The Crabtree Effect: A Review

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Recently much interest has been shown in the inhibition of respiration induced by glucose addition to cells. This inhibition is often called the reversed or inverted Pasteur effect or the Crabtree effect. In this paper the term Crabtree effect has been utilized to describe the inhibition observed after the addition of any hexose or hexose analog capable of inhibiting respiration in any tissue. Unfortunately, the studies on the Crabtree effect are scattered throughout a large variety of journals and often appear incidental to other work. This review is an attempt to bring many of the publications which deal with the Crabtree effect together so that it might be easier to visualize the scope, mechanism, and possibly the meaning of this competition between catabolic pathways. Apparently, no previous attempt has been made to review this field of interest.

THE CHARACTERISTICS OF THE EFFECT IN EHRLICH ASCITES TUMOR CELLS

Although work had been done since 1929, when Crabtree (24) first reported the effect, modern workers were stimulated by the publications of El'tsina and Seitz (31) and Kun et al. (56). Both of these groups of investigators employed the Ehrlich ascites tumor cell, and since then the majority of recent studies on the Crabtree effect have been made with this cell. Therefore, it is convenient to refer to results obtained with the Ehrlich cells as a standard of comparison.

Respiratory inhibition can be induced by glucose, fructose, mannose (12, 21, 56, 80, 88) and 2-deoxyglucose (48, 76). The course of inhibition with glucose is quite constant and has been studied in bicarbonate (31, 37, 88), phosphate (Pi) (10, 12, 21, 43, 56, 76, 88, 95), and Tris buffer systems (10, 50). There is an initial stimulatory period lasting from 20 to 120 seconds (15, 76). This stimulatory period is followed by an inhibitory period, which, after equilibration, is constant and equal to about 30 per cent of the endogenous rate and lasting until all the glucose is utilized (31, 48). About 25 seconds after the glucose disappears the cells will be released from inhibition and will consume oxygen at a rate which can be as great as the initial pre-inhibitory rate (48). The earlier literature, in which manometric technics were utilized, reported that low levels of glucose stimulated rather than inhibited respiration (71, 80). However, the use of oxygen electrode technics has shown that extremely minute quantities of glucose can cause inhibition (19, 50). The earlier workers overlooked this inhibition, since it was released, owing to the full utilization of glucose, before the first manometric reading had been made. The reported stimulation was probably caused by the availability of extra lactate, formed from the glucose, which will prevent the slight decline in the endogenous oxygen consumption rate (50). The glucose-induced inhibition is released by uncouplers of oxidative phosphorylation such as 2,4-dinitrophenol (DNP) (3, 19, 56, 80, 85, 97), dicoumarol (15, 19), dibromophenol (42), n-butyl-3,5-diido-4-hydroxybenzoate (19), and dinitroresol (21). The DNP studies had been carried out in media with phosphate concentration varying from zero to 55 mm. Kvamme (58) reported that DNP did not release...
the inhibition when the Pi level of the media is less than 20 mM. This observation may have been in error because of the lack of control of pH change (50). Methylene blue also releases the inhibition (37, 56, 80, 85). It has been shown by Racker (80) and Wenner et al. (96) that the methylene blue effect may be due to the extramitochondrial stimulation of glucose oxidation.

An important point about which there is controversy is the effect of inhibitors of triose dehydrogenase on the Crabtree effect. Several investigators have found that additions of iodoacetic acid (IAA) in quantities which inhibited aerobic glycolysis did not remove the inhibitory effect of glucose on oxygen consumption (19, 42, 45, 48, 57, 80, 84, 102). On the other hand, it has been reported that IAA (90), DL-glyceraldehyde (90), or bromoacetate (81) stimulated respiration in the presence of glucose, but not endogenously, thus partially releasing the Crabtree effect. Seelich and Letnansky (86) claim that IAA releases the Crabtree effect only in the presence of phosphate. However, El'tsina and Seitz (81) obtained a release of inhibition in bicarbonate buffer, whereas other workers (48, 80) who obtain no effect of IAA on respiration employed phosphate buffers. Theoretically, these differences in findings could be attributed to the fact that the glycolytic inhibitors prevent the accumulation of H^+, which also inhibits respiration. However, the data of the workers who find a release of the Crabtree effect by an inhibitor of the triose dehydrogenase show no evidence of a large pH change caused by lactate accumulation. In fact the kinetic data of El'tsina and Seitz (81) suggest that the pH was well controlled. Possibly the results vary because of the use of a different strain of Ehrlich ascites tumor cells.

The endogenous respiration appears to be more susceptible to hydrogen ion (26, 50, 85) and fluoride ion (47) than is the respiration in the presence of glucose. Therefore, these agents tend to release the Crabtree effect. Cells which have grown in the animal for a shorter time have a smaller rate of endogenous respiration than do cells withdrawn from the animal at a later period after inoculation; yet the respiration in the presence of glucose remains constant (3).

It has been reported that, by increasing the phosphate concentration in the medium, the per cent of inhibition of oxidation caused by glucose can be decreased (12, 51, 102). More recently, it has been demonstrated that the effect of phosphate is caused by three different factors and that, when Pi in the media is greater than 10 mM, the direct effect of phosphate on respiration is probably insignificant (50). When the supplementary buffer capacity is low and the glucose concentration high, Pi decreases the effect of glucose on respiration by maintaining the pH more constant (50, 85). When the Pi in the media is less than 5 mM there may be a direct effect of this ion on oxidation (50). However, the majority of the non-buffer effect of Pi is due to the influence of this ion on glycolysis (50). Decreased glucose utilization was reflected in the oxygen consumption values due to prolongation of the Crabtree effect. Another example of the lack of a direct effect of phosphate on respiration is shown in the data of Seelich et al. (88), who find comparable oxygen consumption values in phosphate and in bicarbonate buffers for both the endogenous respiration and respiration in the presence of glucose. Concentrations of Pi higher than 80 mM have been reported to be inhibitory to respiration (71).

