THE EFFECT OF BERYLLIUM ON CERTAIN ENZYMES*

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Within recent years a number of reports have appeared demonstrating the toxicity of beryllium in humans and animals. Relatively little is known, however, about the mode of action of beryllium in the body. Klemperer, Miller, and Hill (1), as well as Grier, Hood, and Hoagland (2), have demonstrated an inhibitory action of beryllium on alkaline phosphatase and have suggested the possibility that this inhibition might form the basis of the toxicity of this metal. DuBois et al. (3), who have confirmed the beryllium inhibition of phosphatase, have also stated that adenosinetriphosphatase was inhibited by beryllium. In view of these reports, it became desirable to study a larger number of enzymes in order to ascertain whether or not the inhibitory action of beryllium is confined to phosphatase.

EXPERIMENTAL

Respiration—The effect of beryllium salts on the respiration of tissue slices was examined. Guinea pig liver slices were suspended in phosphate-buffered Ringer’s solution containing 0.2 per cent glucose and the oxygen uptake was measured by the Warburg technique. Beryllium chloride, which was added from the side arm, had no effect on the oxygen consumption even in concentrations of 1 mM per liter. Under these conditions, beryllium phosphate precipitated; replacement of phosphate buffer by borate, however, did not alter the results, as is shown by the following experiment: Rat liver slices were incubated in Warburg vessels containing borate-buffered medium according to Feinstein and Stare (4), to which 0.2 per cent glucose was added. Oxygen consumption was measured, and, after 20 minutes, beryllium chloride was added from the side arm. The final concentration of beryllium was $10^{-4}$ mole per liter. During the initial period, the $Q_O_2$ was $-11.2$, and following the addition of beryllium it was $-10.3$. The oxygen uptake closely paralleled that of a control vessel to which no beryllium was added.

Succinic Oxidase—Since impermeability of cellular membranes to beryllium might have been responsible for this apparent lack of sensitivity of

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respiratory enzymes to the metal, the effect of beryllium on the succinic oxidase system was tested. The enzyme preparation used was a fresh homogenate of guinea pig liver, and the oxidation of succinate was measured manometrically at 38\(^\circ\). Table I demonstrates that the succinic oxidase system was activated by beryllium. Included in Table I are data showing the activation of this enzyme system by aluminum, which was first described by Horecker, Stotz, and Hogness (5).\(^1\)

**Glycolysis**—The possible action of beryllium on the glycolytic reactions of the Embden-Meyerhof cycle was examined. Glycolysis was measured by the usual manometric technique, acetone powder prepared according to the directions of Green et al. (7) being used as enzyme preparation. This enzyme solution was extensively dialyzed. The detailed experimental conditions are given in Fig. 1.

**Table I**

*Activation of Succinic Oxidase by Beryllium and Aluminum*

<table>
<thead>
<tr>
<th>Additions</th>
<th>None</th>
<th>BeCl(_2), 5 \times 10^{-4}) M per liter</th>
<th>Al, 5 \times 10^{-4}) M per liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Q_{O_2})</td>
<td>0.5</td>
<td>1.9</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Each vessel contained 25 mg. of guinea pig liver homogenate, 6 \times 10^{-2} mole of Na succinate per liter, and 1.6 \times 10^{-4} mole of cytochrome c per liter. Phosphate buffer, pH 7.4.

Fig. 1 shows that, in the case of the complete system containing magnesium, there was no significant effect of beryllium on the enzymes involved in glycolysis.

Several of the enzymes of the glycolytic cycle depend for their action on the presence of magnesium. Enolase, as well as some enzymes which effect transfer of phosphate (8, 9), belongs to this group. The question whether beryllium can replace magnesium in activating these enzymes

\(^1\) Because of the similarity of action of aluminum and beryllium on succinic oxidase and because of the suggestion of Ball and Cooper (6) that the activation of succinic oxidase by aluminum depended on inhibition of alkaline phosphatase, the effect of aluminum on alkaline phosphatase was tested. It was found that under the conditions described previously (1) no inhibition occurred in the presence of 10^{-5} mole per liter of aluminum chloride. 38 per cent inhibition was caused by a concentration of aluminum of 10^{-4} mole per liter. Maximal inhibition of 88 per cent occurred with 10^{-3} mole per liter of aluminum. The inhibition of alkaline phosphatase by aluminum resembles that caused by beryllium in that it can be counteracted by magnesium. The concentration of aluminum necessary for comparable inhibition, however, is nearly 100 times greater than that of beryllium.
was investigated. That this is not the case is demonstrated by the lower curves of Fig. 1 which show that the rate of glycolysis in the absence of magnesium was not increased by the addition of beryllium.

