EFFECTS OF HEXAN-1:6-BIS (-TRIMETHYLAMMONIUM BROMIDE) ON THE OXIDATION OF SEVERAL SUBSTRATES

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INTRODUCTION

Hexan-1:6-bis (-trimethylammonium bromide) (C₆) has been extensively studied about its pharmacological properties such as ganglionic blockade by Barlow, Ing. (1) (1948), Paton, Zaimis (2) (1951) and Wien, Mason (3) (1951) but less known about the mode of action in the biochemical sense.

On the other hand it has been well known that nicotine action is antagonized by C₆. The study of the effect of such drugs on enzyme systems in vitro presents an opportunity to broaden our understanding of the significance of the relation between enzyme inhibition and pharmacological action.

In this present work, the effects of C₆ on the oxidation of several substrates and on the succinoxidase system were studied.

MATERIAL AND METHOD

Homogenates: Tissues (rat-brain, -liver, -kidney and -spleen) were homogenized by Potter-Elvehjem's glass homogenizer, in Krebs-Ringer phosphate solution with cooling, to give suspensions containing 100 mg tissue per 1 ml. respectively.

Extracts: The tissues were homogenized in a Waring blender with Krebs-Ringer phosphate solution to give suspensions containing 200 mg tissue per 1 ml. respectively and centrifuged for 10 minutes at 3000 r.p.m.. The supernatant fluids were employed.

Green brei: It was prepared following Green's method (4). Rabbit heart muscle was washed with cold distilled water and homogenized in a mortar with a small amount of 0.05M phosphate buffer, pH 7.5, followed by centrifugation for 5 minutes at 2000 r.p.m.. The supernatant fluid was made pH 5.2 by addition of 1M acetate buffer, pH 4.6 and centrifuged for 10 minutes at 3000 r.p.m.. The precipitate was employed as a preparation of succinoxidase system. The preparation was diluted with 0.1M phosphate buffer.

Assay Procedures: Substrates oxidation were measured manometrically with the conventional Warburg apparatus at 37.5°C, pH 7.2, and the atmospheric condition. Succinoxidase activity was also measured manometrically. Succinic dehydrogenase was measured in two ways, viz. by manometrical method in the presence of cyanide and by Thunberg method. Methylene blue was employed for both methods as an acceptor. Cytochrome oxidase was
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measured manometrically using endogenous cytochrome c and p-phenylenediamine as a reductant.

RESULTS

I. Effects of C6 on the oxidation of glucose, lactate, pyruvate, succinate and glutamate in brain

The endogenous respiration was not affected by C6 in concentrations 0.01M to 0.005M. Table 1 shows that C6 has no effect on the oxygen uptake by brain homogenate with glucose, lactate, pyruvate, glutamate as substrates, but it affects on the oxygen uptake with succinate as a substrate and inhibits 10% and 7% at its concentrations of 0.01M and 0.005M respectively.

II. Effects of C6 on the oxidation of succinate in liver, kidney and spleen

1) Homogenate: Effects of C6 on the oxygen uptake by various tissue homogenates with succinate as a substrate are shown in Fig. 1. C6 inhibited the oxygen uptake about twice as much in kidney as in liver, brain and spleen. No significant relation between succinate oxidation activity and the degree of inhibition was found.

2) Extract: The oxidation of succinate by extract was more sensitive towards C6 than by homogenate (Fig. 1).

III. Effects of C6 on the succinoxidase system

1) Complete succinoxidase system: The effects of C6 on the complete succinoxidase system were examined (Fig. 2). C6 inhibited by about 50% at 0.01M and by about 40% at 0.005M,

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**TABLE 1.** The effects of C6 on the oxygen uptake with glucose, lactate, pyruvate, succinate and glutamate as substrate by brain homogenate. Each flask contained the following: 0.5 ml. of Krebs-Ringer phosphate solution; 0.5 ml. of 10% brain homogenate; 0.5 ml. of substrates*; drug concentrations as shown, in the side arm (0.5ml of 0.1M phosphate buffer, pH 7.2, was added to control flask); 0.3ml of 20%KOH, in the center well. The gas phase was air and the temperature 37.5°C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Control</th>
<th>C0.01M</th>
<th>C0.02M</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>76</td>
<td>76</td>
<td>80</td>
</tr>
<tr>
<td>Lactate</td>
<td>97</td>
<td>99</td>
<td>101</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>104</td>
<td>107</td>
<td>104</td>
</tr>
<tr>
<td>Succinate*</td>
<td>129</td>
<td>108</td>
<td>120</td>
</tr>
<tr>
<td>Glutamate</td>
<td>69</td>
<td>73</td>
<td>73</td>
</tr>
</tbody>
</table>

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* 0.33M glucose and sodium glutamate, 0.2M sodium lactate and sodium pyruvate, 0.1M sodium succinate.

