THE BLOOD LACTATE-PYRUVATE RELATION AND ITS USE IN EXPERIMENTAL THIAMINE DEFICIENCY IN PIGEONS

BY ELMER STOTZ AND OTTO A. BESSEY

(From the Department of Biological Chemistry, Harvard Medical School, Boston, and the Biochemical Laboratory of the McLean Hospital, Waverley, Massachusetts)

(Received for publication, February 20, 1942)

Pyruvic acid occupies an important position in the chain of reactions constituting carbohydrate breakdown in animal tissues. Not only does it appear to be the immediate precursor of lactic acid, but it is probably the substance immediately preceding the further breakdown of the 3-carbon intermediates (1).

Of the various pathways postulated by which pyruvic acid may be metabolized, all appear to involve the use of thiamine diphosphate (co-carboxylase) (2). The actual level of pyruvate in the tissues, however, is dependent on many other factors. Thus it has been recognized that, in addition to thiamine deficiency, even mild degrees of exercise (3) and glucose ingestion (4) cause increases of blood pyruvate. It is, therefore, hazardous to depend solely on a blood pyruvate estimation for detection of a thiamine deficiency. These facts combined with inadequate analytical methods have contributed to the confusion as to the value of blood pyruvic acid analysis for the detection of thiamine deficiency (5–8).

It has been known for some time that exercise (9) and anoxia (10, 11) give rise to increases in blood lactate.

As a result of blood studies preceding and following the exercise and anoxia of electric shock convulsions, a rather close relation was noted between the blood pyruvate and lactate levels. Such a relation also existed in various states of exercise, excitement, anoxia, and food consumption in spite of wide fluctuations of the absolute lactate and pyruvate levels. This would indicate that the equilibrium between these two substances is quite rapidly established, although a recent note by Friedemann and Barborka (12) indicates that, even after mild exercise, a definite period of 5 to 6 minutes may be required to establish normal lactate-pyruvate relations.

With the use of such a relation, it was possible to examine more closely the effect of thiamine deficiency on pyruvate metabolism as reflected in the blood, in spite of fluctuations of the absolute levels produced by difficultly controlled factors of experimentation. A marked change in the blood lactate-pyruvate relationship coincident with thiamine deficiency
has been noted in pigeons, in fact a correlation with the degree of deficiency. Thus in spite of the apparent close relation between lactate and pyruvate under most conditions, the decreased removal of pyruvate in thiamine deficiency leads to establishment of an abnormal lactate-pyruvate relation. The use of the relation is of particular value in experimental animals or subjects in which a controlled state of activity is difficult to maintain.

The object of this paper is to present proof of the constancy of the lactate-pyruvate relations under various conditions in humans, rats, and pigeons, and to illustrate the changes in this relation during controlled acute and chronic thiamine deficiency in pigeons.

EXPERIMENTAL

Blood was collected from the brachial veins of humans, stasis being avoided, and oxalate-iodoacetate was employed as recommended by Bueding and Wortis (13). Rat blood was collected by heart puncture or severing of the neck, with collection directly into oxalate-iodoacetate. Pigeon blood (0.6 cc.) was collected from the wing vein. A 1:5 trichloroacetic acid filtrate of the bloods (1:8 for pigeon blood) was prepared within 5 minutes of blood collection.

Pyruvic acid analyses were run on 3 cc. samples of the filtrate by the 2,4-dinitrophenylhydrazone procedure described by Bueding and Wortis (13). The final color was determined with a Coleman DM spectrophotometer at $\lambda = 440$ m$\mu$; a standard curve prepared with sodium pyruvate standardized by bisulfite-iodine was employed.

Lactic acid determinations were performed on the appropriate dilutions of the trichloroacetic filtrates by the p-hydroxydiphenyl reaction employed by Miller and Muntz (14) and the modification of Koenemann (15). The modifications introduced by Barker and Summerson (16) involving copper lime treatment of the filtrates for removal of glucose and addition of Cu++ to increase the sensitivity of the color reaction were introduced only after this work was completed. We have since compared several bloods by this method and the older method employed in this work, and find that our lactic acid results may be 4 to 7 per cent too high. Although the newer method is to be recommended, the use of the older method involves a negligible correction of the results here reported and does not change their interpretation.

