Glucose, Warburg, Cancer and Pathways

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The Warburg effect has been present for almost a century. It is also called aerobic glycolysis. Namely it is an observation that in cancer cells, even with available oxygen, glycolysis occurs resulting in lactate yet only a small fraction of pyruvate passes through the TCA (Krebs) cycle. We examine recent work in this area and make a proposal of rate limiting in the pathway to explain Warburg. Copyright 2018 Terrence P. McGarty, all rights reserved.

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1 INTRODUCTION

In 1925, almost a century ago, Otto Warburg made the observation that cancer cells did not have the same metabolism that normal cells did. They did not rely upon oxygen as strongly and they in many ways appeared a fermentation. Based upon this observation Warburg built an entire theory of cancer as a process built upon a faulty metabolic system, namely the failure of the mitochondria to function properly. Overall the Warburg theory stipulates that the excess glucose overpowers the normal metabolic process resulting in lactate (lactic acid) which in turn results in aberrant cell behavior.

In 1956 Otto Warburg published a summary paper of his studies which concludes with (some edits for clarity of presentation):

Cancer cells originate from normal body cells in two phases.

(i) The first phase is the irreversible injuring of respiration. Just as there are many remote causes of plague-heat, insects, rats-but only one common cause, the plague bacillus, there are a great many remote causes of cancer-tar, rays, arsenic, pressure, urethane- but there is only one common cause into which all other causes of cancer merge, the irreversible injuring of respiration.

(ii) The irreversible injuring of respiration is followed, as the second phase of cancer formation, by a long struggle for existence by the injured cells to maintain their structure, in which a part of the cells perish from lack of energy, while another part succeed in replacing the irretrievably lost respiration energy by fermentation energy.

Because of the morphological inferiority of fermentation energy, the highly differentiated body cells are converted by this into undifferentiated cells that grow wildly-the cancer cells. To the thousands of quantitative experiments on which these results are based, I should like to add, as a further argument, the fact that there is no alternative today. If the explanation of a vital process is its reduction to physics and chemistry, there is today no other explanation for the origin of cancer cells, either special or general.

From this point of view, mutation and carcinogenic agent are not alternatives, but empty words, unless metabolically specified. Even more harmful in the struggle against cancer can be the continual discovery of miscellaneous cancer agents and cancer viruses, which, by obscuring the underlying phenomena, may hinder necessary preventive measures and thereby become responsible for cancer cases.

Namely, the purist Warburg School asserts that cancer is solely a metabolic disorder, even further, a mitochondrial disorder. The last sentence should be of major concern to Warburg purists. Namely, that we should not be distracted by cancer agents or viruses. In reality we have a great deal more evidence of the latter than of the sine qua non of Warburg.

The Warburg School has many adherents and they often reject the many current understandings of cancer initiation and progression and rely upon a purely metabolic hypothesis. For example, in the paper by Seyfried and Shelton the authors conclude with:

Evidence is reviewed supporting a general hypothesis that cancer is primarily a disease of energy metabolism. All of the major hallmarks of the disease can be linked to impaired mitochondrial function. In order to maintain viability, tumor cells gradually transition to substrate level phosphorylation using glucose and glutamine as energy substrates. While cancer causing germline mutations are rare, the abundance of somatic genomic abnormalities found in the majority of cancers can arise as a secondary consequence of mitochondrial dysfunction. Once established, somatic genomic instability can contribute to further mitochondrial defects and to the metabolic inflexibility of the tumor cells.

Systemic metastasis is the predicted outcome following protracted mitochondrial damage to cells of myeloid origin. Tumor cells of myeloid origin would naturally embody the capacity to exit and enter tissues. Two major conclusions emerge from the hypothesis; first that many cancers can regress if energy intake is restricted and, second, that many cancers can be prevented if energy intake is restricted. Consequently, energy restricted diets combined with drugs targeting glucose and glutamine can provide a rational strategy for the longer-term management and prevention of most cancers[1].

This conclusion is a clear statement of cancer prevention and even management via a metabolic mechanism, namely limitation of glucose. While there is substantial interest in the metabolic elements of cancer, reliance upon one thread amongst many may pose substantial risks. For example the excess of ROS, reactive oxygen species, as may be found in inflammatory cancer initiators may be related in a metabolic manner but are not related as directly as that os the Warburg mitochondrial process[2].

When we examine the work regarding the Warburg effect, we note several factors as recent research has proceeded:

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[1] Seyfried and Shelton Nutrition & Metabolism 2010, 7:7 http://www.nutritionandmetabolism.com/content/7/1/7 Page 15 of 22

[2] Again see Seyfried and Shelton, *In addition to avoiding exposure to established cancer risk factors, the metabolism of ketone bodies protects the mitochondria from inflammation and damaging ROS. ROS production increases naturally with age and damages cellular proteins, lipids, and nucleic acids. Accumulation of ROS decreases the efficiency of mitochondrial energy production. The origin of mitochondrial ROS comes largely from the spontaneous reaction of molecular oxygen (O2) with the semiquinone radical of coenzyme Q, .QH, to generate the superoxide radical O2-. Coenzyme Q is a hydrophobic molecule that resides in the inner mitochondrial membrane and is essential for electron transfer. Ketone body metabolism increases the ratio of the oxidized form to the fully reduced form of coenzyme Q (CoQ/CoQH2). Oxidation of the coenzyme Q couple reduces the amount of the semiquinone radical, thus decreasing superoxide production.*

1. The Warburg effect, namely a rebalancing of reduced pyruvate fed TCA generation or ATP as compared to an enhanced lactate production is most likely an effect of the complexity in pathways in cancer cells.

2. Cancer cells manage to readjust their metabolic systems to enhance their growth and proliferation often in low oxygen environments.

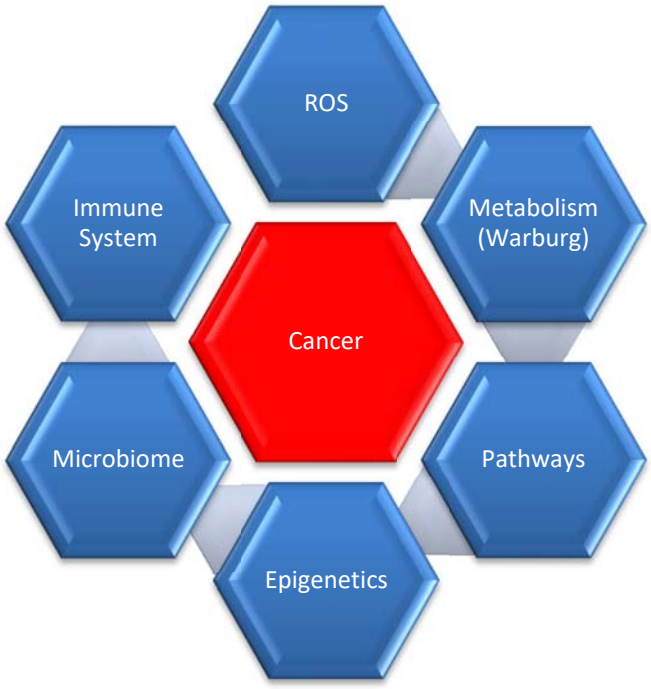
3. There appears to be a set of well-defined pathways that play a strong role in the switch to Warburg like metabolic proliferation.

4. Cancer cells seem to proliferate almost only in a Warburg manner so that blockage of the adjusted pathways may be a means to starve off the cells.

5. There does not appear to be a clearly define set of immune system determinants to target.

Overall, one of the most comprehensive and balanced papers regarding cancer metabolism is the 2016 paper by Pavlova and Thompson. Unlike many of the papers which blindly support the Warburg thesis, these authors provide a well-balanced summary of the facts and where they may lead.

There are a multiplicity of "causes" of various cancers. We depict the usual suspects below. More than likely as the various forms of the disease are better understood, some of these factors may become just a consequence of the disease. Some may be a cause, a consequence, and a random coincidence.



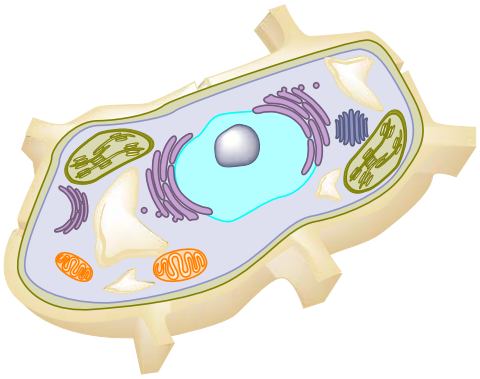
Our focus herein is the metabolic path although we have considered many of the others in some detail. The metabolic path typically is broad in cancers covered. Unlike say the epigenetic paths where we have a collection of specific and identifiable cancers related to specific alterations, the Warburg supporters throw a broad net across almost any and all cancers. In effect the world view of those on the metabolic front often reflect a more classic early 20th century view of cancers, namely a commonality amongst all organs.

2 ENERGY, ATP AND CELL THERMODYNAMICS

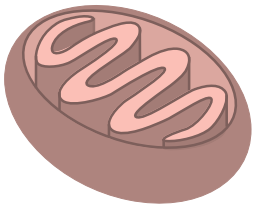
Energy in a cell is generated by the conversion of glucose to ATP. ATP then is a molecule which can give up a phosphate and release energy. That energy then is used throughout the cell. Thus ATP dynamics is at the heart of the metabolic process. We provide but the briefest overview here so as to allow recall. There is a significant amount of literature on these topics[3].

2.1 THE CELL

The cell is depicted below. Key to the metabolic process is the mitochondria. We see the nucleus, the Golgi, the mitochondria, and various other cell elements.



The mitochondria is depicted below. It is a double walled cell and this double wall plays an important role in the dynamics of ATP production.

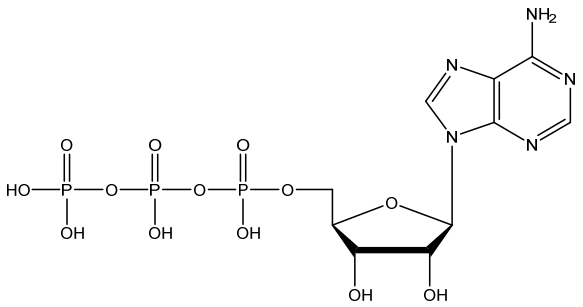


All of the metabolic processes are conducted within this element. It should also be remembered that the mitochondria contains its own DNA and that this DNA id maternally derived. This may be a key point of observation regarding the ultimate assessment of the Warburg Theory.

2.2 ATP

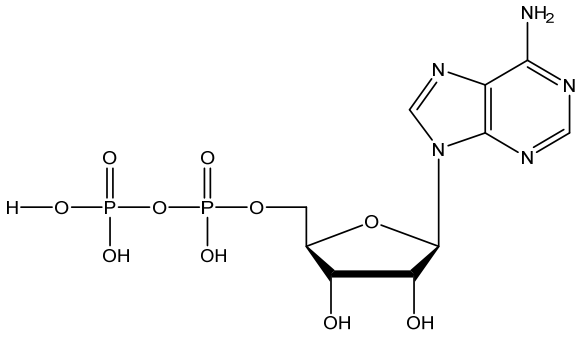
ADP is shown below. It has the adenine on the right, connected to a ribose, the combination is then an adenosine and then the three phosphate groups, often the OH groups are lacking the H and thus are charged.

