Tissue Metabolism Studies on Bone Marrow

Consideration in Relation to Tumor Metabolism

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Bone marrow cells, both myeloid and erythroid, are among the few that exhibit active multiplication in the adult. The metabolism of these cells is consequently of interest in connection with studies of normal and abnormal growth. It is the purpose of this paper to review certain pertinent data on bone marrow metabolism and to add new data permitting a consideration of bone marrow metabolism in relation to tumor metabolism.

Metabolism of Myeloid and Erythroid Cells

Normal rabbit bone marrow consists, in addition to fat cells, of a mixture of myeloid and erythroid cells in approximately equal numbers. The former, however, average some 4 times the size of the latter, so that by far the larger part of the nonfatty tissue is myeloid. When slices of such normal marrow are suspended in serum average Q values, expressed in terms of fat-free dry weight calculated from nitrogen analyses, are $Q_{o_2} = -7$, $Q_{o_2}^N = 3$, $Q_{N_2}^N = 14$. It is possible, however, to distinguish between the metabolism of the myeloid and erythroid cells by comparing predominantly myeloid with predominantly erythroid marrows and making small extrapolations to 100 percent myeloid and 100 percent erythroid cells (22).

The results are given in Table I, which also lists several of the derived metabolic quotients.

<table>
<thead>
<tr>
<th>Cell type</th>
<th>$Q_{o_2}$</th>
<th>$Q_{o_2}^N$</th>
<th>$Q_{N_2}^N$</th>
<th>Meyerhof oxidation quotient</th>
<th>Absolute Pasteur effect</th>
<th>Fermentation excess (U)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythroid cells</td>
<td>-9</td>
<td>0</td>
<td>7</td>
<td>2.3</td>
<td>7</td>
<td>-11</td>
</tr>
<tr>
<td>Myeloid cells</td>
<td>-6</td>
<td>9</td>
<td>22</td>
<td>6.5</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>Approximate limits</td>
<td>2-10±</td>
<td>0-15±</td>
<td>8-20±</td>
<td>3-6±</td>
<td>8-15±</td>
<td>5 to + 25±</td>
</tr>
</tbody>
</table>

It is at once apparent that the myeloid cells are characterized by relatively active glycolytic mechanisms, both aerobic and anaerobic, whereas the erythroid cells exhibit a predominantly oxidative type of metabolism. It is also notable that the myeloid cells so closely meet the requirements of a tumor type of metabolism, as recently defined by Burk (2) and also listed in the table. Two additional criteria, however, the succinate and p-phenylenediamine tests of Craig, Bassett, and Salter (4) and the R. Q. are not shown in the table, but will be discussed in detail below.

Before passing on to these criteria, however, it appears desirable to comment briefly upon the aerobic glycolysis of marrow. Three points are worthy of note: In the first place, aerobic glycolysis is a relatively small but constant feature of the metabolism of normal marrow that appears to be due entirely to the metabolism of the myeloid cells (22). Secondly, it is not due to injury to the cells; see discussion in Fleischmann's paper (8). Lastly, it has about the same value in Ringer's solution as in serum, whereas respiration and anaerobic glycolysis are considerably higher in the latter medium (21). This makes aerobic glycolysis a disproportionately large feature of the metabolism in Ringer's solution and indicates the necessity of considering the suspension medium when attempting to draw a distinction between normal and tumor type metabolism.

Succinate and p-Phenylenediamine Tests

It is clear from the remarks above, that myeloid bone marrow cells exhibit metabolic characteristics that
closely resemble those of most malignant tumors. Craig, Bassett, and Salter (4) have put forward a criterion of malignancy based on the effect upon oxygen consumption of additions of succinate or p-phenylenediamine. These tests are interpreted as indicating “the gross effectiveness of the succinic dehydrase and cytochrome systems” in the cells. It had already been shown (6, 7, 17) that the activity of these enzyme systems is relatively low in certain tumors. The Salter group found that their normal tissues exhibited increases in $Q_{O_2}$ of the order of 100 to 300 per cent upon addition of succinate and 200 to over 400 per cent upon addition of p-phenylenediamine, while most tumors studied, including the “artificially benign” tumors, exhibited poor responses. They noted also, however, that normal mammary tissue and both normal and leukemic leukocytes yielded poor responses to succinate, while exhibiting good responses (though very variable in the case of mammary tissue) to p-phenylenediamine.