Lactate-Pyruvate Relations in Humans and in the Rat

The data collected from experimentation with humans were on normal, depressed, and schizophrenic subjects, no difference being noted between the groups. The values above 60 mg. per cent of lactic acid were in gen-
eral obtained in patients 5 to 10 minutes after electric shock convulsions, in which a condition of exercise and anoxia both play a rôle in elevating the blood lactate. In some cases, successive samples were taken on the same individual during the period following the convulsion. The lower values were obtained on both normal and psychotic patients at various degrees of rest, following mild exercise, and following consumption of glucose or ordinary meals. Since under these various conditions a fixed relation was found between lactate and pyruvate, all the data have been included in a single graph (see Fig. 1). Our object in this paper has been to include a large number of individuals (58 subjects) under the varied conditions likely to be encountered rather than to make an intensive study of a few subjects.

The data collected on rats were on normal animals under various conditions of rest. In these animals, a controlled state of rest is more difficult to attain than in humans. Some of the lower lactate values were obtained only after cord resection, the higher ones after exercise and mild degrees of anoxia. The results are plotted in Fig. 2.

An examination of Figs. 1 and 2 shows that, in spite of individual variations and the varied experimental conditions referred to, there is a definite relation between the blood lactate and pyruvate levels. Above a blood lactic acid of 20 mg. per cent, the points fall on a line which would, in the
case of humans, intercept the axis at 1.05 mg. per cent of pyruvic acid, in the case of the rats, at 1.6 mg. per cent. Below 20 mg. per cent of lactic acid, the points fall on a line extending to the origin. It is possible that above 20 mg. per cent of lactic acid the muscles determine in large part the lactate-pyruvate relations, and below 20 mg. per cent, the other tissues.

Since points falling anywhere on these two straight lines must be considered "normal" for the various states of activity encountered, only points removed from the lines can be considered indicative of more fundamental changes in the metabolism of lactate or pyruvate.

In a consideration of thiamine deficiency it is convenient to express these relations as pyruvic acid values. Because the lines representing the lactate-pyruvate relation have different slopes, two expressions must be

### Table I

**Blood Pyruvic Acid Excesses (PA excess) in Pigeons during Thiamine Deficiency**

$PA_{\text{excess}} = PA_{\text{found}} - PA_{\text{sole}}$. The values are given in mg. per cent.