[3] See Berg, Biochemistry.



ATP

ADP, the diphosphate member, is shown below. It can in turn be reduced to a monophosphate as well which we do not depict.



ADP

Now we follow Atkins (pp 237-239) on a brief description of the ATP biological activity via its thermodynamics. Specifically we see the reaction:

$$ATP(aq) + H_2O \rightarrow ADP(aq) + P_i^-(aq) + H^+(aq)$$

The phosphate release can then be used for energy. More importantly for this reaction we have:

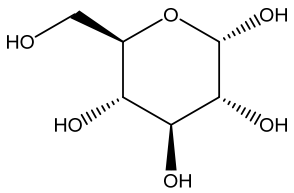
$\Delta G = -30kJ / mol$
$\Delta H = -20kJ / mol$
and
$\Delta S = +34J / K - mol$

Recall that

$$\Delta G = \Delta H - T\Delta S$$

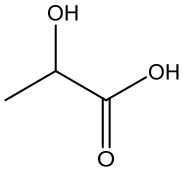
and thus we have a very reactive and energy rich source with ATP.

Now we can recharge the ADP in an aerobic or anaerobic manner. Aerobic requires oxygen and anaerobic is without oxygen, as we see in fermentation. The anaerobic reaction ends in lactate as shown below:

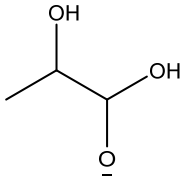


Glucose

gets converted to:



Lactic Acid



Lactate

Lactate is the negatively charged version of lactic acid found in aqueous solutions such as a cell. The reaction is as follows:

$$\text{Glucose} + 2P_i^- + 2ADP \rightarrow 2Lactate^- + 2ATP + 2H_2O \quad \Delta G = -158kJ/mol$$

Now this reaction converts glucose to ATP, as well as lactate and the Gibbs free energy shows it moves forward and thus glucose can be a strong source of ATP. Albeit only 2 ATP units per glucose.

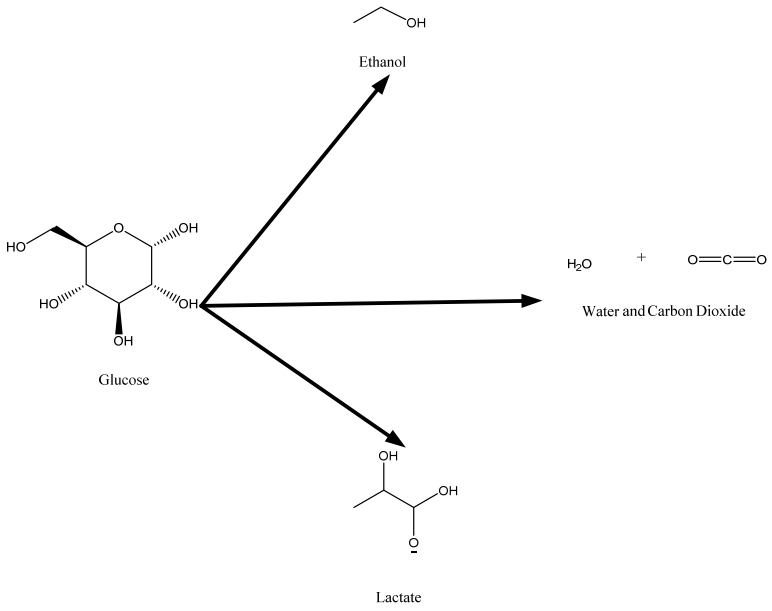
Now the aerobic source, which we shall discuss shortly is much more energy intensive generating many more ATPs. Specifically:

$$Glu\cos e \rightarrow\sim 38ATP \quad \Delta G = -2880kJ/mol$$

This is the complete process that takes glucose to pyruvate through the Krebs cycle. It uses oxygen to accomplish this change and does not progress down the path of lactate. Note that the number of ATP varies considerably from one source to another and thus we have used Atkins number from the time frame of the text. We shall examine it in detail next.

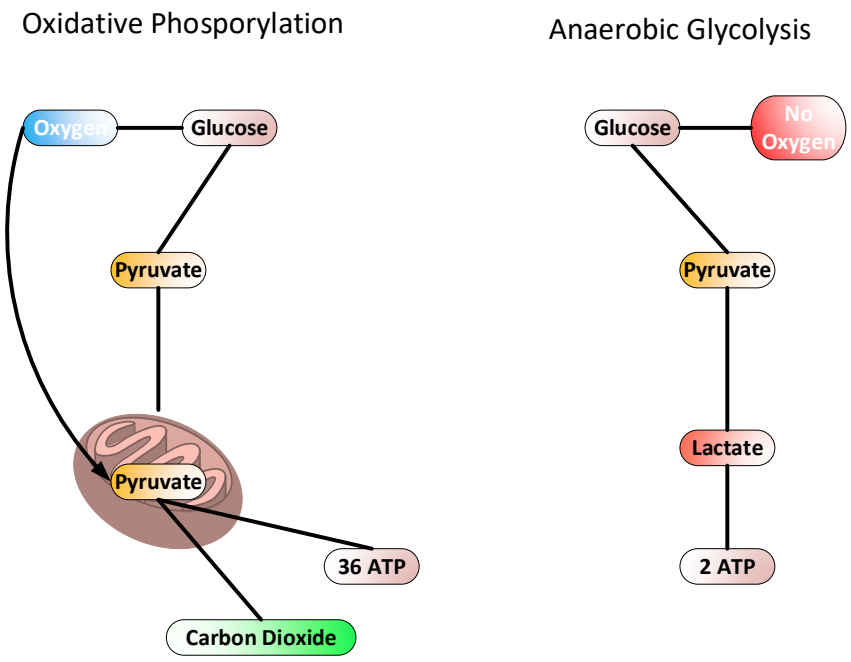
3 METABOLIC PATHWAYS

Cells require a continual source of energy. One primary source is glucose, but there are many other sources as well. As regards to glucose as it applies to Warburg, there are three generally accepted paths in which we see the breakdown of glucose. Glucose can proceed as follows:

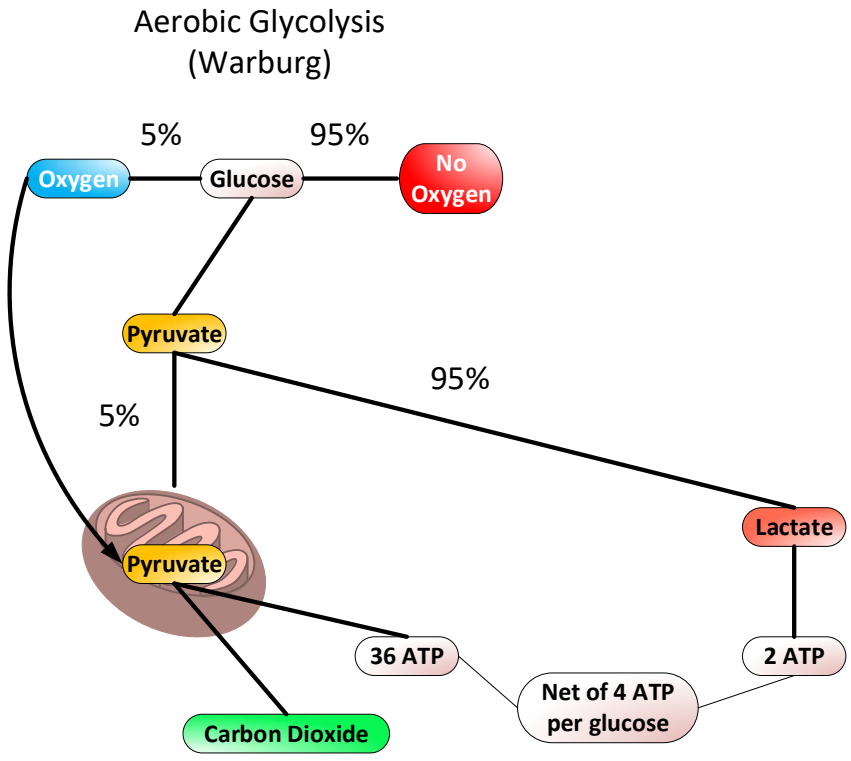


Fermentation and its product alcohol will not be examined. However the water and carbon dioxide path is the oxygen consuming path of normal cellular metabolism and the lactate path is the oxygen depleted path. Warburg came to the conclusion of a third or if you will a fourth (assuming you include fermentation) type of path, one which uses some oxygen but not that much. It was the depleted aerobic path that is the heart of the Warburg construct.

The two classic paths that we focus upon are shown below: with and without oxygen. The following two paths are the typical two we often consider.



In contrast the Warburg path is shown below. Note that it somehow uses both approaches. The explanation for this bifurcation seems obscure. Later we attempt to provide a rationale that can explain it.



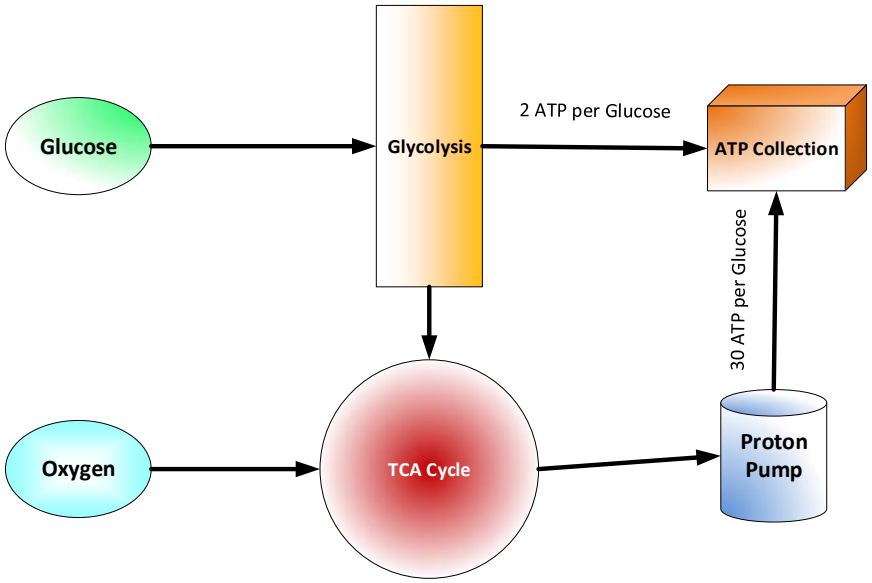
Note that both paths work but the classic oxygen path only slightly. The ATP production is slightly better than the anaerobic path. The details as to how this bifurcated pathway mechanism

functions is yet to be determined. One key question which should be kept in mind is: Can the genes controlling this pathway be themselves controlled and then if this pathway is then disturbed does it have a therapeutic effect on cancer cells. If Warburg effects are merely consequential the answer is there is no effect. If the answer is that there is a therapeutic effect, then Warburg may be correct.

We will now examine some details on each part of these.