In the hope that these tests would enable us to make a clear differentiation between a normal and tumor type of metabolism we have applied them to various types of marrow, with the results shown in Table II.

The first part of the table shows the results obtained with the slices suspended in Ringer’s solution, exactly after the technic described by Craig, Bassett, and Salter. Very poor increases in $Q_{O_2}$ are noted upon addition of succinate, and even the responses to p-phenylenediamine are well below the normal criterion of about 200 per cent. Lest these results be attributed to some error in technic we repeated the procedures with rat liver, and obtained responses in the same range as those reported by the Salter group. Finally, on the basis that the marrow might be damaged in Ringer’s solution we repeated the tests in serum, with the even poorer responses noted in the table. We are forced to conclude that with normal bone marrow, as with normal leukocytes, the succinate and p-phenylenediamine tests are apparently of no value in aiding the distinction between a normal and tumor type of metabolism.

In further consideration of the general applicability of these tests it seemed desirable to study the responses of two other normal tissues, heart and kidney, in which particularly active cytochrome systems have been demonstrated (17). Two p-phenylenediamine tests with rabbit cardiac tissue yielded increases in oxygen consumption of 620 and 535 per cent, but the responses with kidney were definitely poor. In 8 experiments, 3 with rat and 5 with rabbit kidney slices, the average increase in oxygen consumption upon addition of succinate was 68 per cent (37 to 120 per cent) and with p-phenylenediamine 65 per cent (46 to 90 per cent). This result suggests an alternate interpretation of the tests; namely, that they measure the extent to which the enzyme systems concerned are saturated with substrate under the conditions of the experiment, rather than the “over-all activity” of these systems. One would then account for the results described above on the basis that whereas both tissues contain active cytochrome systems, in kidney but not cardiac tissue these are well supplied with substrate without addition of succinate or p-phenylenediamine. The implications of these findings will be considered in the discussion.

### Table II: Succinate and p-Phenylenediamine Tests on Marrow

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Increase in oxygen consumption with succinate, per cent</th>
<th>Increase in oxygen consumption with p-phenylenediamine, per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>45</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td></td>
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<tr>
<td>6</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>82</td>
<td></td>
</tr>
<tr>
<td>10*</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>16*</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>22*</td>
<td>51</td>
<td></td>
</tr>
<tr>
<td>23*</td>
<td>55</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>

* Predominantly myeloid marrows; others normal.

### The Respiratory Quotient of Bone Marrow Cells

Included in Burk’s list of criteria for a tumor type of metabolism is an R. Q. between 0.75 and 0.9. The qualifications attending this inclusion will be discussed below; attention must first be given to the problems involved in measuring the R. Q. of marrow cells.

In a recent publication (24) the R. Q. of 5 normal marrows in serum, measured in Summerson differential manometers (18), was reported as varying between 0.91 and 1.13. Previously (21), with a less satisfactory apparatus, values from 0.88 to 0.94 were obtained in 5 experiments. Upon reinvestigation of this subject with Summerson manometers, for the present publication, values from 0.95 to as high as 1.26 were obtained, and we had also occasionally observed R. Q.’s as high as 1.2 in the past. While some of these figures

