The Metabolism of Pyruvate by Normal and Leukemic White Cells*

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In previous investigations of the metabolism of thiamine by leukocytes (7, 1), abnormally high contents of the vitamin were found in leukemic cells. The probable explanation for this abnormality was shown to be an impaired utilization of the vitamin and not its increased ingestion, the faulty excretion, or the apparent youth of the cells involved. No explanation for the impaired utilization was advanced.

Since thiamine in the form of the pyrophosphate ester (cocarboxylase) acts as a coenzyme in some decarboxylating systems, an explanation for the impaired utilization of thiamine by leukemic cells was thought to be that: (a) thiamine did not exist as cocarboxylase in those cells, or (b) the cocarboxylase was formed but was not used because of a lack or inactivation of the enzymes involved. These possibilities now have been investigated.

CLINICAL MATERIAL

White cells were obtained for study from the following groups of persons:

1. Twenty-eight normal adults. For most experiments it was necessary to pool the cells of from 4 to 6 subjects to provide sufficient material.

2. Fourteen patients with chronic leukemia. Of these, 5 had the myeloid and 9 the lymphoid type of the disease. None were febrile.

3. Five patients with acute infections. Of these, 1 had thrombophlebitis, 1 acute pharyngitis, 1 ulcerative glossitis and septicemia, 1 had postabortal sepsis, and 1 a cellulitis of the face. All had polymorphonuclear leukocytosis.

4. Finally, marrow cells were obtained from the resected ribs of 3 patients subjected to thoracoplasty for chronic pulmonary tuberculosis.

METHODS

1. The methods used to procure the marrow and white cells have been described in previous communications (7, 1). All blood samples were obtained from subjects in a fasted state.

2. Thiamine was measured by an adaptation of the technic of Atkin, Schultz, and Frey (7).

3. Cocarboxylase was determined by the method of Goodhart and Sinclair (6), which was modified in two respects: (a) cysteine, 150 mgm. per cent was included in the medium (8), and (b) the yeast halozymase was washed with warmed (30°C) alkaline phosphate.

4. The technics used to demonstrate aerobic and anaerobic decarboxylation of pyruvate were those of Banga, Ochoa, and Peters (2), and Lohmann and Schuster (9), respectively. The latter method was modified only in that the system was adjusted to pH 7.4 with Ringer phosphate buffer.

5. Pyruvate was measured by the technic of Bueding and Wortis (5).

6. The ability of the white cells to convert pyruvate to lactate was ascertained by an adaptation of the technic described by Bueding and Goodhart (4); the conversion of pyruvate to lactate was stopped by the addition of 20 per cent trichloracetic acid.

7. Lactate was measured by the method of Barker and Summerson (3).

RESULTS

A. Measurement of cocarboxylase.—If the inability of the leukemic white cells to metabolize thiamine was due to the fact that the vitamin existed in an unusual form, then the ratio of cocarboxylase to total thiamine compounds in these cells would be abnormal. Accordingly, measurements of these compounds were made in 4 samples of pooled white cells each obtained from the bloods of 4 normal subjects, and in the white cells of 4 patients with leukemia. From 65 to 101 per cent of the thiamine present in both normal and leukemic leukocytes was found to exist in the form of cocarboxylase (Table I).

B. Failure to demonstrate a pyruvic decarboxylase in white cells.—Since the leukemic white cells contained normal proportions of cocarboxylase, it became
necessary to ascertain whether or not the enzymes (which might require coenzyme A for activation) were absent from the leukemic tissue.

Three samples of normal and 3 of leukemic white cells were used as a possible source of enzymes in: (a) a system in which it is possible to demonstrate aerobic pyruvic decarboxylation by brain and kidney (2), and (b) a system used to manifest the activity of yeast carboxylase (9), but adjusted in these studies to pH 7.4 with Ringer-phosphate buffer. In neither system did the added pyruvate undergo decarboxylation.

Samples of the white cells of normal and leukemic individuals then were homogenized to "liberate" the enzymes, which thus might come into more intimate contact with the components of the above systems, (a) and (b). Nevertheless, pyruvate decarboxylation could not be demonstrated in these preparations.

The possibility existed that no increased O₂ utilization or CO₂ production followed the introduction of pyruvate into the enzyme systems studied because the cells already contained sufficient amounts of the substrate. Chemical determinations of 5 specimens of normal and 5 specimens of leukemic white cells failed to reveal any pyruvate. The absence of this substance from other actively metabolizing tissue (brain and liver) likewise has been observed in this laboratory.