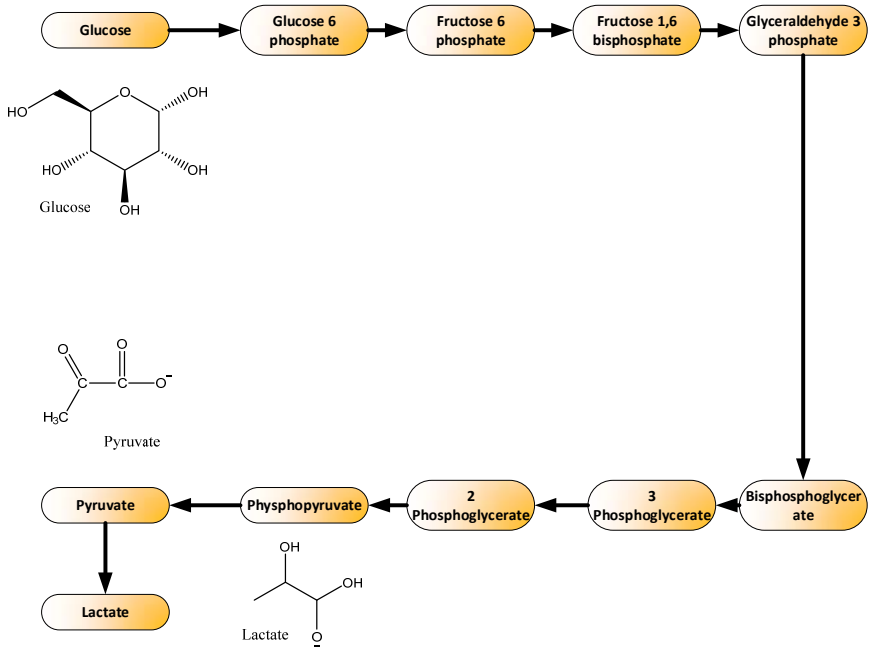
3.1 OXIDATIVE PHOSPHORYLATION

The classic path goes from glucose to water and carbon dioxide. It is a very efficient path and energy rich pumping out well over 30 ATP molecules per glucose molecule. Thus it is the path we see in normal metabolism. An overall summary of the key elements is shown below. This includes three fundamental steps: (i) glycolysis which is the breakdown of glucose to pyruvate producing 2 ATP per glucose molecule, (ii) TCA cycle producing ATP and its precursors NAD and FAD related molecules, (iii) the proton pump mechanism which converts NAD and FAD to ATP. Phosphorylation is a key process whereby energy is transferred. We see this in many pathways and this is but one. The Figure below is a summary of this process.



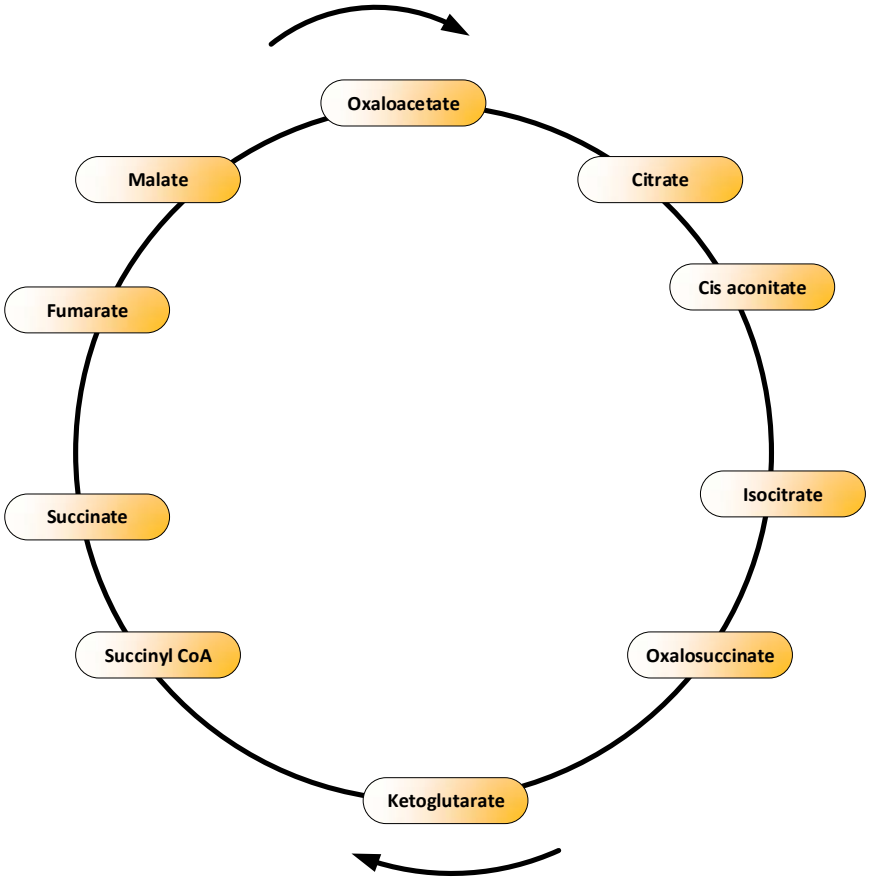
Glycolysis is shown below where we go to pyruvate and then to lactate. We do not present the full details since they are well known and available elsewhere[4].

[4] See Rodwell et al, Biochemistry, McGraw Hill (New York) 2915, pp 160-170, Ferrier, Biochemistry, 6th Edition, Lippincott (New York) 2014 pp 109-120.



In a similar fashion we show below the simplified version of the TCA or Krebs cycle below. We have not shown the inputs and the outputs but only the main elements[5]. This is the most productive of all parts of this process in the direct and indirect production of ATP and it is the section that requires oxygen. Without oxygen this cycle does not function.

[5] Details of the TCA can be found in the references.



The combinations above produce 32 to 36 ATP molecules.

3.2 ANAEROBIC GLYCOLYSIS

This process is the previous one but with no operative TCA. This is because there is no oxygen available. Thus we are limited with ATP generated solely by glycolysis.

3.3 AEROBIC GLYCOLYSIS

This is the Warburg construct. We have shown it above. The current understanding is that it is a small amount of the oxygen based cycle and a dominant amount of the oxygen poor cycle. This is a cycle that has some small oxygen contribution, that a small contribution from a TCA and is dominated by the first step glycolysis.

3.4 DYNAMICS OF PROCESSES

What we reviewed above was the three mechanisms of ATP generation. However we have not described their dynamics, namely the rate at which each of these processes act. Let us look at oxidative phophorylation, the classic path if you will, as a two step process. Namely step one is glycolysis and step two is TCA combined with the proton pump. Let us further assume we can

get 2 ATP from glycolysis and 32 from the second process. That is fine but what of the dynamics. How many ATP can we get per second for example. If we have a very "hungry" cell and a rich glucose environment, or even a cell which can scavenge its environment better than any others, the cell will have lots of food and then it starts the process. However we are faced with a rate problem. If step 1, glycolysis, can just run at any rate, say a rate R, which generates say R ATP cycles per second, or 2 times R actual ATP, then we have a fast generator. Now assume the second path combined can run at a much lower rate. It can generate 32 for each cycle but it is limited to a maximum number of cycles per second which is substantially less that the glycolysis. We have the following model:

R_1 = rate of process 1 (say glycolysis) (cycles/sec)

R_2 = rate of process 2 (say TCA) (cycles/sec)

R_3 = rate of process 3 (say proton pump) (cycles/sec)

then

G = Generate Rate of ATP per sec

$$G = \sum_{n=1}^{3} G_i R_i$$

where

$G_1 = 2$

$G_2 = 2$

$G_3 = 30$

Now this is the rate analysis. It is the maximum rate analysis. If the rate of the second steps is say three orders of magnitude that of the first step, then the effective rate dominates and the process pumps out ATP and the second steps does the same at a higher number but at a much smaller rate. Thus the total number is dominated by the first and rate limited by the second. This is similar to an enzymatic rate limited system. In fact this totally explains the Warburg effect.

We would now have to examine the driver of this process. Namely the supply of glucose. We assume a glucose supply of S molecules per second. We further assume a capacity C for each step in terms of cycles per second, namely processing a glucose molecule or its product. We also assume a yield Y of so many ATP per cycle.

Then:

If N(glucose/sec)

$$If: C_{glycolysis} > C_{TCA} > C_{Proton} > N$$

then

$$Y = Y_{glycolysis}N + Y_{TCA}N + Y_{proton}N$$

$$If: C_{glycolysis} > C_{TCA} > N > C_{Proton}$$

$$Y = Y_{glycolysis}N + Y_{TCA}N + Y_{proton}C_{proton}$$

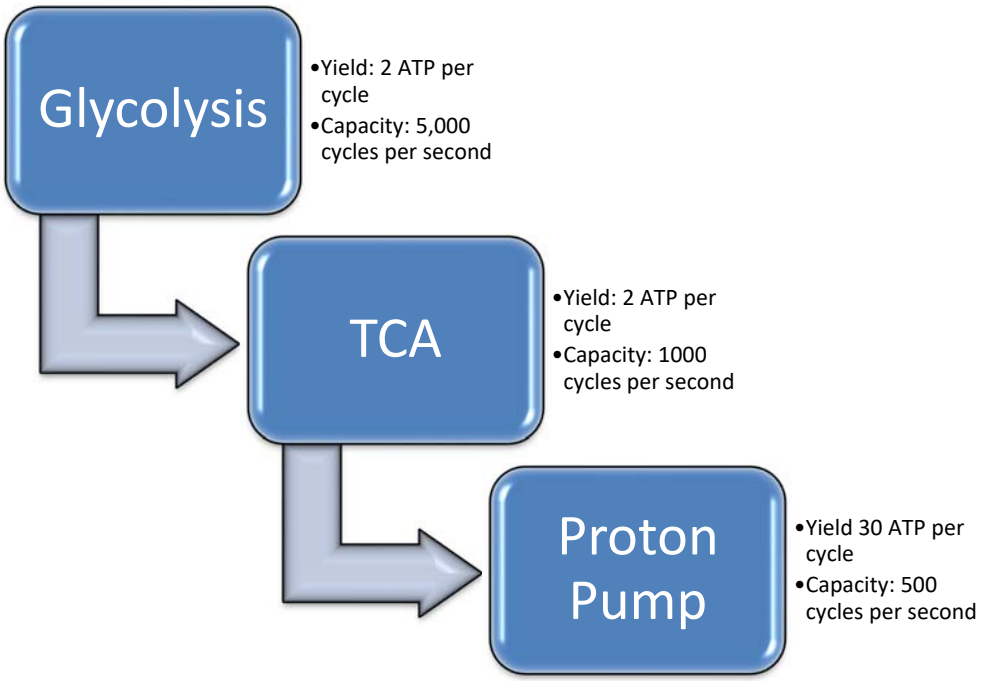
$$C_{glycolysis} > N > C_{TCA} > C_{Proton}$$