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2 Craig, Bassett, and Salter do not state precisely what is to be considered the normal range of response to the succinate and p-phenylenediamine tests. The criteria used in the present paper are those that appear to be warranted from their data.
could be discounted on the basis of experimental error, the high trend was disturbing until the recent paper of Mirski (13) pointed out that certain adipose tissue in serum may have an R. Q. as high as 1.25 associated with incomplete breakdown of carbohydrate, although a synthesis of fat from carbohydrate should perhaps also be considered. This suggested that in normal marrow, with its high content of fat cells, similar processes might be occurring. Accordingly, the experiments now reported (Table III) were made with suspensions of marrow cells prepared by teasing the marrow into small pieces in Ringer's solution, shaking vigorously in a test tube, centrifuging lightly, and removing the supernatant containing most of the fat cells before resuspending the marrow cells in serum. These procedures appear not to damage the cells appreciably (24), and with such material we have never obtained R. Q.'s above 1.03, as the table shows.

### TABLE III: R. Q. OF RABBIT BONE MARROW SUSPENSIONS

<table>
<thead>
<tr>
<th>Experiment No.</th>
<th>Predominantly erythroid marrows</th>
<th>Predominantly myeloid marrows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Erythroid cells, per cent</td>
<td>R. Q.</td>
</tr>
<tr>
<td>5</td>
<td>88</td>
<td>0.96</td>
</tr>
<tr>
<td>3</td>
<td>80</td>
<td>1.02</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>1.00</td>
</tr>
<tr>
<td>8</td>
<td>67</td>
<td>0.97</td>
</tr>
<tr>
<td>9</td>
<td>64</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Average</td>
<td>0.95</td>
</tr>
</tbody>
</table>

In order to determine whether the R. Q.'s of the myeloid and erythroid cells differ appreciably, the measurements were made mostly on marrows containing a preponderance of one cell type. Predominantly erythroid marrows were obtained by inducing a hemolytic anemia with phenylhydrazine (40 mgm. per kg. intraperitoneally). The animals were not sacrificed until 5 days after the drug had been injected. Under these conditions the marrow respiration and glycolysis are not affected except by virtue of the resulting erythroid hyperplasia (22). Predominantly myeloid marrows were obtained by producing intrapleural abscesses by the injection of 0.25 cc. of a 5 per cent solution of croton oil in olive oil 4 to 7 days before sacrifice of the animals.4

The R. Q. values listed in duplicate in Table III vary over the 0.9 to 1.03 range without significant re-

lationship to the dominant cell type. One may con-
clude that the R. Q. of the myeloid cells is outside the 0.75 to 0.9± range included by Burk in his list of criteria for a tumor type of metabolism.

### GLYCOLYSIS AND GROWTH

Metabolic studies on the erythroid (immature nucleated red) cells in bone marrow yield data pertinent to the frequently discussed relationship between glycolysis and growth. Whereas these cells under normal conditions are actively multiplying and maturing to supply the replacement demand for erythrocytes, under conditions of stress such as are occasioned by hemorrhage, hemolytic anemia, and low oxygen tension, the marrow becomes hyperplastic with erythroid cells that exhibit frequent mitotic figures and other evidences of multiplication and growth. Such tissue exhibits low rather than high glycolytic activity, and the small amount of aerobic glycolysis present may be attributed to the myeloid cells (22). Typical Q values for a marrow containing about 80 per cent erythroid cells are $Q_{O_2} = -8.0$, $Q_{CO_2} = 1.5$, $Q_{G} = 11.0$. This is true whether the impetus to hematopoiesis is supplied by hemorrhage, hemolytic anemia, or low oxygen tension (23) and it is also notable that when such marrows are exposed in vivo to lowered oxygen tension there is no increased tendency to form lactic acid. Such marrows are, in fact, comparable to regenerating liver (14) since both exhibit active respiratory rather than glycolytic mechanisms. Erythroid marrow differs, however, in having an R. Q. of nearly unity, and is hence a good example of an actively growing tissue exhibiting low glycolysis and high R. Q. The data presented here provide further support for the view (1, 2, 14) that growth and glycolysis are not of necessity related.