The addition of glucose to ascites cells also upsets the endogenous equilibrium by sparing the oxidation of lipid (73, 74, 90) and amino acids (73, 81). In addition to providing substrate for oxidation, glucose also is utilized as a building block for many intermediates and apparently transforms the cell from a catabolic to an anabolic condition (29, 30).

Although there is an initial re-equilibration period after glucose addition, during which the adenosine triphosphate (ATP) level is greatly reduced and the adenosine diphosphate (ADP) level is correspondingly raised, these values quickly return to levels approaching those found before glucose addition (44, 48, 102). When 2-deoxyglucose is added, all the adenine nucleotides other than adenylic acid (AMP) appear to be quickly utilized and remain at low levels for an extended period (48). The same is true when glucose is incubated in the presence of IAA (102). Interestingly enough, there is no initial upward surge in the ADP level when 2-deoxyglucose is phosphorylated (48). This is possibly owing to the much lower rate of phosphorylation of 2-deoxyglucose relative to glucose and the use of the ADP so produced by the mitochondrial oxidations (48).

Some workers (3, 29, 57) find a slightly increased ATP level in cells incubated in the presence of glucose relative to those depending upon endogenous substrate, whereas others find that the ATP content remains constant (44, 48, 49, 102). The incorporation of P^32 into ATP is not affected by glucose (25, 29). This is true despite the observation (29) of an increased amount of ATP being formed in the presence of glucose and a decrease in the ATP level endogenously. The decrease of ATP is felt to be due to utilization of this compound into macromolecules, whereas the
increase found in the presence of glucose was due to the de novo synthesis of ATP from ribose (29). The endogenous decrease might be related to nucleoprotein synthesis which may be greater in bicarbonate buffer, in which a decrease of ATP was noted (3, 29), than in phosphate buffer in which no decrease of ATP was noted (48). Creaser et al. (25) have shown that de novo synthesis is increased by ascites fluid. Alternatively, the endogenous decrease may occur during the extensive washing employed to remove P₃₂₀ after incubation and before analysis. Ibsen et al. (48) did not find an increased ATP content but did note an increased level of pentoses and of total adenine nucleotides. The latter was transitory. It appears that, whether or not glucose can be shown to increase the ATP content, one must conclude, in agreement with Quastel and Bickis (79), that the energy derived through glycolysis is equal to the energy lost from the decreased rate of oxygen consumption.

Sorbose, maltose, glucosamine, lactose, sucrose, galactose, trehalose, raffinose, xylose, ribose, glycine, hydroxyproline, DL-lactate, L-aspartate, glycolate, pyruvate, succinate, L-glutamate, oxalacetate, DL-alanine, citrate, α-ketoglutarate, and malate do not either affect or stimulate respiration from the endogenous level (56, 71, 80, 85). The longer fatty acids have been reported to depress (38, 56, 85) or to have no effect on the oxygen consumption (73). Of the carbohydrates listed above the only one which has been shown to be glycolyzed is glucosamine₁ (85). This compound is metabolized at one-quarter the rate of glucose.₁ Some investigators report that the glucose-inhibited respiration can be slightly stimulated by succinate (92), lactate (10), and pyruvate (10), whereas others have indicated that citrate (12), succinate (13, 17), or α-ketoglutarate (19) had no effect on respiration. The Crabtree effect has also been reported to be almost overcome by the addition of malate to the glucose-inhibited cell (5). Kvamme (59) has found that, when cells are partially blocked with malonate, fumarate overcame the Crabtree effect, but citrate or α-ketoglutarate did not.

There is less information concerning the pattern of inhibition when it is caused by fructose, mannose, or 2-deoxyglucose. The degree of inhibition is the same for equal concentrations of glucose, fructose, or mannose when measured manometrically for 1 hour (12, 80). Equal quantities of glucose or fructose appear to hold the cell in inhibition equally as long, whereas mannose maintains the inhibition longer (12). This difference may indicate that mannose is glycolyzed more slowly. The depth of inhibition caused by 2-deoxyglucose is also the same as that caused by glucose (48). However, the inhibition lasts longer in the presence of 2-deoxyglucose probably because this compound is phosphorylated at a slower rate than glucose (48). The fructose and mannose inhibition is released by dinitroresol in a manner similar but not identical to glucose (21), whereas DNP releases the 2-deoxyglucose inhibition, but only briefly (47). It has been shown also that mannose (76) and 2-deoxyglucose (47, 76) stimulate respiration more than glucose does during the early pre-inhibitory period. The effects of fructose addition seem to be delayed, since Packer and Golder could show no effect with this sugar during the few minutes' duration of their studies (76). This delayed effect may be due to the relatively low affinity of fructose toward the hexokinase of this cell (56).

### THE PRESENCE OF A CRABTREE EFFECT IN TISSUE OTHER THAN EHRlich ASCITES

Table 1 summarizes some of the essential factors concerning the characteristics of the Crabtree effect in a variety of tissues. The tissues listed in the table are divided into three groups. Group A includes those normal tissues which have shown an effect under the conditions studied and are not known to need special treatment, before or after removal from the body, in order to make their respiratory system sensitive to glycolysis. Group B includes those non-neoplastic tissues which show a Crabtree effect only when they are pretreated or incubated under special conditions or when the animal of their origin needs to be stressed in a particular manner. Group C includes all the neoplastic tissues which show a Crabtree effect regardless of the treatment of the cell. The leukocytes are treated separately in Table 2 because of lack of agreement among the various investigators.