$$Y = Y_{glycolysis}N + Y_{TCA}C_{TCA} + Y_{proton}C_{proton}$$

$$N > C_{glycolysis} > C_{TCA} > C_{Proton}$$

$$Y = Y_{glycolysis}C_{glycolysis} + Y_{TCA}C_{TCA} + Y_{proton}C_{proton}$$

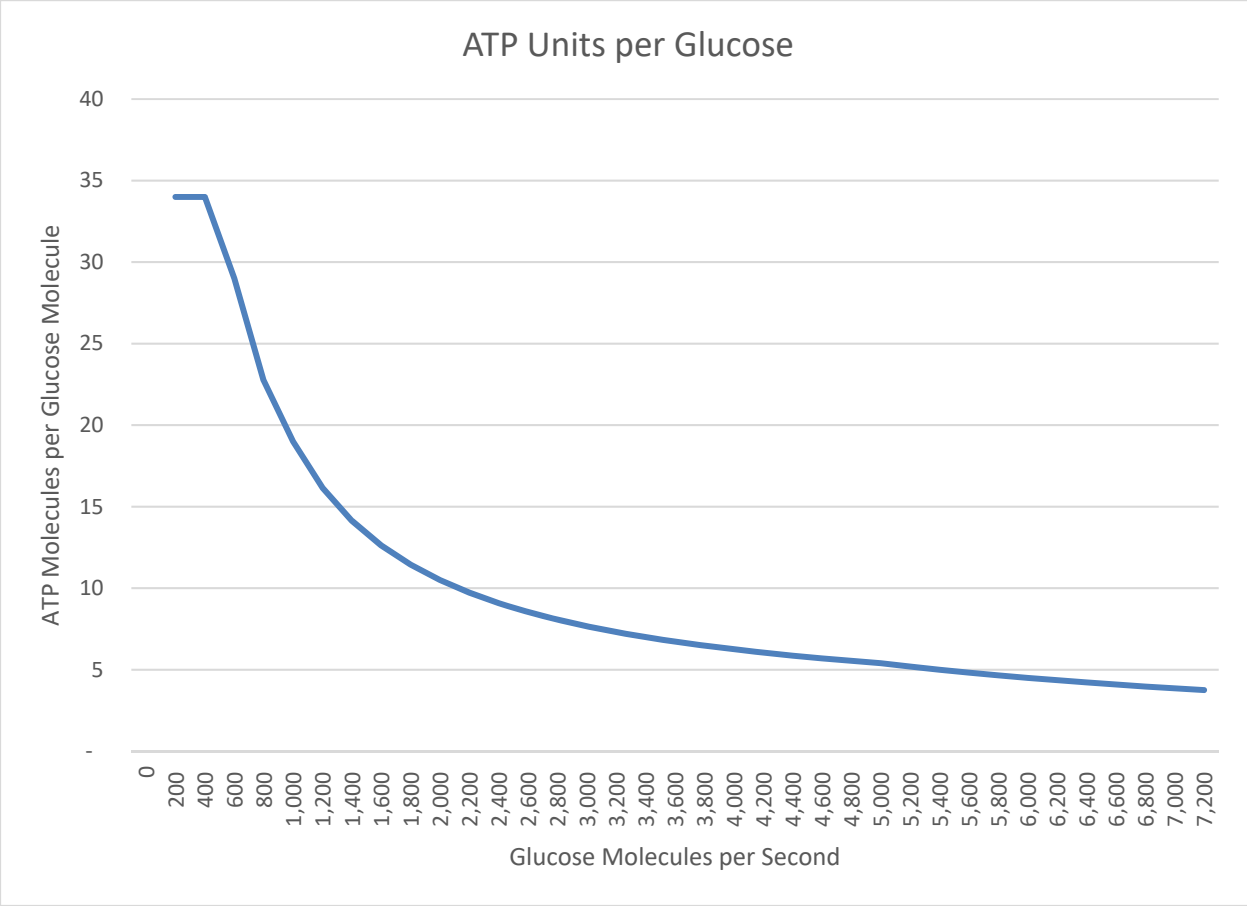
We can depict this below:



Now let us consider a simple example. Choose the following constants:

Capacity Gly	5,000	cycle/sec	ATP Rate	2
Capacity TCA	1,000	cycle/sec	ATP Rate	2
Capacity Proton	500	cycle/sec	ATP Rate	30
				34

Then we obtain the following yield curve:



Note the yield is 4 ATP per glucose. This is identical to the model proposed by…

This is a model which explains the Warburg effect. Namely there are rate limiting steps in the glycolysis, TCA proton pump model and ultimately the fastest controls. In fact if we make this fast enough we drive it to at best 2 ATP per glucose and that is just for the glucose that are processed.

We can now take this a step further and examine the rate processes. We know from the Gibbs free energy results how well each reaction works. We also have data on reaction rates which we can use for each step. Then we can use classic reaction rate theory to ascertain if the data used in the above example is reflective and what changes should be made.

Let us look at classic reaction rate example.

$$A \to B \to C$$

or

$$S_1 \to S_2 \to .... \to S_{10}$$

$$S_1 = glu\cos e$$

$$S_k .....$$

$$S_{10} = pyruvate$$

We assume we know the reaction rate for each reaction. Let us focus just on the three step example. We can then extend it to the glycolysis and TCA directly. We can follow Moore (pp 345-347) for this simple example. For reactions we have:

$$A \underset{k_1}{\to} B \underset{k_2}{\to} C$$

Thus for rate equations we have given the rate constants k:

$$x = [A]$$

$$y = [B]$$

$$z = [C]$$

$$\frac{dx}{dt} = -k_1 x$$

$$\frac{dy}{dt} = k_1 x - k_2 y$$

$$\frac{dz}{dt} = k_2 y$$

Moore then solves these equations as follows:

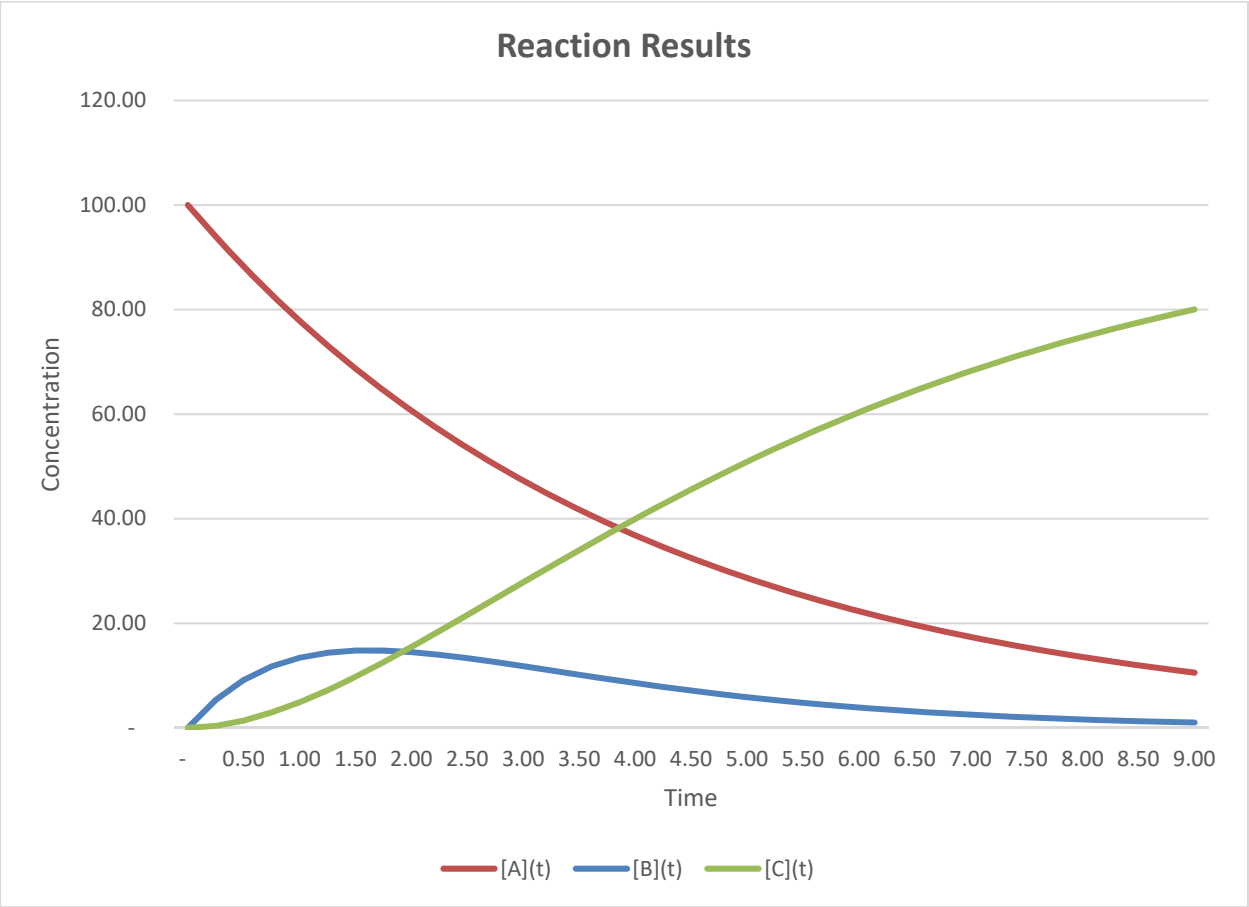
$$x(t) = C_0 \exp(-k_1 t)$$

$$y(t) = \exp(-k_2 t)\left[\frac{k_1 C_0 \exp((k_2 - k_1)t)}{k_2 - k_1} - \frac{k_1 C_0}{k_2 - k_1}\right]$$

$$z(t) = C_0\left[1 - \frac{k_2 \exp(-k_1 t)}{k_2 - k_1} + \frac{k_1 \exp(-k_2 t)}{k_2 - k_1}\right]$$

We can now perform a simple analysis with these equations to demonstrate one more detail below in a simplified example. Here we plot the concentrations of A, B and C as a function of time. Initially we have all A, say glucose. Then an intermediate B is generate but it is used up, and then C the end product appears. The time required to go from A to C is the critical factor. We could then apply this to the glycolysis and TCA chains and demonstrate such rate limiting. It should be noted that we can use IUPAC data on reaction rates for each element in these chains as

contained in the Serjeant and Dempsey tables. We have done this for a few steps and it appear achievable and can then be used for verification.



Note that [A] decays exponentially, [B] increases and then decreases, and then [C] reaches a maximum. This concept then applies for the speed of any one of the three elements.

4 WARBURG'S HYPOTHESIS

Otto Warburg studied the process of cancer in terms of the ATP generation under limited or no oxygen conditions. To an extent this was the state of fermentation, but not one leading to the production of alcohol. As for his background, the details on the Nobel Prize site state[6]:

Warburg was born on October 8, 1883, in Freiburg, Baden. His father, the physicist Emil Warburg, was President of the Physikalische Reichsanstalt, Wirklicher Geheimer Oberregierungsrat. Otto studied chemistry under the great Emil Fischer, and gained the degree, Doctor of Chemistry (Berlin), in 1906. He then studied under von Krehl and obtained the degree, Doctor of Medicine (Heidelberg), in 1911. He served in the Prussian Horse Guards during World War I. In 1918 he was appointed Professor at the Kaiser Wilhelm Institute for Biology, Berlin-Dahlem. Since 1931 he is Director of the Kaiser Wilhelm Institute for Cell Physiology, there, a donation of the Rockefeller Foundation to the Kaiser Wilhelm Gesellschaft, founded the previous year.