<table>
<thead>
<tr>
<th>Thiamine daily during chronic stage</th>
<th>Bird No.</th>
<th>Normal; Mar 11-15, 1941</th>
<th>9 days, no thiamine (acute); Mar. 26</th>
<th>14 days, no thiamine (acute); Mar. 31</th>
<th>12 days on low thiamine (chronic); Apr. 12</th>
<th>25 days on low thiamine (chronic); Apr. 25</th>
<th>4 days after repair with 100 γ thiamine daily; April 29</th>
<th>11 days after repair with 100 γ thiamine daily; May 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 γ</td>
<td>52</td>
<td>-0.14</td>
<td>4.84*</td>
<td>5.02</td>
<td>7.36</td>
<td>0.23</td>
<td>0.14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>67</td>
<td></td>
<td></td>
<td>5.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>54</td>
<td>-0.29†</td>
<td>1.21</td>
<td>2.87†</td>
<td>5.94</td>
<td>0.08</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>62</td>
<td>0.12</td>
<td>1.07†</td>
<td>2.82§</td>
<td>2.77</td>
<td>3.45†</td>
<td>0.60</td>
<td>0.30</td>
</tr>
<tr>
<td></td>
<td>59</td>
<td>-0.08, 0.06</td>
<td>2.51</td>
<td>4.16</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>0.20</td>
<td>1.66</td>
<td>1.85§</td>
<td>6.02†</td>
<td>5.71</td>
<td>0.70</td>
<td>0.36</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>0.29†</td>
<td>2.85</td>
<td>2.81</td>
<td>4.29</td>
<td>5.54</td>
<td>1.30</td>
<td>2.00</td>
</tr>
<tr>
<td>25 γ</td>
<td>58</td>
<td>-0.02, -0.26†</td>
<td>0.38§</td>
<td>2.44</td>
<td>4.97</td>
<td>0.22</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td></td>
<td>57</td>
<td>0.09</td>
<td>2.53</td>
<td>2.32</td>
<td>1.55</td>
<td>1.27</td>
<td>0.90</td>
<td>-0.21</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.0</td>
<td></td>
<td>5.14§</td>
<td>2.66</td>
<td>2.15</td>
<td>0.57</td>
<td>1.40</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td></td>
<td></td>
<td>1.04§</td>
<td>8.00</td>
<td>2.35</td>
<td>0.79</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>73</td>
<td>-0.13, 0.05†</td>
<td>1.61†</td>
<td>3.70§</td>
<td>2.38</td>
<td>3.40</td>
<td>0.57</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>68</td>
<td>0.07†</td>
<td>2.71</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>61</td>
<td>0.21</td>
<td>2.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>69</td>
<td>2.06†</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>74</td>
<td>1.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In mild opisthotonus.
† Exercised.
‡ In noticeably better condition than other birds in the same group.
§ Repaired the day before with 20 γ of thiamine.
formulated. If we let LA equal the lactic acid value found, then for humans at LA < 20 mg. per cent, \( P_{\text{calc}} = \frac{LA}{12.2} \); at LA > 20 mg. per cent, \( P_{\text{cal}} = 1.05 + 0.0264LA \). For rats at LA < 20 mg. per cent, \( P_{\text{calc}} = \frac{LA}{9.44} \); at LA > 20 mg. per cent, \( P_{\text{calc}} = 1.60 + 0.0264LA \).

\( P_{\text{calc}} \) is then the pyruvic acid value calculated from the normal relations of pyruvic and lactic acid illustrated in the graphs. The figures 1.05 (in humans) and 1.60 (in rats) represent the points of intercept of the lines with the pyruvic acid axis, and the figure 0.0264 the slope of the line.

In normal cases the pyruvic acid value calculated from the above formulas should be the same as the value found experimentally; that is, \( P_{\text{calc}} = P_{\text{found}} \). In thiamine-deficient subjects, the difference between the pyruvic acid value found and that calculated from the normal relations (pyruvic acid excess) might represent the degree of thiamine deficiency; that is, \( P_{\text{excess}} = P_{\text{found}} - P_{\text{calc}} \).

Such expressions have been valuable in assigning a single figure for the degree of disturbance of pyruvate metabolism in the thiamine-deficient pigeons.

**Thiamine Deficiency in Pigeons**

Blood lactate and pyruvate studies were made on a group of twelve pigeons on the usual grain diet (17). Some were exercised to observe a wider range of lactate and pyruvate values. So called resting lactic acid values ranged from 23 to 45 mg. per cent and upon exercise could be elevated to as much as 130 mg. per cent. All of the lactate-pyruvate values fell on a line with an intercept of 2.0 on the pyruvic acid axis. In the case of normal pigeons \( P_{\text{calc}} = 2.0 + 0.0135LA \). Thus the slope of the line in the case of pigeons is only about one-half that found in the case of humans and rats. We have not presented a plot of the data in the case of pigeons but have summarized the \( P_{\text{excess}} \) values in the normal birds and in various states of thiamine deficiency in Table I. In the normal birds the \( P_{\text{excess}} \) values fall from \(-0.29\) to \(+0.29\) mg. per cent.

The birds were then placed on a thiamine-free diet and tube-fed to assure uniform caloric intake, in a manner previously described in publications from this laboratory (17). After 9 days on this diet, blood studies were again made. It is significant that at this time no symptoms other than occasional regurgitation of food immediately after feeding could be observed in the birds. Nevertheless examination of Table I reveals that large increases in the \( P_{\text{excess}} \) values may be of use even in detecting mild degrees of thiamine deficiency.