It is impossible to evaluate these heterogeneous data statistically, and therefore a critical view is highly desirable. Particularly questionable are the data which suggest that rat liver, diaphragm, or yolk sac show a glucose-induced inhibition. In none of these cases did the authors themselves indicate that the data represented a real effect, but they are reviewed for the sake of completeness.

Furthermore, glucose may be observed to have no inhibitory effect on respiration in a tissue which potentially could show a Crabtree effect. The effect could be missed for many reasons. Some of these, such as the hormonal balance of the animal or choice of the buffer system, are essentially a
<table>
<thead>
<tr>
<th>Tissue</th>
<th>Approx. per cent depression of oxygen consumption</th>
<th>Remarks</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Non-neoplastic (under no specified necessary condition)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat liver</td>
<td>2-10</td>
<td>Questionable significance</td>
<td>(44, 97, 98, 99)</td>
</tr>
<tr>
<td>Rat yolk sac</td>
<td>4</td>
<td>&quot;</td>
<td>(27)</td>
</tr>
<tr>
<td>Rat diaphragm</td>
<td>8</td>
<td>&quot;</td>
<td>(99)</td>
</tr>
<tr>
<td>Human reticuloeytes</td>
<td>14</td>
<td>&quot;</td>
<td>(81)</td>
</tr>
<tr>
<td>Human thrombocytes</td>
<td>19</td>
<td>Not released by DNP but released by bromoacetate (pH effect?)</td>
<td>(67)</td>
</tr>
<tr>
<td>Bull spermatozoa</td>
<td>42</td>
<td>Succinate and pyruvate stimulate respiration in presence of glucose. Mannose but not fructose also inhibits respiration</td>
<td>(83)</td>
</tr>
<tr>
<td>Bovine articulate cartilage</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast</td>
<td>30</td>
<td>Preincubation with a substrate</td>
<td>(8)</td>
</tr>
<tr>
<td>Human synovial mem-brane</td>
<td>30</td>
<td>Possibly from rheumatoid subjects only</td>
<td>(77)</td>
</tr>
<tr>
<td>Monkey heart</td>
<td>30</td>
<td>From tissue culture only. Not released by IAA</td>
<td>(4)</td>
</tr>
<tr>
<td>Monkey kidney</td>
<td>35</td>
<td>From tissue culture only. Partially released by IAA. Released by methylene blue</td>
<td>(41)</td>
</tr>
<tr>
<td>Developing retina</td>
<td>32-40</td>
<td>Only if incubated in bicarbonate-free buffer</td>
<td>(24, 75)</td>
</tr>
<tr>
<td>Adult retina</td>
<td>0-10-30</td>
<td>Only if incubated in bicarbonate-free buffer</td>
<td>(46, 75)</td>
</tr>
<tr>
<td>Rat thymus</td>
<td>30</td>
<td>Only from adrenalectomized animals</td>
<td>(82)</td>
</tr>
<tr>
<td>Rat mesenteric lymph nodes</td>
<td>30</td>
<td>&quot;</td>
<td>(82)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. Neoplastic tissue</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jensen rat sarcoma</td>
<td>1-15</td>
<td>Effect appears to be more marked in phosphate than in bicarbonate buffer. Fructose stimulates respiration</td>
<td>(24, 27)</td>
</tr>
<tr>
<td>Gardner lymphosarcoma</td>
<td>0-27</td>
<td>The Crabtree effect appears to be dependent on the phosphate level of the buffer. In the absence of phosphate there is no effect2</td>
<td>(6, 28)</td>
</tr>
<tr>
<td>Philadelphia #1</td>
<td>5-10</td>
<td>Value relative to the oxygen consumption in the presence of xylose</td>
<td>(24)</td>
</tr>
<tr>
<td>Crocker sarcoma</td>
<td>10</td>
<td></td>
<td>(24)</td>
</tr>
<tr>
<td>Chicken tumor #13 of Stubs and Furth</td>
<td>14</td>
<td>Octanoate releases, due to greater inhibitory effect on endogeneous respiration</td>
<td>(85)</td>
</tr>
<tr>
<td>Mouse sarcoma 2146</td>
<td>14-19</td>
<td>DNP &amp; IAA inhibit respiration in presence or absence of glucose</td>
<td>(100)</td>
</tr>
<tr>
<td>Mouse sarcoma S-37</td>
<td>20</td>
<td>Partially released by IAA (5%)</td>
<td>(4)</td>
</tr>
<tr>
<td>HeLa (cultured)</td>
<td>20</td>
<td></td>
<td>(98)</td>
</tr>
<tr>
<td>H Fp #2 (cultured)</td>
<td>24</td>
<td></td>
<td>(98)</td>
</tr>
<tr>
<td>Walker rat sarcoma 319 (cultured)</td>
<td>23</td>
<td></td>
<td>(98)</td>
</tr>
<tr>
<td>Flexner-Jobling rat sarcoma</td>
<td>27</td>
<td></td>
<td>(98)</td>
</tr>
<tr>
<td>Ehrlich ascites carcinoma</td>
<td>25-50</td>
<td>Also inhibited by 2-deoxyglucose</td>
<td>(99)</td>
</tr>
<tr>
<td>Hepatoma</td>
<td>35</td>
<td>Also inhibited by 2-deoxyglucose, fructose, and mannose</td>
<td>(98, 100)</td>
</tr>
<tr>
<td>Krebs-2 ascites</td>
<td>35</td>
<td></td>
<td>(98, 100)</td>
</tr>
<tr>
<td>Yoshida ascites rat sarcoma</td>
<td>25-52</td>
<td>Partially released by phosphate2</td>
<td>(29, 54, 86)</td>
</tr>
<tr>
<td>Chicken tumor #11 of Furth</td>
<td>40</td>
<td></td>
<td>(13)</td>
</tr>
<tr>
<td>Ross sarcoma</td>
<td>30</td>
<td></td>
<td>(61)</td>
</tr>
<tr>
<td>Rhabdomyosarcoma ascites cells</td>
<td>32-50</td>
<td></td>
<td>(34)</td>
</tr>
<tr>
<td>S A ascites mammary carcinoma</td>
<td>50</td>
<td>DNP releases</td>
<td>(32)</td>
</tr>
</tbody>
</table>

