THE EFFECT OF LEAD ACETATE ON OXYGEN UPTAKE OF RAT LIVER SLICES

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In vitro studies of the effect of lead acetate on oxygen uptake of liver slices have led to contradictory results. Jowett and Brooks (1) found 20 per cent acceleration, while Dolowitz, Fazekas, and Himwich (2) found 79.3 per cent inhibition. The suggestion is made by these latter workers that possibly this striking inhibition of tissue enzymes is part of the mechanism of lead poisoning in animals.

In work soon to be published we have studied the oxygen uptake of rat livers from lead-poisoned animals and find very little change from the normal.

In view of this contradiction of the results of in vitro work we have repeated these experiments and present our results in this paper.

EXPERIMENTAL

Oxygen uptake measurements were made with Warburg manometers according to the accepted methods described by Dixon (3). The flasks contained 2.0 cc. of suspending fluid, 0.4 cc. of 20 per cent potassium hydroxide with papers, and an atmosphere of oxygen. The average oxygen uptake for the hour was found graphically by plotting the corrected manometer readings for six 10 minute intervals.

1 The figure given was 50 per cent but from the data it is obvious that an error in calculation had been made. The average of three controls was 237 c.mm. of O₂ per hour and the average of three samples with lead 49.

\[ \text{Inhibition} = \frac{(237 - 49) \times 100}{237} = 79.3\% \]
Two methods of exposure of tissues to lead acetate were used: (1) the direct, in which lead acetate was added to the suspending fluid of the flask, and (2) the indirect, in which the tissues were first immersed in lead acetate solutions and then transferred to the flasks containing Ringer-phosphate solution.

**Direct Method**—This is the method used by both groups of workers mentioned. Jowett and Brooks used Locke's solution at pH 7.0 to which lead acetate was added so that the final concentration was 0.001 M, 0.0005 M, and 0.00025 M. No mention was made of the pH after the addition of lead acetate or of the formation of insoluble lead salts. We would expect the precipitation of lead hydroxide at pH 7.0 and a reduction of pH. Dolowitz, Fazekas, and Himwich used Ringer-phosphate solution and added 0.33 per cent lead acetate solution. Each respiration flask contained 5.0 mg. of lead and 100 mg. of tissue. Most of the lead was precipitated as phosphate and chloride. The pH of the suspensions was not given but our experience would indicate that the final pH of such mixtures would be about 5.0.

We have used three sets of solutions in this direct method. Table I shows the results obtained. Solutions A and B are identical except that Solution B contains enough acetic acid to lower the pH from 7.1 to 5.1. There is no lead in either solution and the 78.8 per cent reduction in $Q_{o_2}$ is due to the acid. This experiment emphasizes the importance of pH control.

Solutions C and D are similar to Solutions A and B with respect to pH but the lower pH of Solution D is due to hydrolysis of the lead acetate added. It will be noted that Solution D showed a 57.3 per cent inhibition of $Q_{o_2}$, but in view of the previous experiment this inhibition cannot be attributed entirely to the lead acetate but rather in part at least to the difference in acidity.

Solutions E, F, and G contain phosphate and Solutions F and G contain lead acetate in addition. These latter two solutions are essentially suspensions of lead phosphate at pH 4.1 and 7.4 respectively. It will be observed that the inhibition found in Solution F is entirely missing in Solution G. Moreover Solution G shows a slight acceleration in oxygen uptake over Solution E. If we may assume that the solutions used by Dolowitz and co-workers were similar to our Solutions E and F, their results are confirmed. We must disagree with them, however, in their in-
interpretations. The inhibition in this case must have been due to the acid factor and not to the lead.

If the acceleration of oxygen uptake in Solution G is significant, then the results of Jowett and Brooks are confirmed. The amount of unprecipitated lead in Solution G must be very much less than the amount of lead in their solutions, since there is an excess of phosphate in our solutions. It is altogether probable that the slight acceleration observed by Jowett and Brooks and by us is not due to the lead but to some other difference in the solutions. These data are too meager for any definite conclusions on this point.

From these experiments we may draw the conclusion that this technique is limited by the peculiarities of lead salts. If one tries to control the lead ion concentration, the pH becomes too low for measuring oxygen uptake, and if one controls the pH, the lead concentration decreases to levels at which no effect is produced. It is highly probable that the results for liver reported by Dolowitz,

### Table I

Effect of pH and Lead Acetate on Oxygen Uptake of Rat Liver Slices by Direct Method

<table>
<thead>
<tr>
<th>Concentration</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.1</td>
<td>5.1</td>
<td>7.2</td>
<td>5.4</td>
<td>7.4</td>
<td>4.1</td>
<td>7.4</td>
</tr>
<tr>
<td>Inhibition, %</td>
<td>78.8</td>
<td>57.3</td>
<td>77.0</td>
<td>13.0</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The $Q_0$ values in Solutions A, B, C, and D are averages of six slices each, while those in Solutions E, F, and G are averages of twelve slices each.
Fazekas, and Himwich are due to differences in pH between the lead solution and the control and that lead either has no effect on oxygen uptake or that lead increases the $Q_{O_2}$ of liver as Jowett and Brooks found.