His award of the Nobel declares:

"for his discovery of the nature and mode of action of the respiratory enzyme" in the field of cell physiology, metabolism for the work as follows:

In our cells nutrients are broken down so that energy is released for the construction of cells. This respiration requires enzymes, substances that facilitate the process without being incorporated in the final products. Otto Warburg studied the respiration of sea urchins and other organisms at an early stage of development. By measuring oxygen consumption in living cells and studying which enzymes reacted, in 1928 he concluded that the respiration enzyme he was looking for was a red ferrous pigment related to the blood pigment, hemoglobin.

Thus despite having been awarded the Nobel, it was not for his work in cancer. Yet Warburg seems most well-known for his cancer conjectures.

In Warburg's 1956 Science paper he begins by noting:

Since it is known how much adenosine triphosphate can be synthesized by respiration and how much by fermentation, we can write immediately the potential, biologically utilizable energy production of any cells if we have measured their respiration and fermentation. With the ascites cancer cells of the mouse, for example, we find an average respiration of 7 cubic millimeters of oxygen consumed per milligram, per hour, and fermentation of 60 cubic millimeters of lactic acid produced per milligram, per hour. This, converted to energy equivalents, means that the cancer cells can obtain approximately the same amount of energy from fermentation as from respiration, whereas the normal body cells obtain much more energy from respiration than from fermentation. For example, the liver and kidney of an adult animal obtain about 100 times as much energy from respiration as from fermentation.

[6] https://www.nobelprize.org/nobel_prizes/medicine/laureates/1In effect Warburg931/warburg-bio.html

In effect, Warburg examined cells from ascites, the fluid produced frequently from metastasized cancer in the liver. He then examined how much lactate is produced, namely the end product of anaerobic production of ATP. Strangely is appeared that these cancer cells had a combination of anaerobic plus normal metabolism which he called aerobic. Thus Warburg noted that cancer cells almost exclusively obtain their energy not from a TCA method primarily but from some small mix of a TCA plus mostly but not exclusively from what he termed fermentation, or anaerobic paths. Warburg then concluded that this aerobic path was the "cause" of cancer, not just an artifact of a cancerous process.

Warburg then goes on to state:

Clinical experiences along these lines are innumerable: the production of cancer by intermittent irritation of the outer skin and of the mucosa of internal organs, by the plugging of excretory ducts of glands, by cirrhosis of tissues, and so forth. In all these cases, the intermittent irritations lead to intermittent circulatory disturbances., Probably chronic intermittent oxygen deficiency plays a greater role in the formation of cancer in the body than does the chronic administration of respiratory poisons. Any respiratory injury due-to lack of energy, however, whether it is produced by oxygen deficiency or by respiratory poisons, must be cumulative, since it is irreversible. Frequent small doses of respiratory poisons are therefore more dangerous than a single large dose, where there is always the chance that the cells will be killed rather than that they will become carcinogenic.

Frankly there is no basis for any of the above assertions. Warburg's observations of lactate excess in cells which may have an abundance of oxygen, and primarily cancer cells, is just that, an observation. The generalizations emanating therefrom are at best speculation. As we shall note, however, there has arisen an almost cult like group who have taken Warburg's observations and correlations to extremes.

Notwithstanding the speculation, the observation clearly has merit, merit as a distinguishing characteristic. However the statements made by Warburg such as:

It follows from this that there would be no cancers if there were no fermentation of normal body cells, and hence we should like to know, naturally, from where the fermentation of the normal body cells stems and what its significance is in the body. Since, as Burk has shown, the fermentation remains almost zero in the regenerating liver growth, we must conclude that the fermentation of the body cells has nothing to do with normal growth.

On the other hand, we have found that the fermentation of the body cells is greatest in the very earliest stages of embryonal development and that it then decreases gradually in the course of embryonal development. Under these conditions, it is obvious-since ontogeny is the repetition of phylogeny-that the fermentation of body cells is the inheritance of undifferentiated ancestors that have lived in the past at the expense of fermentation energy.

Warburg's claim of "ontogeny recapitulates phylogeny" can be rephrased as "what comes first, the chicken or the egg?" For one must accept the Bacon like observation of this metabolic effect, however one must be wary of Galen like logic that is phenomenologically limited.

4.1 ORIGINAL

We first examine some current understandings of the classic Warburg hypothesis. As Liberti and Locasale note:

During the 1920s, Otto Warburg and colleagues made the observation that tumors were taking up enormous amounts of glucose compared with what was seen in the surrounding tissue. Additionally, glucose was fermented to produce lactate even in the presence of oxygen, hence the term 'aerobic glycolysis'. However, it was also noted that respiration alone could maintain tumor viability. Therefore, it was concluded that, to kill tumor cells by depriving them of energy, both glucose and oxygen had to be eliminated. Subsequently, in 1929, an English biochemist, Herbert Crabtree, extended Warburg's work and studied the heterogeneity of glycolysis in tumor types.

Note that the conclusion regarding killing cancer cells was via starvation. Unfortunately that would likely cause death of all cells around.

He confirmed Warburg's findings, but further discovered that the magnitude of respiration in tumors was variable, with many tumors exhibiting a substantial amount of respiration. Therefore, Crabtree concluded that not only do tumor cells exhibit aerobic glycolysis, but that there is also variability in fermentation, presumably due to environmental or genetic influences. Contrary to the findings of these previous works and for reasons unclear to these authors, Warburg later proposed that dysfunctional mitochondria are the root of aerobic glycolysis. Warburg further hypothesized that this event is the primary cause of cancer.

The dysfunctional mitochondria is one os a possible number of explanations. As we have discussed and shown, another explanation could be rate limiting processes, and one suspects there are other mechanisms as well.

This phenomenon was then termed the Warburg Effect during the early 1970s by Efraim Racker, who also pointed out that previous data showed respiratory capability of tumors. Racker developed his own theories about the origins of the Warburg Effect, ranging from imbalances in intracellular pH to defects in ATPase activity. It was later observed by Racker, Jeffrey Flier, and Morris Birnbaum that aerobic glycolysis was a controllable process that can be directly regulated by growth factor signaling.

By that time, the discovery of oncogenes led to the conclusion that aberrant regulation of growth factor signaling is an initiating event in oncogenesis. Thus, their observations brought newfound significance to Warburg's hypothesis in cancer biology. Nevertheless, it remained unclear whether the Warburg Effect was a bystander in cancer pathogenesis until more recently, when genetic and pharmacological studies conclusively showed that the Warburg Effect was required for tumor growth.

Coming back to the original findings on tumor metabolism, it is now apparent that targeting both aerobic glycolysis and mitochondrial metabolism may be required . Throughout this history, the function of the Warburg Effect has remained controversial. Here, we discuss several of the major

proposals and argue that the functions of the Warburg Effect for tumor growth remain unknown even today.

Indeed it is a controversial observation. We will discuss latter the work by some who see this as a panacea for cancer treatment. Namely the massive blocking of glucose and in turn the aerobic metabolic pathway. As a side note, it is also know that excess lactate has deleterious effects, but that may at best be a sidebar.

4.2 CURRENT VIEW

We now examine the current view. We start with the recent work of Levine and Kutter who note:

Rapidly dividing cells require favorable energetics, that is, higher ATP/adenosine diphosphate (ADP) and ATP/adenosinemonophosphate (AMP) ratios. Many cancer cells satisfy this problem by taking up much larger amounts of glucose than do normal cells. This results from facilitated glucose transport by one or more of several isozymes of membrane glucose transporters (GLUT 1 to 9). Once inside the cell, glucose is phosphorylated by one of several hexokinase enzymes (the first step in glycolysis) to keep it in the cell because of the charge added to glucose. The high concentrations of glucose in the cells of a cancer may be observed by positron emission tomography (PET) scans of radioactive F-19-2-deoxyglucose (FDG is not metabolized but is located in the cell), which is indicative of enhanced glucose uptake by cells.

Many, but not all, cancers have this property of increasing glucose uptake, and this is a confirmation of the Warburg effect. With large amounts of glucose available in a cell, glucose is metabolized through the PPP, producing nucleosides and generating NADPH. The NADPH is essential for fatty acid synthesis, along with acetyl-CoA (which is made from some of the pyruvate in mitochondria that is not converted to lactate). NADPH also contributes to a proper redox control and protects the cell from ROS. There are several ways the cell responds to lower ROS (reactive oxygen species) levels, but by far the major molecule involved is glutathione (GSH), which eliminates ROS by accepting an electron and is converted to its oxidized form, GSSG (glutathione disulfide).

The enzyme glutathione reductase uses NADPH to reduce GSSG to GSH. Thus, NADPH is a major source of cellular "coolant" when oxidative reactions run too "hot" (high ROS levels) by using large amounts of glucose to produce both substrates and energy. However, high levels of ROS can be advantageous for cancer cells when they allow for the stimulation of cell proliferation, induction of genetic instability, and evasion from senescence.

As Thompson, who has done extensive recent work in cancer cell metabolism, noted (see Riccio):

After the initial step in glucose metabolism—glycolysis, conversion of glucose to two molecules of pyruvate—mitochondrial oxidative phosphorylation usually proceeds to yield ATP. But even in the presence of oxygen, many cancer cells divert pyruvate to fermentation, producing lactate. This less rewarding mode of ATP production demands a relatively high rate of glycolysis. Otto Warburg described this shift toward "aerobic glycolysis" in cancer cells in 1924. The molecular

and genetic basis of the Warburg effect, however, has only recently come to light. Contrary to Warburg's hypothesis that mitochondrial defects necessitate this shift, most cancer cells maintain the ability to execute oxidative phosphorylation and do fully catabolize a small amount of glucose.

Cancer cells are genetically differentiated from normal cells, but it is now clear that the metabolic shifts they exhibit are also partly required for division of normal cells. In a quiescent cell, maximum ATP production yields enough energy for cellular machinery, and at least 50% of free energy is used for ion transport across the membrane. When a cell divides, glycolytic intermediates are diverted from the tricarboxylic acid (TCA/Krebs) cycle to reserve carbon and nitrogen for fatty acid synthesis and for production of nonessential amino acids. DNA replication demands de novo nucleotide synthesis, beyond the supply garnered from recycling pathways in a non-dividing cell. Ribose, serine, and glycine (byproducts of glucose metabolism), as well as glutamine for pyrimidine production, are needed for nucleotide synthesis.