* The necessary conditions are listed under the column "Remarks."
matter of chance. The effect of phosphate appears variable, apparently increasing the inhibition in the Jensen sarcoma (37) and Gardner lymphosarcoma, while decreasing the inhibition in the Yoshida ascites (86). Retina shows a glucose effect only in the absence of bicarbonate (46). In other instances the effect may be missed because of utilizing a too high cell-to-glucose ratio, in which case the glucose might be fully utilized before the effect was evaluated. An effect may also be glucose stimulated, rather than inhibited, respiration. A final way in which the Crabtree effect may be missed is by allowing the cell to utilize its endogenous substrate before beginning the experiment. Such a case was illustrated in sperm by Lardy and Phillips (60). Actually, glucose, if metabolized, probably would stimulate respiration in any starved cell; therefore, by pre-feeding yeast cells Belitzer (8) was able to prime them so that glucose might then cause a respiratory in-

**Table 2**

<table>
<thead>
<tr>
<th>Approx. per cent Crabtree effect</th>
<th>Incubation medium</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Leukocytes from normal donors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Phosphate</td>
<td>Human</td>
<td>(87)</td>
</tr>
<tr>
<td>14</td>
<td>Phosphate</td>
<td>&quot;</td>
<td>(87)</td>
</tr>
<tr>
<td>30</td>
<td>Phosphate</td>
<td>&quot;</td>
<td>(87)</td>
</tr>
<tr>
<td>?</td>
<td>Phosphate</td>
<td>Guinea pig</td>
<td>(35)</td>
</tr>
<tr>
<td>No effect</td>
<td>Phosphate</td>
<td>Rat</td>
<td>(33)</td>
</tr>
<tr>
<td>0</td>
<td>Serum</td>
<td>Human</td>
<td>(86)</td>
</tr>
<tr>
<td>0</td>
<td>Bicarbonate</td>
<td>Human lymphatic leukemia</td>
<td>(93)</td>
</tr>
<tr>
<td>8</td>
<td>Phosphate</td>
<td>&quot;</td>
<td>(87)</td>
</tr>
<tr>
<td>19</td>
<td>Phosphate</td>
<td>&quot;</td>
<td>(87)</td>
</tr>
<tr>
<td>30</td>
<td>Phosphate</td>
<td>Lymph nodes of adrenalectomized rats only</td>
<td>(94)</td>
</tr>
<tr>
<td>C. Leukocytes from patients with myeloid leukemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>Bicarbonate</td>
<td>Human</td>
<td>(69)</td>
</tr>
<tr>
<td>0</td>
<td>Phosphate</td>
<td>&quot;</td>
<td>(87)</td>
</tr>
<tr>
<td>26</td>
<td>Serum</td>
<td>&quot;</td>
<td>(66)</td>
</tr>
<tr>
<td>29</td>
<td>Bicarbonate</td>
<td>&quot;</td>
<td>(87)</td>
</tr>
<tr>
<td>D. Chicken myeloblasts</td>
<td>Gey’s saline</td>
<td>Short-time culture</td>
<td>(7)</td>
</tr>
</tbody>
</table>

missed because of the decreased rate of endogenous respiration with time. The data of Hopkinson and Kerly (46) show how the Crabtree effect in the retina would have been missed, as it had been previously (55), had they reported the results on the basis of only 1-hour values. In 40 minutes 280 μmoles O₂ were utilized endogenously, whereas in 60 minutes only 307 μmoles were utilized. However, in the presence of 15 mm glucose 245 μmoles O₂ were used in 40 minutes and 364 μmoles in 60 minutes. Therefore, if in this case only 1-hour values were reported, it would appear as if the inhibition. It is possible that a more correct impression of the inhibitory effect of glucose could be ascertained by adding glucose to a cell already stimulated by another oxidizable substrate.

In cells which produce lactate aerobically the glucose-induced respiratory inhibition may be caused by the increased hydrogen ion concentration in the media. Such a pH effect is not the metabolically significant Crabtree effect which is the primary cause of the respiratory inhibition in the Ehrlich ascites cell. Therefore, an inhibition caused by acid can be termed a "quasi" effect. That the inhibition caused by decreased pH of the

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\[ W. E. Hull, private communication. \]
medium is not the primary cause of the respiratory inhibition in Ehrlich ascites cells can be demonstrated by the following facts: (a) glucose inhibition does not increase progressively with incubation and is immediately removed upon utilization of the glucose (48); (b) titration of the formed acid with alkali does not relieve the inhibition (71); (c) glucose in concentrations too low to affect pH appreciably still causes an inhibition of respiration (19, 50); (d) glucose plus monooiodoacetic acid and 2-deoxyglucose can inhibit oxygen consumption while not changing the pH (48, 80); and (e) DNP releases the inhibition but does not decrease the pH change found in the presence of glucose alone (48). To say that the respiratory inhibition is not caused by the pH change of the media does not necessarily mean the metabolically significant effect is not caused by the production of acid at a specific site within the cell. This possibility is discussed in the next section.