In order to eliminate the difficulty of precipitation of lead as hydroxide or as phosphate in the suspending fluids we introduced sodium citrate into Ringer's solution instead of phosphate.

The reaction between lead salts and sodium citrate is interesting. If lead acetate is added to citric acid solutions, a heavy white precipitate of lead citrate is formed, $\text{Pb}_3(\text{C}_6\text{H}_5\text{O}_7)_2 \cdot \text{H}_2\text{O}$ (4). If sodium hydroxide is now added, the precipitate dissolves and a clear solution is obtained. The reactions appear to be similar to those occurring in mixed Fehling's solution.

The advantage of using such mixtures of citrate and lead acetate is that the tissues may be exposed to a soluble and presumably diffusible compound of lead at pH levels compatible with normal oxygen utilization. It is uncertain whether the lead is physiologically active when combined with citrate in this soluble form, since many of the ordinary precipitation reactions of lead salts are absent. However, phosphate produces an immediate precipitation of lead phosphate from the complex and this we have considered evidence for at least partial availability of the lead.
Table II and Fig. 1 show the results obtained. The mean $Q_{O_2}$ for slices in lead citrate mixtures is 9.4 and for the controls 10.1. Statistical analysis\(^2\) of the data shows a critical index of 4.64 which is considered adequate to prove that the difference found is significant.

**Indirect Method**—Because of the insolubility of lead hydroxide and lead phosphate and the uncertainty regarding the availability of lead when combined with citrate in the soluble complex the direct methods are unsuited to measurement of oxygen uptake.

![Bar chart showing frequency distribution of $Q_{O_2}$](chart.png)

**Fig. 1.** Frequency distribution of $Q_{O_2}$ with and without lead acetate in Ringer-citrate solution.

Better control of the various factors is obtained by first immersing the slices in Ringer's solution containing lead acetate and then transferring them to Ringer-phosphate in the Warburg flasks. 30 minutes exposure to lead was arbitrarily chosen as sufficient. The lead solutions are acid, owing to hydrolysis, and we must therefore prepare a control mixture of the same acidity but lacking lead. Under the conditions chosen the acid injury was about 90 per cent reversible and we have corrected the lead inhibitions

\(^2\) We wish to thank Dr. C. A. Hammond of this division for assistance in these calculations.
Oxygen Uptake of Liver

accordingly. Table III shows the averages obtained with five different livers all giving similar values.

The net inhibition 1.8 per cent obtained with 0.001 \( \text{m} \) lead acetate is probably not significant but that obtained with 0.002 \( \text{m} \), 13.8 per cent, shows definitely that lead inhibits oxygen uptake under the conditions chosen.

That such conditions prevail in the intact animal is not probable. At physiological pH, 7.4, lead is probably present as insoluble lead phosphate (5).

| TABLE III |
| Effect of Previous Immersion in Lead Acetate on QO\(_2\), Determined in Ringer-Phosphate |

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>( \text{m per l.} )</td>
<td>cc.</td>
</tr>
<tr>
<td>Ringer's solution</td>
<td>100</td>
</tr>
<tr>
<td>0.15 Disodium phosphate</td>
<td>10</td>
</tr>
<tr>
<td>0.10 Lead acetate</td>
<td>0</td>
</tr>
<tr>
<td>Acetic acid, glacial</td>
<td>0</td>
</tr>
<tr>
<td>pH</td>
<td>7.4</td>
</tr>
<tr>
<td>( QO_2 ), average of 15 slices</td>
<td>8.7</td>
</tr>
<tr>
<td>Inhibition, %</td>
<td>12.0</td>
</tr>
<tr>
<td>Corrected for control Solution D</td>
<td>1.8</td>
</tr>
</tbody>
</table>

**SUMMARY**

1. Oxygen uptake of rat liver slices has been determined by the Warburg method in the presence of lead acetate and after previous immersion of the slices in lead acetate solutions.

2. The former procedure is found to be unsuitable, owing to the precipitation of insoluble lead salts and to acid produced by hydrolysis.

3. The \( QO_2 \) appears to be slightly accelerated when liver is shaken in lead phosphate suspensions at pH 7.4 but it is doubtful whether this can be attributed to the action of lead ions on tissue enzymes.

4. In the presence of soluble lead citrate complex at pH 7.30, the \( QO_2 \) is slightly decreased. The activity of lead ion in these solutions is not known but it is probably higher than that in lead phosphate suspensions which contain an excess of phosphate.
5. By separating the lead exposure from the oxygen uptake determination a better control of the various factors is obtained. In this type of experiment the $Q_{O_2}$ is slightly decreased by previous exposure to 0.002 M lead acetate but not by exposure to 0.001 M lead acetate.

BIBLIOGRAPHY

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