Adenosinetriphosphatase—The effect of beryllium on adenosinetriphosphatase was investigated. The enzyme was myosin prepared by the method of Greenstein and Edsall (10). Sodium adenosinetriphosphate was prepared from the commercial barium salt (Armour) by means of cation exchange as described by Polis and Meyerhof (11). All solutions were prepared with glass-redistilled water. The experimental conditions were as follows: 0.2 ml. of 0.1 N buffer was combined with the additions given in Table II, and the volume was brought to 0.55 ml. with water. Then 0.1 ml. of a solution containing 1.0 mg. of myosin in 0.5 M potassium chloride and 0.01 M potassium cyanide was added, and after temperature equilibration the reaction was initiated by quickly delivering 0.15 ml. of a 0.015 M solution of sodium adenosinetriphosphate. After 5 minutes the reaction was terminated by the addition of 0.1 ml. of 50 per cent trichloroacetic acid. The inorganic phosphate liberated was determined by the method of Fiske and Subbarow (12). Under these conditions, the amount of phosphate split was proportional to the concentration of the enzyme.
The data given in Table II demonstrate that in the presence of calcium there was no significant effect of beryllium on adenosinetriphosphatase. This is in disagreement with the data of DuBois et al. (3) who found inhibition of the enzyme in $8 \times 10^{-4}$ M solutions of beryllium. When the experimental conditions of these authors were duplicated and the reaction time was extended to 15 minutes, gradual precipitation of myosin in the tubes containing beryllium occurred, with subsequent loss of enzyme activity. Precipitation of the enzyme apparently had not been observed by these authors because unpurified tissue homogenate had been used.

Table II demonstrates also that in the absence of calcium in borate solution at pH 9.2 beryllium activated adenosinetriphosphatase. This activation amounted to only 15 per cent, while that brought about by calcium was nearly 200 per cent. At greater hydrogen ion concentrations, no activation of adenosinetriphosphatase by beryllium was observed.

Table III demonstrates the lack of interaction of beryllium with a number of enzymes. All rates are expressed in per cent of the maximal rates observed in the presence of the required activators.

**Table II**

<table>
<thead>
<tr>
<th>Buffers</th>
<th>pH</th>
<th>Additions</th>
<th>P split</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No Be</td>
</tr>
<tr>
<td>Veronal</td>
<td>7.6</td>
<td>Ca, 3 mM per liter</td>
<td>21.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>7.6</td>
<td>None</td>
<td>16.5</td>
</tr>
<tr>
<td>&quot;</td>
<td>8.3</td>
<td>Ca, 3 mM per liter</td>
<td>8.2</td>
</tr>
<tr>
<td>Borate</td>
<td>9.2</td>
<td>Ca, 3 mM per liter</td>
<td>29.2</td>
</tr>
<tr>
<td>&quot;</td>
<td>9.2</td>
<td>None</td>
<td>10.8</td>
</tr>
</tbody>
</table>

*Carboxylase*—Carboxylase was prepared from yeast and freed from coenzyme and magnesium by repeated precipitation with ammoniacal ammonium sulfate according to the method of Green et al. (13). Its activity was determined by measuring carbon dioxide production from pyruvate under the conditions defined by these authors. It is seen that $10^{-4}$ mole per liter of beryllium did not inhibit the enzyme in the presence of co-carboxylase and magnesium, nor could it replace magnesium as an activator. When the concentration of beryllium was raised to 1 mM per liter, visible precipitation of the enzyme took place with complete loss of activity.
Arginase—The enzyme was prepared by extracting commercially defatted and dried liver powder (Viobin) with water and the extract was dialyzed against water. The activity of the enzyme was determined by the method of Hunter and Downs (14). Table III shows that arginase is neither inhibited by beryllium nor activated in the absence of manganese.

Carbonic Anhydrase—Meldrum and Roughton (15) have reported that beryllium had no effect on carbonic anhydrase. In their experiments, the liberation of carbon dioxide from bicarbonate was measured in the presence of phosphate buffer. Because of the extreme insolubility of beryllium phosphate, the question arose whether precipitation of beryllium might have accounted for the apparent insensitivity of this enzyme to beryllium. In order to avoid the presence of phosphate, we have employed the method of Philpot and Philpot (16), and measured the time of hydration of carbon dioxide in the absence of buffers. No visible precipitation occurred in the presence of beryllium. The data of Table II demonstrate that there was no inhibition of enzymatic activity by beryllium.