Despite the fact that the “quasi” effect will always be added to the true metabolically significant Crabtree effect whenever there is a pH drop and respiration is pH-sensitive, the degree of the two effects can be estimated. If the “quasi” effect is minimal, the rate of oxygen consumption after release from inhibition will be equal to the pre-inhibitory rate (48). On the other hand, kinetic data will also demonstrate a case which is truly due to the accumulation of H⁺ in the media; in this case the onset of inhibition will be delayed; the effect will be accumulatory and will not be released when all the glucose is utilized.

It follows, therefore, that, if in any tissue a kinetic study was done and the glucose effect is immediate and not accumulatory, if acid production can be stopped without releasing the inhibition, or if respiration can be stimulated without decreasing the pH change, then the Crabtree effect is probably due to an effect other than pH production in the media and can be called a metabolically significant effect.

Although Crabtree originally thought that the effect of glucose on respiration was a characteristic of tumors only (24), it is obvious from Table 1 that this effect is not limited to neoplastic tissues; yet there is a pronounced tendency toward such a relationship. Of all the studies found, in which the respiration of neoplastic tissue was compared in the presence and absence of glucose, only the Walker 256 (28) and the DBA mouse ascites thymoma (62) were reported to have no Crabtree effect. However, many non-neoplastic tissues including brain (27, 28, 55), testis (27, 28), kidney (24, 27, 28, 41), spleen (27), adipose tissue (53), and rat, rabbit, and chicken embryos (27) have been reported to show no Crabtree effect. Furthermore, still other tissues, such as liver and diaphragm, show an effect which is of doubtful statistical meaning, or which may be due to a pH effect such as in the reticulocyte or thrombocyte. Finally, the majority of non-neoplastic tissues which show an effect do so only under rather specific conditions as demonstrated in Table 1B. One of these conditions, tissue culturing, is known to cause several other neoplastic-like alterations (cf. 41). It has also been found that the induction of a neoplastic growth in a tissue which previously showed no Crabtree effect can induce the effect in this tissue (61). It has been suggested (66) that there is a positive relation between the amount of aerobic glycolysis and the Crabtree effect. Although this relationship does appear to be true in general, it may not explain all the facts. For instance, testicular cells which show no Crabtree effect have a high rate of aerobic glycolysis (27). Also, adrenalectomy which induces a Crabtree effect in several tissues does not stimulate glycolysis in these cells (82).

It has been suggested (2, 48) that the existence of a Crabtree effect in Ehrlich ascites cells is related to the location of the hexokinase in the mitochondria. In this regard, it is of interest to note that culturing creates a tendency for the hexokinase of cells to become more active and to relocate into the mitochondria (36, 41).

MECHANISM OF THE CRABTREE EFFECT

Ehrlich ascites cells.—Several mechanisms have been proposed to explain the Crabtree effect in the Ehrlich ascites cells. Of these the suggestion that a pH effect caused the inhibition has a minimum of experimental support. Tiedemann (92) first noted that the respiration was pH-sensitive and that the endogenous metabolism could be reduced to that of the respiration in the presence of glucose by H⁺. He suggested that the observed respiratory inhibition induced by glucose was caused by the concomitant production of H⁺ through glycolysis. As previously discussed, it is very unlikely, if not impossible, that the inhibition is due to the general pH decrease of the medium. Bloch-Frankenthal and Weinhouse (11), however, refined the pH concept and suggested that the production of H⁺ at specific sites within the cell may cause the inhibition. Such a concept has gained some experimental support, since it has been found that the pH change may be greater within the cell than in the media (26). Nonetheless, to explain the inhibitory effect of deoxyglucose or of glucose in the presence of IAA, it becomes necessary to assume that in
these cases a different mechanism operates or that the critical pH change is associated with the early phosphorylations. Furthermore, it becomes necessary to assume that DNP somehow protects these sites, since DNP does not inhibit the production of H⁺ by glucose. Emmelot and Bos (92) present further evidence and a lucid discussion on the unlikelihood that H⁺ induces the Crabtree effect in S3A ascites cells.

As early as 1936 Belitzer (8) suggested that the Crabtree effect was due to a competition between glycolysis and respiration for a common intermediate. Since DNP has been shown to uncouple phosphorylation from oxidation (63) and does release the Crabtree effect, it was suggested that this competition might be for inorganic phosphate or adenine nucleotide. The competition would then be removed, since the rate-limiting mitochondrial phosphorylations would become uncoupled from oxidation by DNP and the substrates of phosphorylation could no longer limit the respiration, which would then proceed at a greater rate.

Besides the DNP effect, the evidence which suggested a Pi involvement is: (a) the Pi level is lowered during glycolysis, (3, 43, 48, 80, 101); (b) raising the Pi level of the medium reduces the Crabtree effect (12, 102); and (c) limitations of Pi can cause a respiratory inhibition in reconstituted systems (40).