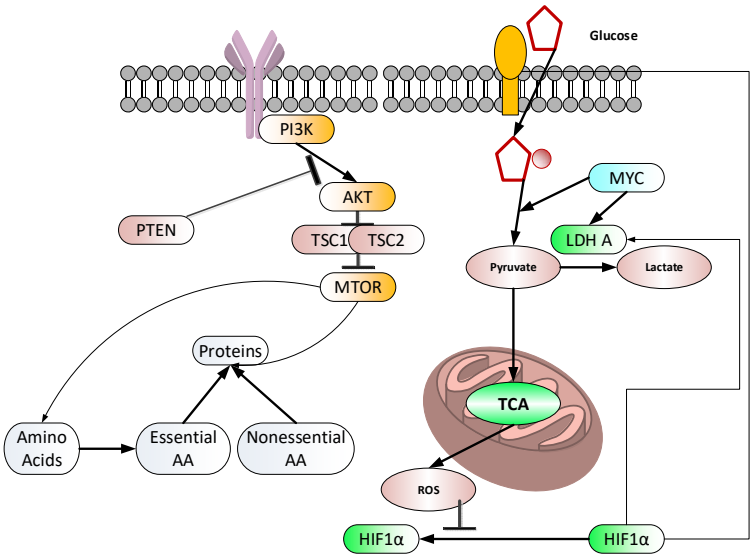
Finally Thompson notes the active role genetic alterations play. This is sharp contrast to the abject rejection of such by Warburg and his followers. Specifically Thompson notes:

The most commonly mutated gene in cancers is KRAS. The KRAS protein, a GTPase, normally functions as a molecular switch, relaying signals received by receptor tyrosine kinases and other receptors of extracellular signals. Two of its main targets include the MAPK and PI3K signal transduction cascades. But many indirect targets of KRAS are involved in cellular metabolism, including glucose transporters that are positively regulated by the PI3K/Akt pathway. Glutamine-addicted tumors are often characterized by the oncogenic expression of Myc, a transcription factor that promotes the expression of glutamine transporters as well as metabolic enzymes needed for biosynthesis. Constitutively activated KRAS thus primes a cell to undergo aerobic glycolysis by ensuring a steady influx of glucose.

Namely the Warburg effect is not standard for cancer cells. Thus the mass of conclusions based upon this faulty construct a truly built with feet of sand. We shall detail this further.

5 PATHWAYS AND METABOLISM

Warburg factors relate to the metabolism of the cell, focusing on the mitochondria. However there are many cell factors which in turn control this process for better or worse. There has been a great deal of study examining the various genetic pathways and their controls on the metabolic actions that are a focal point of the Warburg effect. The following Figure depicts the complex interaction of pathways and metabolism.



The paper by DeBerardinis et al from which we have abstracted the above demonstrates several of the pathways control elements which may be prominent in controlling the metabolic processes. They state regarding the above diagram:

The model shows some of the prominent aspects of metabolism in proliferating cells, including glycolysis; lactate production; the use of TCA cycle intermediates as macromolecular precursors; and the biosynthesis of proteins, nucleotides, and lipids. The PI3K/Akt/mTOR pathway, HIF-1a, and Myc participate in various facets of this metabolic phenotype. The binding of a growth factor (GF) to its surface receptor brings about activation of PI3K and the serine/threonine kinases Akt and mTOR.

Constitutive activation of the pathway can occur in tumors due to mutation of the tumor suppressors PTEN, TSC1, and TSC2, or by other mechanisms. Metabolic effects of the PI3K/Akt/mTOR pathway include enhanced uptake of glucose and essential amino acids and protein translation. The transcription factor HIF-1a is involved in determining the manner in which cells utilize glucose carbon. Translation of HIF-1a is enhanced during growth-factor stimulation of the PI3K/Akt/mTOR pathway.

In the presence of oxygen, HIF-1a is modified by prolyl hydroxylases, which target it to a ubiquitin ligase complex that includes the tumor suppressor VHL. This association results in constitutive normoxic degradation of the HIF-1a protein. Hypoxia, mutation of VHL, or accumulation of reactive oxygen species (ROS) or the TCA cycle intermediates succinate and

fumarate impair HIF-1a degradation, allowing it to enter the nucleus and engage in transcriptional activity. Transcriptional targets include genes encoding glucose transporter 1 (GLUT1), LDH-A, and PDK1. The combined effect on glucose metabolism is to increase both glucose utilization and lactate production, as PDK1 inhibits conversion of pyruvate to acetyl-CoA by pyruvate dehydrogenase (PDH). The transcription factor Myc increases expression of many metabolic enzymes, including glycolytic enzymes, LDH-A, and several enzymes required for nucleotide biosynthesis.

Abbreviations: PI3K, phosphatidylinositol 3-kinase; PTEN, phosphatase and tensin homolog; TSC, tuberous sclerosis complex; mTOR, mammalian target of rapamycin; glc-6-P, glucose-6-phosphate; 3-PG, 3-phosphoglycerate; PDK1, pyruvate dehydrogenase kinase 1; SDH, succinate dehydrogenase; FH, fumarate hydratase; HIF-1a, hypoxia-inducible factor 1a; VHL, von Hippel-Lindau.

Now there are multiple other papers which depict variants on the above and referenced herein. One suspects that this analysis is still a work in progress and as such we expect a considerable amount of complexity. The full details of the interaction and control are yet to be specified. This does raise the issue; is the Warburg effect an artifact or a separate entity? The observations of it being an artifact are currently compelling.

Thompson in EMBO notes regarding the work of Pate et al:

In this issue of The EMBO Journal, Pate et al identify Wnt signaling as a mechanism that suppresses pyruvate oxidation in the TCA cycle and promotes rather than inhibits cell proliferation. As such, Wnt signaling is a candidate for the signal transduction pathway that could synergize with PI3K/AKT signaling in proliferating cells. Wnt signaling is already well characterized as a regulator of cell proliferation.

First, Wnt induced LEF/TCF/b-catenin transcription complexes have been implicated in controlling cell proliferation through the induction of Myc and Cyclin D. Cyclin D levels are critical to cell cycle progression through G1, and Myc has been implicated in the stimulation of glutamine metabolism and nucleotide synthesis necessary to support S-phase. Pate et al identify additional transcriptional targets of LEF/TCF/b-catenin complexes.

They report that the genes connected to metabolism are the most highly overrepresented category of Wnt-target genes. Two relevant Wnt targets were identified: pyruvate dehydrogenase kinase 1 (PDK1) and the lactate transporter (MCT-1). These two proteins along with the Myc induced gene LDH-A allow Wnt-activated cells to divert glycolytic pyruvate away from the TCA cycle by converting it into lactate and promoting lactate secretion from the cell. Induction of PDK1 was found to be required for Wnt-induced aerobic glycolysis, in vivo tumor cell accumulation, and VEGF independent angiogenesis.

Pate et al did not test for whether Wnt induction of Myc and/or glutamine metabolism contributes to Wnt-induced tumor cell accumulation. However, it is reasonable to suspect that Wnt induction of PDK1 cooperates with Myc-induced glutaminolysis to facilitate a cellular transition from growth to proliferation since Myc is a well-characterized effector of Wnt signaling.

The combined effects of Wnt-facilitated aerobic glycolysis and Myc-induced glutaminolysis provide the cell with a potent ability to engage in de novo nucleotide biosynthesis

This observation would modify the above diagram by adding a WNT receptor and having it drive MYC. Thus we now would understand the driver of MYC and the importance of the WNT connection. This is an added example of the progression of the modifications and understanding of the metabolism which seems to be tightly controlled by various pathways, and pathways which we know to be subject to attack in many cancers. Thus the argument that the Warburg proponents make that the pathways are irrelevant seems to be destroyed in these studies.

Finally in a recent paper by Pavlova and Thompson we have:

Tumorigenesis is dependent on the reprogramming of cellular metabolism as both direct and indirect consequence of oncogenic mutations. A common feature of cancer cell metabolism is the ability to acquire necessary nutrients from a frequently nutrient-poor environment and utilize these nutrients to both maintain viability and build new biomass. The alterations in intracellular and extracellular metabolites that can accompany cancer-associated metabolic reprogramming have profound effects on gene expression, cellular differentiation and the tumor microenvironment.

In this Review, we have organized known cancer-associated metabolic changes into six hallmarks:

(1) deregulated uptake of glucose and amino acids,

(2) use of opportunistic modes of nutrient acquisition,

(3) use of glycolysis/TCA cycle intermediates for biosynthesis and NADPH production,

(4) increased demand for nitrogen,

(5) alterations in metabolite-driven gene regulation, and

(6) metabolic interactions with the microenvironment.

While few tumors display all six hallmarks, most display several. The specific hallmarks exhibited by an individual tumor may ultimately contribute to better tumor classification and aid in directing treatment.

In the above paper the authors fill in further details of internal gene pathway controls on the cell metabolic process.

6 OBSERVATIONS

We can now make a few observations which have merit when examining the Warburg effect.

1. IF CANCER CELLS NEED GLUCOSE TO PRODUCE ATP, BUT THEY PRODUCE AT BEST 4 ATP PER GLUCOSE, WHEREAS NORMAL CELLS PRODUCE 30-36, THEN IF WE REDUCE GLUCOSE DRAMATICALLY, TO A BASAL AMOUNT FOR NORMAL CELLS, WOULD THAT "STARVE" CANCER CELLS. EXAMPLE IS METFORMIN AND PROSTATE CANCER.

This has been the standard conjecture that advocates of Warburg effects articulate. As Levine and Kutter noted:

Cells from some tumors use an altered metabolic pattern compared with that of normal differentiated adult cells in the body. Tumor cells take up much more glucose and mainly process it through aerobic glycolysis, producing large quantities of secreted lactate with a lower use of oxidative phosphorylation that would generate more adenosine triphosphate (ATP), water, and carbon dioxide.

This is the Warburg effect, which provides substrates for cell growth and division and free energy (ATP) from enhanced glucose use. This metabolic switch places the emphasis on producing intermediates for cell growth and division, and it is regulated by both oncogenes and tumor suppressor genes in a number of key cancer-producing pathways. Blocking these metabolic pathways or restoring these altered pathways could lead to a new approach in cancer treatments.

For example, we have examined the use of metformin in prostate cancer and the result can be a diminution of the malignancy.

2. SINCE WE HAVE INTERNAL CELL PATHWAYS TO CONTROL METABOLIC PATHWAYS CAN WE IDENTIFY SPECIFIC PROTEINS TO BLOCK TO STARVE THE AEROBIC PATHWAY? IF SO, THEN WHAT HARM MAY THAT CAUSE OTHER THAN STARVING THE CANCER?

Pathway control of the Warburg effect has been examined by several recent studies. We have referred to them and have discussed them in some detail. However their use is still and open question. Secondary harms are all too often the controlling factor. By starving the patient of glucose do we create a plethora of secondary and unintended but deleterious consequences? One suspects that to be the case.

As Tisdale noted when examining cachexia in cancer:

Most cancer cells use glycolysis as the principal method to generate ATP, and this phenomenon is called the Warburg effect. The increased glucose uptake by tumors is the basis of the [18F]fluorodeoxyglucose positron emission tomography (FDG-PET) tumor diagnostic method, which is based on the assumption that cancer tissue has a higher rate of glucose uptake than normal tissue (29). In addition, glycolytic inhibitors have been suggested as being useful to specifically target the slow-growing cells of a tumor, which would complement currently used

chemotherapeutic agents and radiation, which target rapidly growing cells. Several reasons have been suggested to explain this phenomenon including dysfunctional mitochondria, which exhibit frequent mutations in the DNA which would prevent their use of the tricarboxylic acid cycle, preventing the total combustion of pyruvic acid. Since mitochondrial DNA codes for 13 components of the respiratory chain, it is likely that such mutations would cause malfunctions in respiration. Indeed, respiration-deficient cells with deletions in mitochondrial DNA show an increased dependency on glycolysis, increased NADPH and activation of the Akt survival pathway, resistance to antitumor drugs, and a survival advantage in hypoxic conditions. Other alterations include overexpression of the "low Km" form of hexokinase, type II hexokinase, due to gene demethylation, resulting in tumor glucose utilization at normal blood sugar levels, oncogenic signals, such as ras and src, which increase dependence on glucose, and tumor hypoxia due to growth beyond the vascular supply. Hypoxia activates a transcription factor called hypoxia- inducible factor 1 (HIF-1), which increases the transcription of the cell-surface glucose transporter GLUT1, and at least one isoform of nearly all the core enzymes of glycolysis.