### DISCUSSION

The foregoing sections dealing with myeloid marrow have shown that this tissue has a respiratory and glycolytic metabolism remarkably similar to that of
most malignant tumors. This would make all the more difficult the effort to demonstrate metabolic differences between myeloid leukemic and comparable normal cells. Victor and Potter (20), Hall and Furth (9), and Burk and his collaborators (3) have shown that with spontaneous, but not methylcholanthrene-induced lymphatic leukemia, this demonstration is facilitated by the relatively low anaerobic glycolysis of normal lymphatic tissue. However, in view of the foregoing considerations, exception may be taken to the following statement of Kempner (10), “Whether leukemic cells are malignant or benign tumor cells, or normal young tissue cells, cannot be decided by morphological investigation. The question can be answered definitely by studies of the metabolic reactions of leukemic blood cells.” The metabolic reactions referred to by Kempner are measurements of aerobic glycolysis, which he considers “may characterize either cancer metabolism or the dying off of any tissue within or without the body.” I would add that it may also characterize various normal tissues, including myeloid cells. On the other hand, I agree that metabolic studies are of the utmost importance in attempting to gain an understanding of the fundamental abnormal processes in cancer, but it would appear desirable, particularly in the cases of myeloid leukemia, to direct attention to specific metabolic processes rather than to over-all respiratory measurements. The recent important paper of Dickens and Weil-Malherbe (5) is an example of this type of approach. These authors show that certain malignant liver tumors, in which respiration is not impaired (although aerobic and anaerobic glycolysis is elevated), nevertheless exhibit greatly impaired ability to carry out specific chemical processes, including urea formation and oxidation of uric acid.

Concerning the results with the succinate and p-phenylenediamine tests, we find that normal rabbit bone marrow and kidney give poor responses (malignant type metabolism) while Craig, Bassett, and Salter (4) report that both normal and leukemic leukocytes give good responses to p-phenylenediamine, though poor responses to succinate. Nevertheless, Roskelley, Horwitt, and Salter (15) find the tests useful in distinguishing normal from malignant human kidney tissue. This confusing state of affairs, in addition to the questionable theoretical basis of the tests, raises some doubt respecting their general applicability as useful supplements of the customary morphologic and metabolic criteria for distinguishing between normal and malignant tissues.

The relatively high R. Q. of myeloid marrow found in this paper is the only metabolic criterion so far studied that places this tissue outside the malignant class. This result should not be interpreted as implying that the R. Q. is generally of greater value than the other metabolic criteria, for the reverse is more nearly true (2). There is, in fact, considerable doubt whether the R. Q. of malignant tissues is usually lower than that of their normal homologues. In the case of hematopoietic tissue this question has been studied principally with reference to leukemia in mice, a subject that has recently been exhaustively reviewed by Burk and his collaborators (3). It seems clear that in some, but not all, spontaneous lymphatic leukemias the R. Q. of the malignant lymphatic tissue is lower than that of the normal. This difference is not found in the induced leukemias so far investigated.

There is a paucity of data on the R. Q. of myeloid leukemic cells. A few high, intermediate, and low values are listed in the review cited above. Kempner and Gaffron (11) report an R. Q. of 0.75 in a single case of myeloblastic leukemia in man. The R. Q. of rabbit exudate leukocytes is about 0.85 (19). It is clear that the available data are both insufficient and too questionable in accuracy to permit a generalization as to the value of the R. Q. in distinguishing normal from leukemic tissue.

SUMMARY AND CONCLUSIONS

Bone marrow respiration and glycolysis have been reviewed with particular reference to the eight criteria of a tumor type of metabolism listed by Burk (2). Myeloid, but not erythroid cells, fulfill seven of the eight criteria—only the relatively high R. Q. (about 0.96) serves to distinguish these cells metabolically from malignant cells. The succinate and p-phenylenediamine tests of Craig, Bassett, and Salter (4) appear not to be of value in this connection and their general applicability is questioned. Also, more evidence is presented to support the view that growth and glycolysis are not necessarily related. The implications of these studies with reference to the metabolism of leukemic cells is discussed.

REFERENCES


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