On the other hand, there is evidence which suggests that Pi is at least not the only factor involved in the Crabtree effect. First, it has recently been established that much of the stimulatory effect which Pi has been reported to cause on the respiration in the presence of glucose was due to the effect of this ion on glycolysis, but not to a direct effect on respiration (50). Kvamme (57) and Bloch-Frankenthal and Ram (10) have found that increasing the Pi level of the medium may even increase rather than reduce the Crabtree effect. Secondly, although all the investigators found an initial drop in the Pi level, Hess and Chance (43) find that the level returns to the endogenous level within 3 minutes, and Ibsen et al. (48) found that in Pi-rich media the intracellular Pi level found in the presence of glucose approached the endogenous value before respiration was released from the inhibition. Furthermore, increased Pi in the media increases the cellular Pi level (25, 48). It has been suggested that, when the Pi level of the medium is above 10 mm, Pi has no effect on respiration and the Crabtree effect, but when the extracellular level is below 5 mm the Pi probably does have a direct effect on respiration (50). Similarly, Creaser et al. (25) found that increasing the Pi of the incubation medium did not influence the amount of P³² incorporated into ATP or ADP, provided the Pi level was over 8 mm.

Kvamme (58) had previously advanced a similar concept of a critical level of Pi in the medium. He felt that, in a buffer with less than 20 mm Pi, the Crabtree effect could be explained by a competition for Pi between glycolysis and phosphorylation(s) at the substrate level in the a-ketoglutarate-succinate reaction. However, this concept was based largely on the observation that DNP did not release the Crabtree effect when the Pi level of the incubation medium was lower than 20 mm. As previously mentioned, this observation had not been made by others (50, 97) and may have been due to the greater change of pH which would be expected as the total buffer capacity was reduced. Such a pH effect is suggested by Kvamme's kinetic data (58, Fig. 2), which show that the inhibition does not begin until almost 20 minutes after glucose addition. This is characteristic of the "quasi" Crabtree effect as discussed previously. Since in these experiments the final pH was about 5.1-5.3, since glycolysis itself is pH-dependent in these cells (36, 50, 85), and since Tris, which has no buffering capacity below pH 7, was used to replace phosphate, it is meaningless to try to evaluate the pH effect by comparing the final pH in different samples. It would be the rate of change of pH which is most important.

There is a considerable body of evidence, apart from the effect of uncouplers, which suggests that ADP limitation may be of importance in the respiratory inhibition. This evidence includes the following: (a) ADP is present in low concentration endogenously and returns to an equally low or lower level shortly after glucose addition (44, 48); (b) in isolated mitochondria ADP controls respiration more effectively than Pi (16); (c) the initial stimulatory phase after glucose addition is very reminiscent of the effect of ADP addition to mitochondria in which respiration was limited by ADP (15, 16, 18, 20); (d) an equal quantity of phosphorylation occurs in the presence or absence of glucose under aerobic conditions (25, 29); (e) Gatt and Racker (40) have shown that limiting ADP can inhibit respiration in reconstituted systems.

Of the evidence given above there is some controversy over point (e). Ibsen et al. (48) were unable to find the initial stimulation but suggested this was because of an inadequate measuring device. In subsequent work (47) it was possible to show the stimulation, although never to the same degree as reported by Chance and Hess (15, 16, 18, 20).
There appears to be, therefore, good reason to suspect ADP as a factor limiting respiration. However, again a competitive mechanism could not explain a Crabtree effect induced by 2-deoxyglucose or glucose and IAA. If the decreased rate of oxygen consumption was indeed due to the decreased availability of ADP at the mitochondrial sites of phosphorylation, it would be necessary to suppose either that the mechanism causing the inhibition was entirely different when it was induced by 2-deoxyglucose or by glucose in the presence of IAA or that the ADP limitation was not due to a direct competition with glycolysis (48). A second reason for suggesting that if ADP became limiting it must do so indirectly is that it appeared as if the oxygen inhibition occurred at a time when the ADP level was much higher than it was endogenously (48). It must be recognized, however, that this observation may be invalid, because the cell concentration was lower when the change of oxygen consumption was measured than when the nucleotides were measured (48).

Two different theories involving the concept of intracellular barriers and a resulting indirect competition for ADP were published. The first of these suggested that ATP became trapped within the mitochondria because of the initial increased rate of oxidation and an associated change in mitochondrial permeability (15, 80). This theory has been supported by Packer and Golder (76), who have demonstrated structural changes in the mitochondria seemingly associated with the early phase of stimulation of oxidative phosphorylation and by Hess and Chance (44), who find more ATP in mitochondria of cells which have been exposed to glucose. This hypothesis can also be used to explain the Pasteur effect along the lines proposed by Lynen and Koenigsberger (68). The second theory of compartmentalization of nucleotides was based upon Siekevitz and Potter’s theory (89) of nucleotide transport through the mitochondrial membrane. It was supposed that, upon initiation of glycolysis, the hexokinase, which is located in the mitochondria (2, 101), utilized a portion of the normal mitochondrial membrane ATP, making it unavailable to aid in the transport of cytoplasmic ADP through the membrane to the sites of oxidative phosphorylation within the mitochondria (48). This theory as formulated was no stronger than the Siekevitz-Potter hypothesis of transport upon which it is based. However, the hypothesis of Siekevitz and Potter has been criticized (14) on the grounds that AMP did not stimulate oxygen consumption as quickly as did ADP. Mitochondria in which the respiratory rate was controlled by limiting amounts of phosphate acceptor were used in both cases. However, in the author’s opinion, there is no suggestion, in the Siekevitz-Potter hypothesis, of a primary role of AMP as the phosphate acceptor in oxidative phosphorylation and therefore no reason to believe that AMP would stimulate respiration as fast as ADP, particularly in the absence of substrate quantities of ATP. In fact, the dual adenylatekinase conversions suggested by Siekevitz and Potter might be regarded as a funnel directing ADP from the cytoplasm to sites of oxidative phosphorylation in the mitochondria. The Siekevitz-Potter hypothesis, therefore, could be considered as a means by which the mitochondria could enhance their capacity to compete with the cytoplasmic reactions for ADP.