Therefore, if normal or leukemic white cells have enzymes analogous to the pyruvate decarboxylase of brain, kidney, or yeast, and which require coenzyme A for their activity, it was not possible to demonstrate the existence of those enzymes by the technics used.

C. Ability of white cells to convert pyruvate to lactate.—Although it was not possible to demonstrate that white cells decarboxylate pyruvate, nevertheless they do utilize that substance at a considerable rate. Observations of a similar nature have been made by Beuding and Goodhart (4) on the utilization of pyruvate by red cells or whole blood.

In order to compare the ability of one sample of white cells to utilize pyruvate with that of another, it first was necessary to demonstrate that the amount of pyruvate utilized in the system studied was a function of the amount of white cells present. Accordingly, the amounts of pyruvate metabolized by different amounts of white cells obtained from 3 groups of normal persons and from 3 patients with leukemia were measured. These values, when plotted against the amounts of cells used, gave almost a linear relationship.

When this fact was established, determinations were made of the comparative ability of normal and of leukemic white cells to utilize pyruvate. Seven speci-
was reduced to lactate. Recent studies elsewhere have shown that the disappearance of pyruvate in whole blood is associated with the appearance of almost equivalent amounts of lactate (4). In 6 of the 7 experiments in which the specimens of normal white cells were studied for their ability to utilize pyruvate, the amounts of lactate that appeared during the course of the experiment were measured and found to be equivalent to from 76 to 120 per cent (average 102) of the pyruvate that disappeared (Table II). In contrast to this finding were the relatively small proportions of lactate that appeared during the pyruvate utilization by the specimens of leukemic cells. Lactate formation by 11 of the 12 samples of leukemic white cells was found to account for from 2 to 11 per cent (average 57) of the pyruvate utilized (Table II). Of these 11 per cent values, 8 were below the lowest normal.

Table III: Experiments to Demonstrate That Lactate Was Derived from Pyruvate

<table>
<thead>
<tr>
<th>Material</th>
<th>Lactate present before incubation, mgm.</th>
<th>Lactate present after one hour incubation, mgm.</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ml. leukemic plasma and 0.135 ml. leukemic</td>
<td>72</td>
<td>81.0</td>
<td>9.0</td>
</tr>
<tr>
<td>white cells</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 ml. leukemic plasma alone</td>
<td>60</td>
<td>68.5</td>
<td>8.5</td>
</tr>
<tr>
<td>Amount lactate from 1.0 ml. leukemic white cells alone</td>
<td>3.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

To establish that the lactate that appeared during the course of pyruvate utilization was formed from the pyruvate, measurements were made of the amounts of lactate formed when no pyruvate was added to the system. In two experiments it was found that normal or leukemic white cells incubated without pyruvate for 1 hour (Table III) produced no significant amounts of lactate.

The impaired ability of leukemic white cells to convert a normal proportion of pyruvate into lactate was considered possibly to be due to the apparent youth of the cells. This supposition, however, was not tenable for the following reasons:

1. The white cells of 5 patients with leukocytosis secondary to acute infections converted normal proportions of the pyruvate utilized into lactate (84 to 106 per cent). The total amounts of pyruvate used were abnormally high (Table IV) in 4 instances.

2. The immature cells obtained from the marrow of 3 patients with chronic pulmonary tuberculosis, and who had normal peripheral blood pictures, resembled normal white cells in the extent of their ability to utilize pyruvate and convert that compound to lactate. Of the 570 to 734 mgm. of pyruvate metabolized per hour by each milliliter of marrow cells, from 84 to 130 per cent was converted to lactate (Table V).

