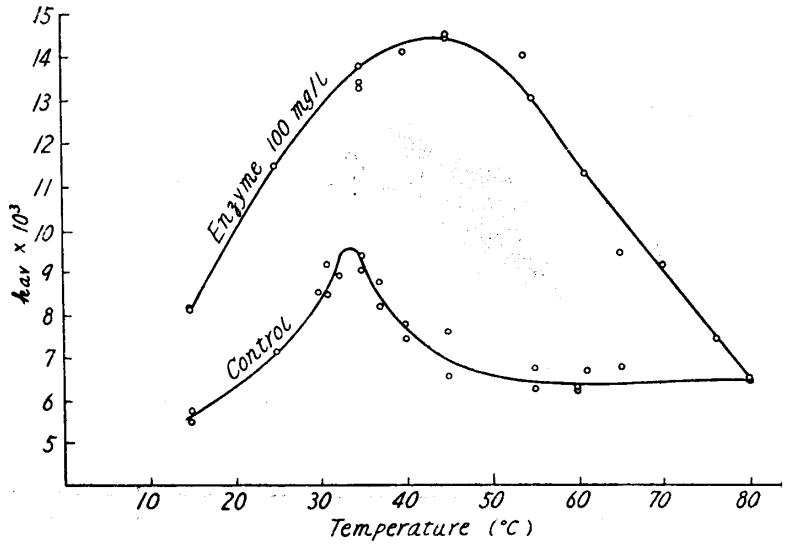

Fig. 6. Effect of Temperature.

kinds of the salts and the concentrations, etc. The results were shown in Fig. 6.

(5) The effect of the addition of red blood corpuscles.

By the above experiments, it was observed that the enzyme had surely the effect on the absorption of carbon dioxide by magnesium oxide. But the preparation of the enzyme was troublesome and expensive. Then the effect of red blood corpuscles was examined. As the material, fresh ox blood was used, which was mixed with a quarter volume of 3.8 per cent sodium citrate solution for preventing coagulation and separated from the serum by a centrifugal machine and washed three times with a physiological solution of salt. It was used in a solution containing magnesium oxide in the rate of 2 g/l at 15°C, perceiving the accelerating action for the absorption velocity. The results were shown in Table 1.

Table 1.

Run No.	Concentration of MgO g/l	Concentration of Corpuscle cc/l	Temp. °C	Initial Pressure of CO_2 mmHg	$k_{av.} \times 10^3$ (90～300 sec.)
1	2	0	15	972	5.5
2	2	0	15	973	5.7
55	2	40	15	1000	6.0
56	2	100	15	978	5.8
57	2	100	15	973	6.2

Conclusion

In the absorption of carbon dioxide by magnesium oxide, carbonic anhydrase acted as an accelerator at the concentration of 2～35.4 g/l of magnesium oxide. The effect of the enzyme was almost independent of the particle size of magnesium oxide. On the temperature, the absorption velocity was maximum at about 45°C. but the enzyme lost its activity at 80°C. The red blood corpuscles slightly accelerated the absorption velocity.

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