The theories of Chance’s and McKee’s groups (15, 48), promulgated as an attempt to explain the Crabtree effect, are similar in many respects. Both groups feel that ADP becomes limiting at the mitochondrial sites of phosphorylation, that this limitation is not due to a direct competition with glycolysis but rather an indirect one involving compartmentalization of the nucleotides, that the mitochondrial deficiency of ADP is in some manner related to the phosphorylations of the carbohydrate, and that this compartmentalization effect may act only during the first few minutes of the total time of depression. By accentuating the effect of phosphorylation, these theories would explain how even 2-deoxyglucose or glucose plus IAA can initiate the Crabtree effect. Nevertheless, it must not be forgotten that it is possible that the Crabtree effect is caused by different mechanisms when induced by different agents.

Although there is a fair amount of evidence suggesting an ADP involvement in the mechanism of respiratory inhibition with additional Pi effects when this ion is present in limiting quantities, an argument can also be offered for the possible imbalance in the oxidative reductive state of the tissue as a cause of the Crabtree effect when it is induced by glucose.

Such an effect of glucose could be proposed to explain the following: the capacity of methylene blue to release the inhibition (41), the reported low specific activity of lactate from C14 glucose (5), the ability of oxamic acid—a lactate dehydrogenase inhibitor—to release the inhibition (78), and the observation that sulfhydryl-containing compounds completely release the inhibition (37). Bennett et al. (9) have found two types of respiratory inhibition in the same cell. At lower lactate levels they find a normal glucose effect; but, when glucose is added to cells respiring in the presence of 0.16 mm lactate, respiration is almost completely
inhibited. This latter inhibition can be overcome by succinate but by no other Krebs cycle intermediate nor by DNP. It is conceivable that, whereas the usual Crabtree effect is dependent mainly upon the nucleotide balance, the latter effect is dependent mainly upon the redox state within the cell.

The specific stimulatory effect of succinate suggests that the variant type of Crabtree effect discovered by Bennett et al. is indeed related to pyridine nucleotide. Also, since DNP does not release the inhibition, the mechanism inhibiting respiration in the presence of excess lactate must be different from the mechanism in operation when lactate is present in lesser amounts. Since glucose can then cause respiratory inhibition to occur by two distinct mechanisms in the same cell under different conditions, it is conceivable that the normal Crabtree effect is not due to one mechanism but to a series of different mechanisms, the relative importance of each being dependent upon the circumstances. If this concept were true it may explain why there is some divergence in the observations reported by different laboratories.

Some evidence can be found which favors such a concept of multiplicity of mechanisms. Chance and Hess (16, 19) have indicated that the inhibitory phase of the glucose effect could be divided into a period of deep inhibition and a period of the regular steady-state inhibition. Lonberg-Holm (64) has data which may suggest even further subdivision of the glucose effect. The more recent studies (50) on the effect of Pi on the Crabtree effect could be best explained as an introduction of an additional limiting factor without suggesting an elimination of the original controlling mechanisms.

In considering the possible effect of glucose on the redox balance of the cell, it is interesting to note that 2-deoxyglucose, which is only phosphorylated, stimulates lactate production from endogenous substrate (48).

**Ascites tumors other than Ehrlich.**—Emmelot and Bos (32) suggested that the Crabtree effect in the S2A ascites cells is due to a lack of Pi or phosphate acceptor because: (a) DNP removes the inhibition of oxidation caused by glucose, (b) exogenous tricarboxylic acid intermediates decrease glycolysis in a manner which seems to be correlated to the amount of phosphorylation associated with their oxidation; and (c) the endogenous respiration can be stimulated by DNP, which indicates that, even in the absence of glucose, Pi or ADP limits oxidation. Furthermore, they find no stimulatory effect of exogenous Pi on respiration and present evidence which suggests that neither the intra- or extra-cellular pH change causes the inhibition (32). Also, as in the Ehrlich cell, glucose replaces fatty acid as a source of substrate for oxidation (33).

Yushok and Batt (103) have found that glucose, 2-deoxyglucose, fructose, and mannose produce an inhibition of oxygen consumption in the Krebs-2 ascites carcinoma cell. Furthermore, they find that glucosone, which has a high affinity for the hexokinase but is very slowly phosphorylated, does not cause a Crabtree effect itself and prevents other carbohydrates from causing the effect. This would appear to relate the hexokinase reaction to the Crabtree effect in these cells. Interestingly, as in the Ehrlich ascites cell, the hexokinase is particular (70).

On the other hand, Seelich and Letnansky (86), who conclude that Pi is not a limiting factor in the Ehrlich cells, think that in Yoshida ascites sarcoma cells the inhibition of oxidation is due to the competitive removal of Pi. Hull has data which suggest that in these cells even the endogenous respiration is grossly stimulated by an increase of the phosphate in the buffering medium. Kieler (54) has found that an atmosphere containing 1 per cent carbon dioxide neutralizes the Crabtree effect in Yoshida but not in Ehrlich ascites cells. However, other investigators (29, 86) have found a marked Crabtree effect in Yoshida sarcoma cells while using a bicarbonate buffer. Kieler attributes the carbon dioxide effect to a possible ADP-sparing effect caused by shuttling phosphoenolpyruvate to oxaloacetic acid via the Utter-Kurahashi reaction.

From the limited data available it may be tentatively assumed that the Ehrlich ascites, the Krebs-2 ascites (103), and the S2A ascites carcinoma cells (32) have their respiration controlled by similar mechanisms, whereas, in the Yoshida ascites sarcoma cell, respiration is more directly controlled by Pi or by other mechanisms.

