LACTATE AND PYRUVATE IN BLOOD AND URINE AFTER EXERCISE

BY R. E. JOHNSON AND H. T. EDWARDS

(From the Fatigue Laboratory, Morgan Hall, Harvard University, Boston)

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The changing lactate content of body fluids is a classical problem in the physiology of muscular exercise (1). Interpretation of results from whole animals has usually been in terms of what is known about glycolysis in isolated tissues and in enzyme preparations. The analysis of anaerobic glycolysis in muscle has proceeded a long way since 1932 (22, 23), and the current schemes suggest that, if the source of the excess lactate found in the body after muscular exercise is the musculature, the concentrations of lactate and various other substances in the body after exercise should vary together. In the opinion of most workers pyruvate is the immediate precursor of lactate in muscle glycolysis. We have, therefore, estimated lactate and pyruvate in the blood and urine after severe exercise.

Our three subjects were healthy young men in moderately good athletic training. We drew a sample of the subject's blood from an antecubital vein without stasis, and took a sample of urine, before he ran. After he had run to exhaustion on a motor-driven treadmill at an 8.6 per cent grade, he lay quietly on a bed. Samples of blood were drawn at the stated intervals from an antecubital vein, and the subject urinated at the times noted. The first subject ran at 311 meters per minute for 55 seconds, the second at 233 meters per minute for 80 seconds, and the third at 311 meters per minute for 56 seconds.

Lactate analyses were made by the method of Friedemann, Cotonio, and Shaffer (11). The urine was prepared for analysis by treatment with copper-lime. The blood filtrates were not, because we have confirmed the statement of Cook and Hurst (5) that its use is unnecessary with tungstate filtrates from blood.
We used Peters and Thompson's (24) modification of the Neu-
berg-Case method for pyruvate estimations. This is a colori-
metric estimation of the pyruvate as the 2,4-dinitrophenylhy-
drazone.

Table I gives the results in two experiments and Fig. 1 those
in a third. All three experiments showed the same features.
The lactate and pyruvate recovery curves for blood had similar

The values for blood are measured in mM per liter; the values for urine
are the total output for stated periods in mM.

<table>
<thead>
<tr>
<th>Time</th>
<th>Lactate</th>
<th>Pyruvate</th>
<th>Lactate</th>
<th>Pyruvate</th>
<th>Period</th>
<th>Lactate</th>
<th>Pyruvate</th>
<th>Lactate</th>
<th>Pyruvate</th>
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<tbody>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before run</td>
<td>2.2</td>
<td>0.18</td>
<td>12</td>
<td></td>
<td>Before run</td>
<td>0.1</td>
<td>0.006</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>After &quot;</td>
<td>5 min.</td>
<td>12.1</td>
<td>0.33</td>
<td>37</td>
<td></td>
<td>After run</td>
<td>6.1</td>
<td>0.01</td>
<td>610</td>
</tr>
<tr>
<td>16 &quot;</td>
<td>11.7</td>
<td>0.60</td>
<td>20</td>
<td>0-22 min.</td>
<td>7.7</td>
<td>0.04</td>
<td>193</td>
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</tr>
<tr>
<td>26 &quot;</td>
<td>9.1</td>
<td>0.41</td>
<td>22</td>
<td>0-50 &quot;</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>40 &quot;</td>
<td>5.1</td>
<td>0.27</td>
<td>19</td>
<td></td>
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Experiment II

<table>
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<tr>
<th>Time</th>
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<th>Lactate</th>
<th>Pyruvate</th>
<th>Period</th>
<th>Lactate</th>
<th>Pyruvate</th>
<th>Lactate</th>
<th>Pyruvate</th>
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<tr>
<td>Before run</td>
<td>0.8</td>
<td>0.11</td>
<td>7</td>
<td></td>
<td>Before run</td>
<td>0.1</td>
<td>0.02</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>After &quot;</td>
<td>5 min.</td>
<td>7.3</td>
<td>0.29</td>
<td>25</td>
<td></td>
<td>After run</td>
<td>0.9</td>
<td>0.04</td>
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<tr>
<td>15 &quot;</td>
<td>7.3</td>
<td>0.36</td>
<td>20</td>
<td>0-10 min.</td>
<td>7.0</td>
<td>0.14</td>
<td>50</td>
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<tr>
<td>25 &quot;</td>
<td>5.2</td>
<td>0.26</td>
<td>20</td>
<td>0-45 &quot;</td>
<td>7.6</td>
<td>0.18</td>
<td>42</td>
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<tr>
<td>41 &quot;</td>
<td>2.6</td>
<td>0.22</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>55 &quot;</td>
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</table>

