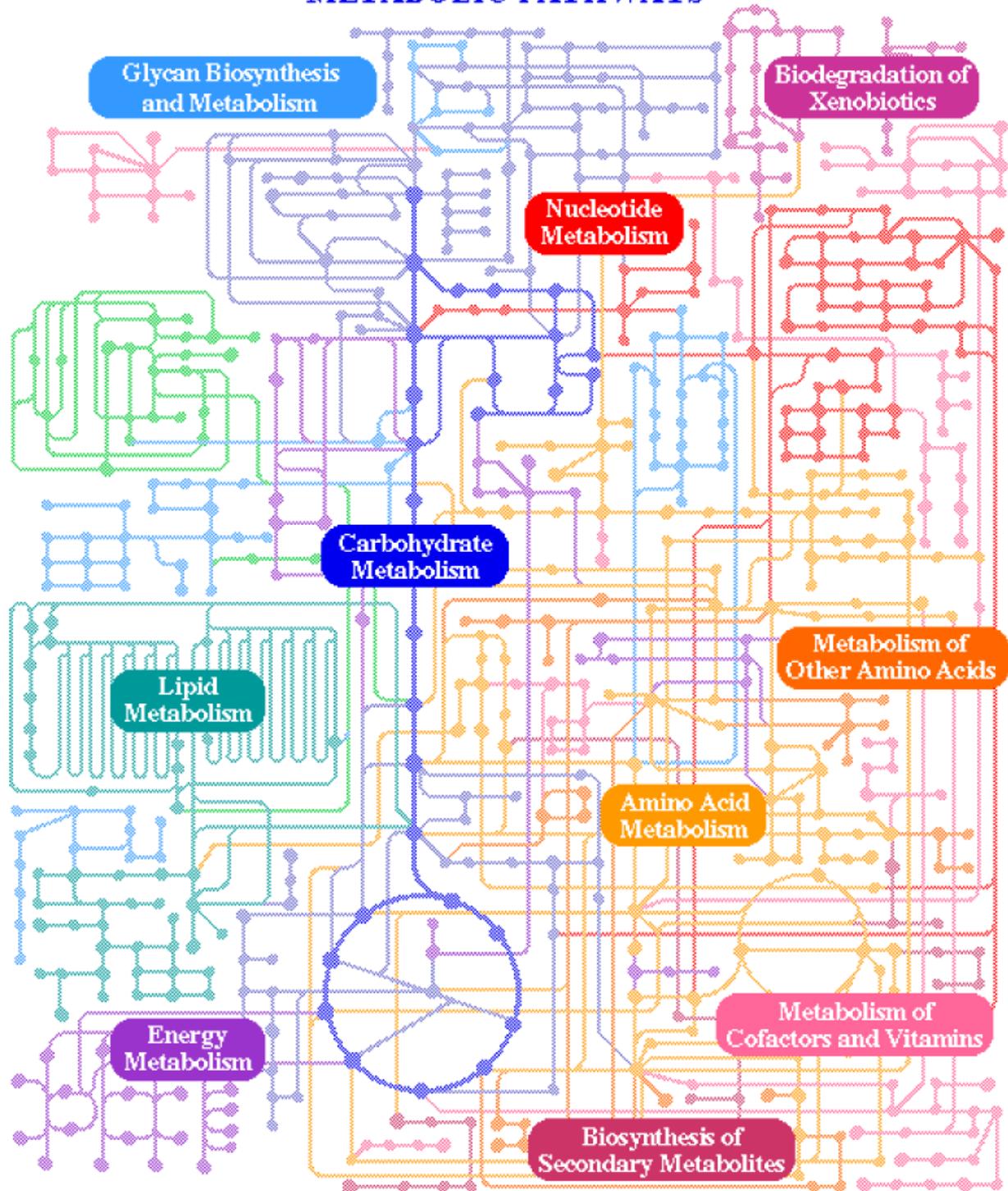


Cellular Respiration

Cells obtain the energy they require from anaerobic or aerobic respiration. It has been postulated that tumour cells rely on anaerobic respiration even in the presence of oxygen and that this is due to some impairment of the mitochondria in these cells. One opportunity for fighting cancer may therefore be to disrupt the glycolytic pathway in cancer cells, thereby depriving them of the energy they need to survive. A medication that has been used to inhibit this metabolic pathway is glutathione. It is claimed that this effect can be amplified by the exposure of tumour cells to radiofrequency waves. For this effect to be researched the exposure to RF needs to be measured in terms of an absorbed dose delivered to the location of the cancer within the patient's body.