**Retina.**—Retina shows a Crabtree effect only when incubated in the absence of bicarbonate (46). Furthermore, only the nonvisual elements of the retina display a Crabtree effect (75). This has been shown by destruction of the visual elements with IAA (75) and by utilizing immature retinas which have not yet developed the visual elements (22, 75).

In addition to removing the glucose effect, bicarbonate greatly stimulates oxidation (46). This stimulation has been felt not to be due to the provision of tricarboxylic acid intermediates alone, but to be due also to the production of oxidized TPN via the TPN-malic dehydrogenase reaction (46). If it is true that respiration is limited by the TPN+ concentration in the absence of bicarbonate,
this limitation might be expected to be made more severe by glucose due to stimulation of the oxidative shunt and perhaps indirectly through the formation of DPNH. Assuming glucose inhibited respiration by decreasing TPN⁺, one would expect that glucose would stimulate respiration in the presence of bicarbonate and that IAA might increase the inhibition. Both these exceptions are met (46), but it is not obvious why DNP released the inhibition (75). It is possible that DNP stimulates visual or other cells not affected by glucose.

Leukocytes.—It is obvious from Table 2 that the leukocyte picture is confused. Seelich et al. (87) found that, under their experimental conditions, increasing Pi levels resulted in a tendency to decrease the per cent of inhibition due to the Crabtree effect in normal leukocytes, although they did not feel that the effect in the absence of phosphate was necessarily significant. Yet McKinney and associates (69, 72) found a large Crabtree effect, even though they employed phosphate buffer, and Luganova et al. (66) found a Crabtree effect in normal cells incubated in serum. These differences may be due to the method of preparation, one method perhaps being richer in a particular cell type.

Seelich et al. (87) found the minimal effect in normal leukocytes but obtained the largest effect in leukocytes obtained from patients with lymphatic leukemia and with chronic myelocytic leukemia. Yet, Luganova et al. (66) found no effect with leukocytes obtained from patients with lymphatic leukemia but reported a large effect in leukocytes from normal individuals and from patients with chronic myelocytic leukemia. These inconsistencies suggest that the lack of agreement is not due to differences in the experimental methodology such as total buffer capacity, etc. The data of Roberts and White (88) show that the corticoid hormonal balance of the animal affects the degree of Crabtree effect in thymus, mesenteric lymph nodes, and lymphosarcoma and suggest that some of the reported differences may be due to variance in the hormonal balance of the leukocyte donors.

THE PSYCHOLOGICAL SIGNIFICANCE OF THE CRABTREE EFFECT

No in vitro study is complete unless its biological meaning, if any, is assessed. In considering what role the Pasteur or Crabtree effects might play in living cells one cannot help observing that, if considered in the reverse manner from which they are studied, they are both excellent emergency measures. The releasing of the Pasteur effect would allow glycolysis to proceed more rapidly when the cell's oxygen level dropped to a low level, thus maintaining a continuous source of energy. Similarly, the release of the Crabtree effect would oxidatively provide needed energy normally supplied by glycolysis when the cell's supply of carbohydrate substrate becomes exhausted. However, one can expect the glucose and oxygen supply to remain relatively constant for any given mammalian cell other than cancer cells or perhaps rapidly dividing fetal cells. Therefore, these emergency mechanisms would be of greater advantage for primitive cells living under less organized conditions. It is possible that both effects are chemical remnants of evolution, the Pasteur effect generally remaining, whereas the Crabtree effect usually remains only in a latent form, to be taken advantage of by those cells which for some reason break away from the controlling mechanisms. Although entirely teleological, this concept could be considered to be supported by the following evidence: (a) the development of a tumor in a tissue has been shown to induce a Crabtree effect in that tissue (61); (b) growing the tissue outside the body in tissue culture causes the induction of a Crabtree effect (4, 41); (c) adrenalectomy can induce a glucose effect in several tissues which normally have no Crabtree effect (82).

On the other hand, it is possible that the energy relations are only a coincidental fact related to the mechanism inducing the inhibitions and that the Pasteur and Crabtree effects either have no physiological meaning or are artificially induced reflections of normal intracellular control mechanisms. It has been demonstrated that the total amount of phosphorylation in the Ehrlich ascites or hepatoma cell remains constant under aerobic conditions in the presence or absence of glucose (25, 29). This should mean that all energy-needing reactions would proceed as well in the absence or presence of glucose. This is true in some instances, such as in amino acid uptake (79); but on the other hand, Acs (1) has found that K⁺ accumulation in the Ehrlich ascites cell depends upon glycolysis. This K⁺ accumulation could not be associated with glyconeogenesis, since these cells have no phosphorylase (91). Similarly, phagocytosis in leukocytes appears to be completely dependent upon glycolysis even under aerobic conditions (39). These facts suggest that the energy from glycolysis may be channeled differently from that energy which is derived by oxidative processes. If there is such a variance of effect from the energy obtained through the different catabolic routes, then the cell needs to have a mechanism capable of shifting from one catabolic
process to another. This shift, magnified by artificial conditions, may be what is being studied as the Crabtree or Pasteur effect.

Since glucose has been shown to be necessary for anabolic metabolism of Ehrlich ascites cells in vitro (25, 29, 31) one would expect that, by increasing the availability of sugar in vitro, the tumor cells' growth would be enhanced. However, the opposite appears to be true, since hyperglycemic mice survive doses of Ehrlich cells significantly longer than do normal mice (32).

ACKNOWLEDGMENTS

The author wishes to thank Drs. Hull, Gal, Garcia, and McKee for their critical appraisal of this paper. He wishes to thank Miss Ardella Sweek for aid in checking the references. And Dr. MeKee for making his reference files available.

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The Crabtree Effect: A Review

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