The birds were continued on the same diet for 5 more days or a total of 14 days on the thiamine-free diet, after which time mild regurgitation of food was common. On the 13th day some of the birds already showed
opisthotonus and were given a dose of 20 γ of thiamine intramuscularly for temporary repair. On the 14th day, blood studies were repeated. During the blood collection, three of the birds (noted in Table I) were in mild opisthotonus. These had the highest PA_{excess} values (3.70 to 5.14). Some of the lowest values occurred in those birds which had received the repairing dose of thiamine the previous day. In general the values were considerably higher than those found at the 9 day stage.

The birds were then divided into two groups, one of which received 20 γ of thiamine daily (intramuscularly) and the other 25 γ daily, all being maintained on the same forced feed régime. After 12 days on this schedule, blood lactates and pyruvates were again determined. With the exception of two birds in which one of us (O. A. B.) had independently noted a less severe deficiency from clinical signs during the acute stage, the birds on the 20 γ level showed definitely higher PA_{excess} values than those on the 25 γ level (average of 5.5 and 2.4 respectively). Likewise after 25 days on the thiamine-low diets, a real difference (now with only one exception) was noted in the PA_{excess} values of the two groups (average of 6.1 and 2.8 respectively). Of importance was the fact that such a separation of the groups on the basis of the PA_{excess} values was not as evident from the pyruvic acid levels alone. This was particularly true, since, as a result of leg weakness developed by a few birds before regular therapy was instituted, some birds remained in a comparatively quiet state, while others struggled vigorously to adopt a standing position, resulting in great variations in the actual lactate and pyruvate levels. Since a distinct difference in PA_{excess} values can be noted between birds receiving 25 γ of thiamine daily and those receiving only 20 per cent less, the use of PA_{excess} values may be useful in determining relatively small differences in degrees of thiamine deficiency in other animals.

The ten birds remaining in the group were then given 100 γ of thiamine daily and blood studies made 4 days later. It was surprising to find that the normal PA_{excess} of zero was reestablished in only three of the birds, although all showed a decrease. Even after 11 days of thiamine therapy, two of the birds still showed abnormal PA_{excess} values. Thus it would appear that even the chemical pathology of chronic thiamine deficiency, not to mention the anatomical, may be slow in returning to normal.

SUMMARY

It has been found that in a variety of conditions such as excitement, exercise, anoxia, and different degrees of fasting, marked fluctuations in

1 This is sufficient thiamine to cure opisthotonus, prevent the development of symptoms of chronic deficiency, and to lead to slow repair of those birds which had previously developed leg weakness.
blood pyruvic and lactic acid values may occur. Nevertheless, a strict relation between the two is maintained, so that a normal relation can be expressed graphically or by formula. This was found true in humans, rats, and pigeons.

Thus although the actual level of pyruvate or lactate individually can serve as a measure of the above factors, and under carefully controlled conditions even reflect true changes in the metabolism of either component, only a deviation in the normal relation between the two components is rigorous proof of a more fundamental disturbance. Therefore the use of this relation eliminates the otherwise difficult decision as to whether a given pyruvate increase is due to a genuine disturbance of pyruvate metabolism or to changes in difficultly controlled experimental conditions.

Such a change in the blood lactate-pyruvate relations has been noted in pigeons during the course of acute and chronic thiamine deficiency, indicating a marked decrease in pyruvate breakdown. The use of this relation has in fact made it possible to note a disturbance of pyruvate metabolism early in acute thiamine deficiency and to distinguish with assurance between relatively small degrees of chronic thiamine deficiency in pigeons.

Since the colorimetric determination of lactic acid is so simple, even as compared to the pyruvate estimation, it is suggested that both lactate and pyruvate levels be considered rather than pyruvate alone to determine fundamental changes in pyruvate metabolism.

BIBLIOGRAPHY

THE BLOOD LACTATE-PYRUVATE RELATION AND ITS USE IN EXPERIMENTAL THIAMINE DEFICIENCY IN PIGEONS
Elmer Stotz and Otto A. Bessey


Access the most updated version of this article at http://www.jbc.org/content/143/3/625.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/143/3/625.citation.full.html#ref-list-1