Overall Tisdale does not relate Warburg that strongly with the overall cachexia process. To some degree this is surprising.

3. Is there a Reverse Warburg Effect?

Can Warburg work in reverse? As Xu et al note:

There is much evidence that the Warburg effect has many questionable points. Based on a mass of research, a new hypothesis is catching people's attention, the reverse Warburg effect. Glycolysis occurs in mesenchymal stoma cells under the activation of neighboring cancer cells. Furthermore, an increased formation of recycled nutrients is produced. This high-energy metabolism is transferred to the neighboring cancer cells by the orientation of transport to participate in the TCA cycle.

The consequence is that the OXPHOS (oxidative phosphorylation) increases enhancing ATP production, thus constituting metabolic coupling. This new model may well explain both the way ATP is produced via a low efficiency method despite extremely high energy demand of the tumor cells, and reasonably explain the 'autophagy paradox' that has long been questioned. Although our focal point on the aerobic glycolysis mirrors the core of the realm, further research is still required on cancer bioenergetics.

We have argued that there is a simpler way to explain Warburg, namely simple rate limiting.

4. Epigenetics can be induced by metabolic pathways. How then does the metabolic issue in and around cancers play with the epigenetic changes?

Epigenetics has become one of the fertile areas for understanding cancers. We use herein a broad definition of epigenetics as one where there is a non-DNA specific factor altering the ultimate expression of a gene. This may range from simple DNA methylation, methylation of acetylation of chromosome complexes, micro RNAs and other related gene control mechanisms. As Yun et al note:

Epigenetics is defined as heritable changes in gene expression without alterations in the underlying genetic material. Modifications include DNA methylation and covalent post-translational modifications of histones such as acetylation, methylation, phosphorylation, ubiquitination, phosphorylation, and crotonylation. Since every cell in the body has the same genetic code, epigenetic regulation of gene expression plays a large role in determining cellular identity. Failure of proper maintenance of cellular epigenetic status can, thus, result in loss of tissue identity or aberrant signaling pathways that lead to developmental defects or dis- ease states such as diabetes and cancer .

It is now well accepted that cancer initiation and progression are driven by a series of genetic and epigenetic alterations that cause either activation of oncogenes or inactivation of tumor suppressor genes. Much of the recent excitement in the field of cancer epigenetics lies in the reversible nature of epigenetic alterations; unlike genomic mutations, these changes can theoretically be reversed by epigenetic therapy.

Recently, four drugs that target the epigenetic machinery have been approved by the FDA for cancer treatment and have demonstrated prolonged survival and lower toxicity than conventional chemotherapy . Despite intensive research and remarkable advances in our understanding of epigenetics, the mechanisms and regulators that trigger pathological epigenetic reprogramming in cancer remains poorly understood.

How these relate to Warburg is unclear. However Bensinger and Christofk have noted regarding miRNA and Warburg the following:

Since the discovery that miRNAs are aberrantly expressed in cancer, accumulating evidence suggests that miRNAs contribute to tumor growth by modulating levels of oncogenes and tumor suppressors. Not surprisingly, some miRNAs have been shown to regulate cancer metabolism. miRNA-23a and miRNA-23b, which are suppressed by MYC, repress mitochondrial glutaminase expression. Therefore, MYC enhances glutaminase and glutamine metabolism, an important carbon and nitrogen source for biosynthesis in cancer cells, by repressing miRNA-23a/b expression. A recent study by Eichner et al. found that ERBB2 signaling leads to miRNA-378 expression, which promotes the Warburg effect by inhibiting expression of ERR, a binding partner for PGC-1, leading to reduced transcription of tricarboxylic acid cycle genes. miRNA-378 expression, which correlates with progression in human breast cancer tissues, causes increased lactate production, decreased respiration, and increased proliferation of breast cancer cell lines. Another recent study has implicated miRNA-210 in metabolic reprogramming in cancer….miRNA-201, which is induced by hypoxia, represses the mitochondrial iron sulfur scaffold protein ISCU resulting in decreased mitochondrial complex 1 activity, aconitase activity, increased lactate production and hypoxic cell survival. Future studies will undoubtedly uncover additional miRNAs important for aerobic glycolysis in cancer.

Thus perhaps epigenetics via the mi RNA path may have a significant role to play.

Pavlova and Thompson also examine the interaction with the epigenetic elements. Namely they state:

Aberrantly activated growth and survival signals that drive tumorigenesis facilitate the reprogramming of cancer cell metabolism to enable increased nutrient acquisition and biosynthesis. However, metabolic networks themselves are not merely passive recipients of growth signals, but quite the contrary, directly transmit the information about the cellular metabolic state to a diverse array of regulatory enzymes, among which are those that mediate the deposition and removal of epigenetic marks from chromatin. A key metabolite that builds up when cells metabolize more glucose than needed for bioenergetic support is cytosolic acetyl-CoA. Cytosolic acetyl-CoA is the obligate substrate for enzymes that acetylate histones and other proteins. The deposition of acetyl marks on histones is associated with the increased accessibility of the genomic DNA for the assembly of transcriptional complexes, and has a rapid turnover rate. Histone acetylation is exquisitely sensitive to alterations in the cellular nutritional and signaling status. Indeed, withdrawal and re-addition of glucose, as well as activation of oncogenic signaling via introduction of an oncogenic KRAS mutant or a constitutively active form of Akt, increase total histone acetylation, which, in turn, promotes the enhanced and broader gene expression.

Overall there appears to be a rich field of examining metabolism and epigenetics.

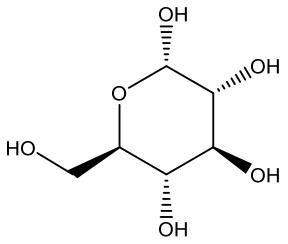
5. GLUTAMINE HAS A SIMILAR EFFECT AS GLUCOSE. ARE THE CELLULAR DYNAMICS THE SAME OR SIMILAR?

Cells use both glucose and glutamine. As Altman et al note:

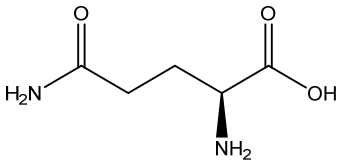
The maintenance of high levels of glutamine in the blood provides a ready source of carbon and nitrogen to support biosynthesis, energetics and cellular homeostasis that cancer cells may exploit to drive tumour growth. Glutamine is transported into cells through one of many transporters, such as the heavily studied SLC1A5, and can then be used for biosynthesis or exported back out of the cell by antiporters in exchange for other amino acids such as leucine, through the L-type amino acid transporter 1 (LAT1, a heterodimer of SLC7A5 and SLC3A2) antiporter. Glutamine derived glutamate can also be exchanged through the xCT antiporter for cystine, which is quickly reduced to cysteine inside the cell. …

The expression of enzymes involved in glutamine metabolism varies widely in cancers and is affected by tissue of origin and oncogenotypes, which rewire glutamine metabolism for energy generation and stress suppression. Of the two glutaminase enzymes28, GLS is more broadly expressed in normal tissue and is thought to have a crucial role in many cancers, whereas GLS2 expression is restricted primarily to the liver, brain, pituitary gland and pancreas36. Alternative splicing adds further complexity, as GLS pre-mRNA is spliced into either glutaminase C (GAC) or kidney-type glutaminase (KGA) isoforms37–39. The two GLS isoforms and GLS2 also differ in their regulation and activity. GLS but not GLS2 is inhibited by its product glutamate, whereas GLS2 but not GLS is activated by its product ammonia in vitro28,29. Although both GLS and GLS2 are activated by inorganic phosphate, GLS (and particularly GAC) shows a much larger increase in catalysis in the presence of inorganic phosphate37. Sirtuin 5 (SIRT5), which can be overexpressed in lung cancer40, can desuccinylate GLS to suppress its enzymatic activity41, whereas SIRT3 can deacetylate GLS2 to promote its increased activity with caloric restriction.

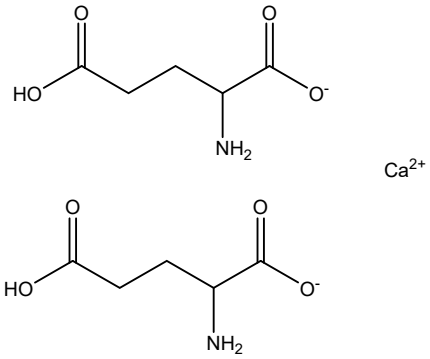
We depict the three key molecules below.



Glucose



Glutamine



Glutamate

The authors continue:

Ninety years ago, Warburg discovered that many animal and human tumours displayed high avidity for glucose, which was largely converted to lactate through aerobic glycolysis. Warburg also suggested that cancers are caused by altered metabolism and loss of mitochondrial function. These dogmatic views have been replaced and refined over the past several decades with the emergence of oncogenic alterations of metabolism, appreciation of the importance of mitochondrial oxidation in cancer physiology and the rediscovery of the role of glutamine in tumour cell growth in addition to the pivotal role of glucose.

In this Review, we provide an updated overview of glutamine metabolism in cancers and discuss the complexity of metabolic rewiring as a function of the tumour oncogenotype as well as the microenvironment, which adds to the heterogeneity found in vivo. In certain types of cancer, such as those driven by MYC, tumour cells seem to depend on glutamine, and hence targeting glutamine metabolism pharmacologically may prove beneficial. Conversely, different oncogenic drivers may result in tumour cells that could bypass the need for glutamine.

However, targeted inhibition of some oncogenic drivers has been reported to rewire cells to become dependent on glutamine, and hence targeted inhibitors could be synthetically lethal with inhibition of glutamine metabolism. Overall, the field of cancer metabolism has made considerable progress in understanding alternative fuel sources for cancers, including glutamine, which under specific circumstances can be exploited for therapeutic purposes.

6. IS THERE A RELATIONSHIP BETWEEN THE IMMUNE SYSTEM AND THE WARBURG EFFECT, AND IF SO CAN IT BE USED TO ADDRESS VARIOUS TYPES OF CANCERS?

There has been a significant amount of recent work examining the relationship between cell metabolism and the immune system. The metabolic factors can result in complex immune response, separate from the more simple issues of cancer cell markers.

Herbel et al have noted:

Conversion of normal cells to cancer is accompanied with changes in their metabolism. During this conversion, cell metabolism undergoes a shift from oxidative phosphorylation to aerobic glycolysis, also known as Warburg effect, which is a hallmark for cancer cell metabolism. In cancer cells, glycolysis functions in parallel with the TCA cycle and other metabolic pathways to enhance biosynthetic processes and thus support proliferation and growth. Similar metabolic features are observed in T cells during activation but, in contrast to cancer, metabolic transitions in T cells are part of a physiological process. Currently, there is intense interest in understanding the cause and effect relationship between metabolic reprogramming and T cell differentiation.