No evidence could be advanced that the reactions involving the utilization of pyruvate either by normal

Table IV: The Ability of White Cells of Patients with Acute Infections and Leukocytosis to Utilize Pyruvate and to Form Lactate

<table>
<thead>
<tr>
<th>Patient</th>
<th>Condition</th>
<th>WBC</th>
<th>Pyruvate used/ml./hr., mgm.</th>
<th>Pyruvate converted to lactate, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>D.T.</td>
<td>Acute pharyngitis</td>
<td>22,000</td>
<td>487</td>
<td>91</td>
</tr>
<tr>
<td>J.H.</td>
<td>Ulcerative glossitis</td>
<td>24,000</td>
<td>2,189</td>
<td>91</td>
</tr>
<tr>
<td>J.K.</td>
<td>Thrombophlebitis</td>
<td>25,000</td>
<td>890</td>
<td>84</td>
</tr>
<tr>
<td>H.B.</td>
<td>Cellulitis of face</td>
<td>17,400</td>
<td>890</td>
<td>105</td>
</tr>
<tr>
<td>M.T.</td>
<td>Postabortal sepsis</td>
<td>21,000</td>
<td>1,755</td>
<td>106</td>
</tr>
</tbody>
</table>

Table V: The Ability of Marrow Cells Obtained from Patients with Chronic Tuberculosis to Utilize Pyruvate and to Form Lactate

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pyruvate used/ml./hr., mgm.</th>
<th>Pyruvate converted to lactate, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>E.L.</td>
<td>734</td>
<td>114</td>
</tr>
<tr>
<td>H.R.</td>
<td>570</td>
<td>130</td>
</tr>
<tr>
<td>J.K.</td>
<td>700</td>
<td>84</td>
</tr>
</tbody>
</table>

Table VI: Effects of Thiamine or Cocarboxylase on the Ability of White Cells to Convert Pyruvate into Lactate

<table>
<thead>
<tr>
<th>Cells</th>
<th>Component added, mgm.</th>
<th>Pyruvate used/ml./hr., mgm.</th>
<th>Lactate used/ml./hr., mgm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pooled normal cells</td>
<td>0</td>
<td>645</td>
<td>600</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.5</td>
<td>587</td>
<td>608</td>
</tr>
<tr>
<td>Thiamine</td>
<td>3.0</td>
<td>612</td>
<td>595</td>
</tr>
<tr>
<td>Cocarboxylase</td>
<td>3.0</td>
<td>560</td>
<td>575</td>
</tr>
<tr>
<td>Leukemic cells</td>
<td>0</td>
<td>900</td>
<td>460</td>
</tr>
<tr>
<td>Thiamine</td>
<td>0.5</td>
<td>1,000</td>
<td>525</td>
</tr>
<tr>
<td>Thiamine</td>
<td>3.0</td>
<td>925</td>
<td>494</td>
</tr>
<tr>
<td>Cocarboxylase</td>
<td>3.0</td>
<td>988</td>
<td>512</td>
</tr>
</tbody>
</table>

or leukemic white cells were dependent upon the presence of thiamine or of cocarboxylase. The addition of from 0.5 to 3.0 mgm. of thiamine or of cocarboxylase to the systems studied had no effect on the pyruvate utilization or lactate formation (Table VI). A similar lack of correlation between the ability of whole blood to utilize pyruvate and the concentration of thiamine in the medium was found by others (4). It must be emphasized that the critical experiment to show a relationship between the concentration of the vitamin and the reaction under study could not be done, namely, to remove or destroy the thiamine and cocarboxylase already present in the
white cells, and then to measure the ability of those cells to convert pyruvate into lactate.

COMMENT

No explanation for the inability of leukemic white cells to metabolize thiamine normally can be advanced at this time. It would appear that those cells do contain normal proportions of cocarboxylase, but the existence of enzyme systems known to require that compound as a coenzyme has not been demonstrated either in normal or neoplastic white cells. Pyruvate, nevertheless, is utilized by the white cells, but that utilization probably does not require O₂ directly, nor is CO₂ produced. Rather, it appears that normal white cells, like erythrocytes or whole blood (4), reduce the pyruvate quantitatively to lactate.

In contrast, it has been found that leukemic cells not only utilize more pyruvate than do normal white cells, but also convert abnormally small proportions of the compound to lactate. What other substances are formed from the pyruvate used by the leukemic cells is not known.

CONCLUSIONS

1. From 65 to 101 per cent of the thiamine present both in normal and leukemic white cells exists in the form of cocarboxylase. Nevertheless, those enzyme systems that are known to utilize cocarboxylase as a coenzyme could not be demonstrated in either the normal or leukemic cells.

2. Qualitative and quantitative differences have been observed for the utilization of pyruvate by normal and leukemic white cells. The normal cells apparently utilize less pyruvate and, in most instances, convert a greater proportion of that compound to lactate than do the leukemic cells. This abnormality of the neoplastic cells probably is not due simply to their apparent youth.

REFERENCES

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