Uricase—This enzyme was prepared from acetone-dried pig liver by borate extraction and acetic acid precipitation as described previously (17). Its activity was measured gasometrically in borate buffer according to the procedure of Klemperer, Trimble, and Hastings (18). Again no inhibition by beryllium was noted.

**DISCUSSION**

In an attempt to correlate the toxic action of beryllium with its possible effect on certain biochemical reactions, it seemed advantageous first
to investigate systems in which the simultaneous action of a large number of enzymes is involved. For this reason respiring liver slices and glycolyzing muscle extracts were studied. These experiments revealed that beryllium has no effect on these systems.

Study of the succinate oxidase system revealed that beryllium in a concentration of 0.5 mM per liter produced a 4-fold increase of the rate of oxygen uptake. This activation appeared similar, although slightly smaller, than that which occurred in the presence of a similar concentration of aluminum.

The activation of succinic oxidase by aluminum has been explained variously by different authors. Ball and Cooper (6) maintained that it is due to inhibition of the destructive effect of phosphatase on a compound essential for the oxidation of succinate. If this were the case, the activation of succinic oxidase by beryllium could be explained by a similar mechanism, since beryllium inhibits alkaline phosphatase. On the other hand, Keilin and Hartree (19) and Bonner (20) have shown that activation of succinic oxidase can be brought about by a variety of colloidal agents or precipitates, including denatured globulin. They conclude, therefore, that the activation by aluminum is due to orientation of enzymes on the surface of the gelatinous precipitate. Since beryllium, under the conditions of the experiment, forms a gelatinous precipitate, similar to that of aluminum, it is likely that the activation of succinic oxidase by beryllium is also dependent on an alteration of the colloidal structure of the enzyme material. It appears that this activation is not specific and hardly of toxicological significance.

In the study of single enzymes attention was given to those which require divalent metals for activation. This was done because our earlier studies on alkaline phosphatase (1) had shown that its inhibition by beryllium could be counteracted by increasing concentrations of magnesium ions, an observation which suggested that the inhibition was based on competition of the divalent metals for the enzyme. The enzymes studied were adenosinetriphosphatase, arginase, and carboxylase. None of these enzymes was inhibited by beryllium unless its concentration was raised to the point at which precipitation of the enzyme resulted. There was slight activation of adenosinetriphosphatase by beryllium when magnesium was absent. Under such conditions, slight activation of phosphoglucomutase by beryllium had been reported by Stickland (21). Beryllium, however, could not replace other divalent metals in their activating function on enzymes of the glycolytic system, arginase, or carboxylase.

The effect of beryllium on carbonic anhydrase was studied because this
enzyme contains zinc and the possibility of competition with this metal seemed possible. No such phenomenon, however, could be demonstrated.

If the inhibition of alkaline phosphatase depended on the acidic property of beryllium hydroxide, it would be conceivable that interaction between this metal and a protein becomes apparent only at low hydrogen ion concentrations favoring the acidic dissociation of the hydroxide. For this reason the effect of beryllium on uricase, which shows maximal activity at pH 9.2, was examined. No inhibition was shown in this case.

The fact that a variety of enzymes was not inhibited even by high concentrations of beryllium while alkaline phosphatase had been shown to be sensitive to concentrations of beryllium as low as $10^{-8}$ M demonstrates a considerable specificity of beryllium for this enzyme, which lends support to the concept that interference with phosphatase action is related to the toxicity of beryllium.

**SUMMARY**

The effect of beryllium on various enzymes was tested.

Beryllium had no effect on the respiration of liver slices.

Succinic oxidase was activated by beryllium. This activation was similar to that which is caused by aluminum.

Beryllium does not inhibit glycolysis in muscle extracts; it cannot replace magnesium as an activator of this reaction.

Adenosinetriphosphatase, carboxylase, arginase, carbonic anhydrase, and uricase are not inhibited by beryllium.

In the absence of calcium there was slight activation of adenosinetriphosphatase by beryllium. Carboxylase and arginase are not activated by beryllium in the absence of other activating metals.

The previously reported inhibitory action of beryllium on alkaline phosphatase appears to be specific.

**BIBLIOGRAPHY**

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