** 0.3ml of 10% homogenate.

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FIG. 1. The activity of succinate oxidation and the degree of inhibition by C6 in kidney, liver, brain and spleen. The experimental flasks contained 0.01M C6. All other components and conditions were the same as listed on Table 1.

- ▲: μl/0.3ml of 10% homogenate/2 hrs,
- △: μl/0.3ml of 20% extract/2 hrs,
- ○: Degree of inhibition at homogenate,
- ●: Degree of inhibition at extract.

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FIG. 2. The activity of succinoxidase system.
To characterize the action of Cs more fully, its inhibitory action on the succinoxidase system was tested in the presence of various concentrations of succinate. It is apparent that increasing the concentration of succinate does not overcome the inhibitory effect of Cs. The degree of inhibition tends to increase as the succinate concentration is increased. This indicates that the inhibitory effect of Cs is not the result of competition with succinate for succinic dehydrogenase (Fig. 3).

2) Succinic dehydrogenase: The effect of Cs was further studied by the observation of its action on some components of the succinoxidase system.

Aerobic condition: The oxygen uptake determined in this method was nearly equivalent to that of complete succinoxidase system. This indicates that methylene blue added as a hydrogen acceptor is able to sufficiently transfer hydrogen. Cs had less effect on the succinic dehydrogenase than on the complete succinoxidase system as shown in Fig. 4.

Anaerobic condition: As shown in Table 2, Cs scarcely failed to affect on the succinic dehydrogenase under the anaerobic condition,
3) **Cytochrome oxidase**: C6 had no appreciable effect on the oxygen uptake with p-phenylenediamine as a reductant of the endogenous cytochrome c (Table 3).

**DISCUSSION**

C6 decreased succinate oxidation by brain homogenate alone, but not on the oxidation of glucose, lactate, pyruvate and glutamate.

On the other hand, we (5) have obtained that nicotine had the inhibitory effects on the oxidation of glucose, lactate, pyruvate and glutamate but no action of the succinate oxidation by rat brain homogenate.

Two compounds above mentioned would affect on different enzymes in the same metabolic chain.

The effects of C6 on the succinoxidase system was further studied because it was noted that C6 inhibited the succinate oxidation alone,
The initial experiments were designed to test the effect of \( C_6 \) on the complete succinoxidase system using Green brei as the source of enzyme. \( C_6 \) caused inhibition at 0.01M by 51%.

The effects of \( C_6 \) on the succinic dehydrogenase were measured in two ways, and gave slight inhibition under aerobic condition but no effect under anaerobic condition. Although it can not be explained exactly the difference of the results under the two ways, methylene blue would be possible to accept also the hydrogen which was transferred beyond the portion where was inhibited by \( C_6 \) in succinoxidase system.

\( C_6 \) had no effect on the cytochrome oxidase in this experiment.

Concerning the mechanism of succinate oxidation, Hopkins (6), Keilin and Hartree (7) obtained evidence that some factor is necessary to connect the two systems, the succinic dehydrogenase system and the cytochrome system. Similar results were obtained by Straub (8), Slater (9, 10). Slater demonstrated following scheme:

\[
\text{succinate} \rightarrow \text{cyt. b} \rightarrow \text{factor} \rightarrow \text{cyt. c} \rightarrow \text{cyt. a} \rightarrow \text{cytochrome oxidase (a_3)} \rightarrow \text{oxygen}
\]

The effects of \( C_6 \) on the succinoxidase system suggest that they would prevent transfer of electrons from cytochrome b to c.

Slater (11) had proved that the components of the CoI \( H_2 \) oxidase system are diaphorase, factor, and cytochrome c, a and oxidase (a_3).

If the inhibitory action of \( C_6 \) on the succinoxidase system depends upon the destruction of factor, it is possible to check the lactate oxidation which requires CoI \( H_2 \) oxidase system, however, we have obtained that \( C_6 \) had no effect on the lactate oxidation.

These results therefore tend to consider that the inhibitory action of \( C_6 \) on the succinoxidase system would be the prevention of hydrogen transfer between cytochrome b and factor.

**SUMMARY**

The effects of hexan-1:6-bis (-trimethylammonium bromide) (\( C_6 \)) on the oxidation of the several substrates by rat brain homogenate and on the succinoxidase system in Green brei have been studied.

1. \( C_6 \) inhibited succinate oxidation, but did not affect the oxidation of glucose, lactate, pyruvate and glutamate in brain.

2. The degree of inhibition of \( C_6 \) on the succinate oxidation was about twice as much in kidney homogenate as in liver, brain and spleen homogenate.

3. \( C_6 \) affected the complete succinoxidase system much more than the succinic dehydrogenase and the cytochrome oxidase.
REFERENCES

2) Paton, W.D.M. and Zaimis, E.J.: Ibid. 6, 155 (1951)
3) Wien, R. and Mason, D.F.J.: Ibid. 6, 611 (1951)
10) Slater, E.C.: Ibid. 46, 484 (1950)