After the recent success of cancer immunotherapy, the crosstalk between immune system and cancer has come to the forefront of clinical and basic research. One of the key goals is to delineate how metabolic alterations of cancer influence metabolism-regulated function and differentiation of tumor resident T cells and how such effects might be altered by immunotherapy. Here, we review the unique metabolic features of cancer, the implications of cancer metabolism on T cell metabolic reprogramming during antigen encounters, and the translational prospective of harnessing metabolism in cancer and T cells for cancer therapy. ...

T cells and cancer cells inexorably share metabolic programs and preferences, and thus there is high competition for nutrients between cancer and T cells within the tumor microenvironment. Nutrient deprivation, increased metabolic waste, and the ability of tumors to express inhibitory ligands impair the metabolic fitness and capacity of T cells to uptake and utilize nutrients. Additionally, metabolism determinants of the tumor microenvironment drive T cells to exhaustion and Treg differentiation programs rather than Teff and Tm phenotypes leading to impaired antitumor responses.

The changes and adaptations in the tumor microenvironment most likely are not limited to solid tumors because leukemia and lymphoma cells have similar metabolic characteristics with solid tumors and often express immunomodulatory ligands. In addition, lymphomas may also contain infiltrating T cells with an exhausted phenotype similar to that identified in chronic viral infections or solid tumors. Thus, drugs that directly target key metabolic enzymes or their upstream regulators will likely interfere with metabolism of both cancer and T cells in which core cell signaling and metabolic pathways converge.

Understanding the similarities and differences of metabolic vulnerabilities of T cells and cancer may lead to the development of single-target or combination-based therapies to modify metabolism of the tumor niche thereby targeting both cancer cells and immune cells. Identification of such specific changes in oncometabolites and immuno-metabolites may define not only novel therapeutic targets but also biomarkers for assessment of therapeutic responses to tumor immunotherapy combined with metabolism-targeting drugs. The ultimate goal is to design metabolism-based treatment strategies to attack and eradicate cancer while promoting effective and sustainable anti-tumor T cell responses.

As Elliott and Head have noted:

It is at this time we must briefly mention the work and contributions of Paul Ehrlich and Otto Warburg. Paul Ehrlich's magic bullet theory has inspired many generations of scientist to

explore numerous molecular cancer therapeutics. He connected chemistry to biology and medicine; and predicted the existence of specific cell recaptors .

Otto Warburg in the 1930s, described a link between defects in mitochondrial physiology and tumorigenesis. He observed a significant increase in glycolysis and lactate production in the presence of oxygen without an increase and an occasional decrease in oxidative phosphorylation. This phenomenon is known as aerobic glycolysis or the "Warburg effect" and is well documented in tumor cells. The work of the above two scietists has contributed much to the field of tumorigenesis, and those of us in the field should be extremely grateful for their contributions.

In 2005 Gottlieb and Tomlinson did a tremendous job reporting on mitochondrial tumor suppressors with a genetic and biochemical update. They mention the work of Warburg, but it was 60 years after Warburg that the first genetic evidence that might explain the mechanisms of aerobic glycolysis was reported. There were many tumors shown to contain somatic mutations in mitochondrial DNA (MTDNA).

It is thought that most are homoplastic and the outcome is non-functional oxidative phosphorylation, causing cells to increase glycolysis, the only other avenue for ATP (adenosine triphosphate) synthesis. However, there is limited evidence that indicates mitochondrial mutations might directly promote tumorigenesis. There are some mitochondrial proteins encoded by nuclear genes that can be tumor suppressors, some are involved in benign and malignant tumors. Two of the proteins are the enzymes succinate dehydrogenase (SDH) and fumarate hydratase also known as fumarase. Both of these enzymes are involved in the Kreb's cycle that connects glucose metabolism in the cytosol to mitochondrial oxidative phosphorylation.

The inhibition of SDH has been linked to the induction of the hypoxicinducible factor (HIF). HIF is a transcription factor induced under low oxygen conditions. SDH inhibition causes an accumulation of succinate, which transmits an oncogenic signal from the mitochondria to the cytosol, which inhibits HIF-α prolyl hydroxylase (PHD) activity leading to the stabilization of the HIF-1α subunit at normal oxygen levels. The result is the transcription of genes involved in tumorigenesis, such as, the angiogenesis factor vascular endothelial growth factor (VEGF). Therefore, succinate has been identified as a new intracellular messenger through discovery of the mitochondrion cyto-sol pathway. Gottlieb and Tomlinson have done a great job of discussing the link of mitochondrial dysfunction to cancer and we will now present some important aspects of their findings.

The TCA cycle (tricarboxylic acid cycle also known as the Krebs cycle) is fundamental to the bioenergetics of cells, however, it is not exactly known how TCA dysfunction leads to cancer. To address that problem, they proposed several models. They included decreased programmed cell death (apoptosis), increased production of reactive oxygen species (ROS), and activetion of a hypoxia-like pathway under normoxic conditions (pseudohypoxia). Though impossible to distinguish between these options as they interact with each other, which leads to a complex grid of tumor regulatory systems. They still provided evidence to support the role for each of these three models in mitochondrial dysfunction induced tumorigenesis.

Overall the potential exists for utilizing the Warburg like responses in conjunction with the immune system although the path is not clear at this point. However Peng et al have recently noted:

Aerobic glycolysis (the Warburg effect) is a metabolic hallmark of activated T cells and has been implicated in augmenting effector T cell responses, including expression of the proinflammatory cytokine interferon-g (IFN-g), via 3′ untranslated region (3′UTR)–mediated mechanisms. Here, we show that lactate dehydrogenase A (LDHA) is induced in activated T cells to support aerobic glycolysis but promotes IFN-g expression independently of its 3′UTR. Instead, LDHA maintains high concentrations of acetyl–coenzyme A to enhance histone acetylation and transcription of Ifng. Ablation of LDHA in T cells protects mice from immunopathology triggered by excessive IFN-g expression or deficiency of regulatory T cells. These findings reveal an epigenetic mechanism by which aerobic glycolysis promotes effector T cell differentiation and suggest that LDHA may be targeted therapeutically in autoinflammatory diseases.

7. THUS THE FINAL QUESTION IS: WHY DOES THE WARBURG EFFECT EVEN OCCUR?

As DeBerardinis et al note:

So why does the Warburg effect occur? Clearly, the high glycolytic rate provides several advantages for proliferating cells.

First, it allows cells to use the most abundant extracellular nutrient, glucose, to produce abundant ATP. Although the yield of ATP per glucose consumed is low, if the glycolytic flux is high enough, the percentage of cellular ATP produced from glycolysis can exceed that produced from oxidative phosphorylation. This may be due to the high rate of ATP production during glycolysis compared to oxidative phosphorylation.

Second, glucose degradation provides cells with intermediates needed for biosynthetic pathways, including ribose sugars for nucleotides; glycerol and citrate for lipids; nonessential amino acids; and, through the oxidative pentose phosphate pathway, NADPH. So the Warburg effect benefits both bioenergetics and biosynthesis.

What remains controversial about the Warburg effect is why the rate of lactate production is so high when more of the pyruvate could presumably be oxidized to enhance ATP production. One explanation is simply that glycolysis outpaces the maximal velocity of pyruvate oxidation, so that cells must instead eliminate pyruvate using high-flux mechanisms.

7 CONCLUSIONS

We have made several observations which can lead to a few reasonable conclusions. They are:

1. Warburg effect is most likely an artifact of the other elements which make up for a malignancy. The classic understanding was that the effect was the cause of cancers even though the cause of the effect itself was unknown.

2. Warburg effect is not a cause nor a sine qua non for cancer and addressing its control would most likely be addressing a secondary consequence not a causal element.

3. The Warburg effect can be explained by a rate limiting process in the overall manner in which glucose produces ATP. In fact it may be that the cancer cells are very "hungry" cells in need of massive amounts of glucose that they saturate the TCA and thus perform aerobic glycolysis.

Finally we pose the questions from Bensinger and Christofk:

1. Can the Warburg effect and cancer metabolism be programmed? While the Warburg effect metabolic phenotype was initially identified in cancer tissue, it is now well appreciated that rapidly dividing normal tissues, such as ES cells and lymphocytes, employ aerobic glycolysis to meet their energetic and biosynthetic requirements during expansion. These observations support the notion that aerobic glycolysis is a preferred metabolic program under conditions of rapid cellular expansion. However, it remains unclear how the Warburg effect is initiated and maintained; these need not be the same signals in cancer versus normal tissues which lack dysregulated signaling. One critical signaling axis for metabolic programming of normal cells is the PI3K/AKT/mTOR pathway downstream of growth receptors. Genetic and pharmacologic models have clearly identified mTOR signaling in controlling cellular growth and metabolism.

This is a clear statement of what is desired but also a clear articulation of what we really do not know about Warburg. mTOR is a powerful control mechanism, yet is it a driver of or driven by Warburg?

2. What is the relationship between the Warburg effect and cancer microenvironment? ....Thus, catabolic programs in normal cells within the tumor parenchyma can play a pivotal role in supporting the anabolic program of cancer. Indeed, recent studies indicate that despite robust anabolic programs in tumors, addition of lipolytic capabilities robustly increase tumorigenesis. Although speculative, the symbiotic relationship between normal cells and tumor cells could help to explain cancer associated cachexia by driving a generalized catabolic program in tumor bearing individuals.

The microenvironment is becoming significant factor on a cancer development and proliferation. Cancer cells have a high demand for nutrients and oftentimes those nutrients are obtained from its microenvironment even to the extant that it cannibalizes its benign neighbors, using their remnants as energy to grow.

3. Does aerobic glycolysis contribute to chemotherapeutic resistance or susceptibility? Given that many cancers exhibit altered metabolism, it should not be surprising that there has been

increased effort to therapeutically target these pathways as a means to decrease tumor growth or alter behavior.

There is a great deal of evidence that Warburg effects do inhibit certain chemotherapies. This is a compelling path to explore since it may enlighten us on Warburg as well as expanding understanding of chemotherapeutic mechanisms.

4. What is the metabolism of cancer stem cells? While the concept of bone fide cancer stem cells (CSCs) remains controversial, there is strong evidence to indicate that a subset of cancer cells are endowed with the capacity to initiate tumor formation. Oft times defined as tumor-initiating cells (TICs), these cells appear to be critical for the ability of tumors to resist conventional radio- or chemotherapeutics and repopulate the tumor during and after treatments. Whether TICs have distinct metabolic programs from the bulk of tumor cells is not well established.

This last question is compelling since it indicates that Warburg is but one presentation of cancer cell metabolism. It also presents the argument that understanding cancer cell metabolism in the whole may provide a fruitful approach to the ultimate control of cancers. However, and this is critical, we must always be fearful of falling into what I have termed the "Warburg Trap", namely the positing of the "silver bullet" cause of all cancers. Clearly if we have discovered anything, it is that cancers have a multiplicity of causes and ongoing support mechanisms, even in the same organ, and even in the same cell types in that organ.

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