shapes, though pyruvate was present in much smaller amounts
than lactate. The ratio of mM of lactate to mM of pyruvate
ranged from 7 to 44. In our three subjects the pyruvate maxi-
mum came later than the lactate, but both curves fell off in the
same way. The shapes of the curves for urine were remarkably
similar. Excretion of both lactate and pyruvate was complete in
about 40 minutes. Here again the amount of pyruvate in relation
to lactate was very small.
Spiro (31) showed that lactate is excreted in the urine after exercise. This has been confirmed many times (3, 4, 8, 9, 12, 13, 16–18, 27, 28, 30). Liljestrand and Wilson (17) have made the most complete study of urinary lactate excretion. They obtained samples of urine every 10 minutes from their subjects and constructed a complete curve for rates of excretion during recovery. They were also the first actually to isolate the lactate from urine after exercise, and to show that it is L-(+)-lactate. Jervell (13) studied lactate excretion in relation to its blood concentration.

We confirm the conclusions of these earlier workers, that excretion of lactate is complete about 40 minutes after work stops, and that increased blood lactate is followed by increased lactate excretion.

There was some doubt that the substance we estimated was actually pyruvate. Acetic acid 2,4-dinitrophenylhydrazide (6), and the 2,4-dinitrophenylhydrazones of such acids as oxaloacetic, glyoxylic, and mesoxalic would, if present, increase the value for pyruvate. Therefore, we prepared pyruvic acid 2,4-dinitrophenylhydrazone from 1 liter of urine and 400 cc. of blood collected from four subjects after they had run to exhaustion. We used the technique that Johnson (14) used for pigeon blood. It worked well for the blood, but we had to purify the urine hydra-
zone further by solution in toluene, precipitation from toluene with petroleum ether, and recrystallization from ethyl acetate with petroleum ether (Table II).

The yields suggest that most of the substance estimated by the Neuberg-Case method under our conditions is in fact pyruvic acid or some unstable precursor.

Fricke (10) failed to isolate pyruvate from the blood and urine of normal and diabetic humans, and Simon and Aubel (29) could not isolate it from dog blood. Berthelot and Amoureux (2) reported its presence in the human small intestine. Mendel, Bauch, and Strelitz (21) used a very indirect method to demon-

<table>
<thead>
<tr>
<th>Source</th>
<th>Amount</th>
<th>M.p., corrected</th>
<th>Mixed m.p., corrected</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>30</td>
<td>216</td>
<td>216</td>
<td>20.80</td>
</tr>
<tr>
<td>Blood</td>
<td>11</td>
<td>215-216</td>
<td>216</td>
<td>20.48</td>
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<tr>
<td>Synthetic</td>
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<td>20.55</td>
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<tr>
<td>Theory</td>
<td></td>
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<td></td>
<td>20.88</td>
</tr>
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</table>

*The analyses were made by Mrs. Wellwood of the Converse Laboratory, Cambridge. It is sometimes difficult to get off the last traces of nitrogen from 2,4-dinitrophenylhydrazones by the micro-Dumas method. We have repeatedly analyzed this sample of synthetic hydrazone ourselves with theoretical results.

strate it in the serum of cancerous humans. It has been isolated as the 2, 4-dinitrophenylhydrazone from the urine of compensated diabetics (25), from pig serum (32), from the blood of pigeons deficient in vitamin B₁ (14), and from the urine of humans with beriberi (26). We (15) have reported it in the urine of normal humans after exercise.

If we assume for both lactate and pyruvate the conditions of diffusion that Margaria, Edwards, and Dill (20) described for lactate, then the total excretion of excess lactate and pyruvate accounts for about 2 per cent of the total excess that the body has to dissipate after hard exercise for a short time.

We looked for but did not find methylglyoxal 2,4-dinitrophenyl-
osazone in any of our estimations. Either methylglyoxal is formed during exercise in the tissues but does not reach the blood stream, or else it is not formed at all. We interpret our data as support for the Embden-Meyerhof scheme for muscle glycolysis, although we realize that the excess pyruvate might be an oxidation product of excess lactate by the liver or some other tissue (19), or might be associated with the rise in blood sugar which follows hard exercise (7). The curves are, however, consistent with the hypotheses that during or immediately after hard exercise, glycolysis takes place in the muscles according to the Embden-Meyerhof scheme; that lactate and at least one of its precursors, pyruvate, are formed in such excess that they appear in amounts greater than the resting value in the blood stream; and that they are removed in similar ways.

SUMMARY

1. The lactate and pyruvate recovery curves for blood and urine in young men after hard running are similar in shape, but in any sample of blood or urine, pyruvate is present in much smaller amounts than lactate.

2. Pyruvic acid from blood and urine collected after running has been isolated as the 2,4-dinitrophenylhydrazone.

3. These facts seem to support the validity of the Embden-Meyerhof scheme for muscle glycolysis in vivo.

BIBLIOGRAPHY