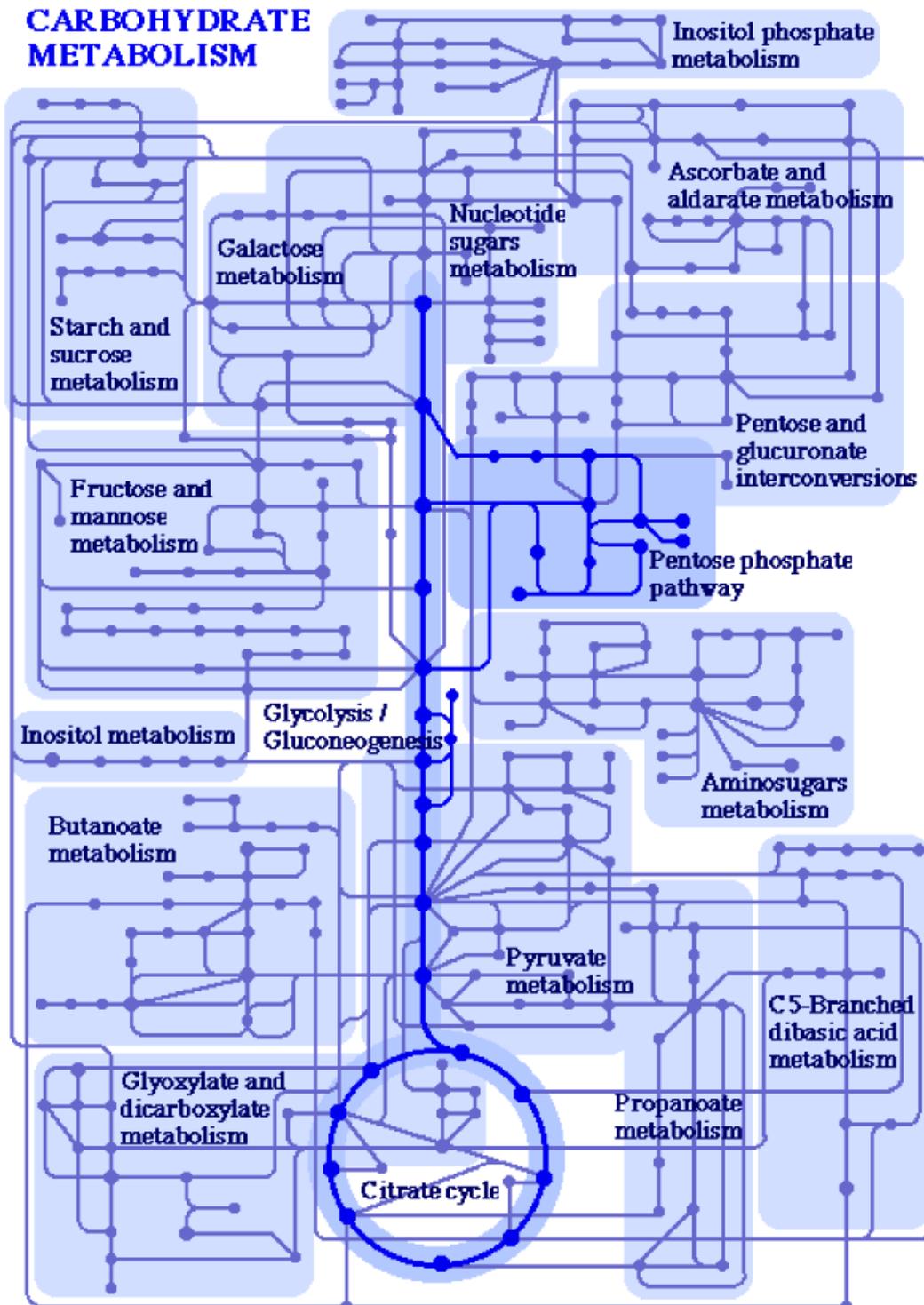
A complex network of chemical reactions are continually at work in cells in order to sustain life. These reactions form the basis of metabolism and are organized into an interconnected series of reactions called metabolic pathways (Figure1). The operation of these pathways is made possible by enzymes, made up mostly of proteins, that act as a biological catalysts; that is, they catalyse (accelerate) chemical reactions. An essential metabolic process within cells is respiration, the breaking down of complex molecules into smaller ones in order to release the energy required to power the cells through catabolic reactions. Animals rely on carbohydrate catabolism (Figure 2) for cellular respiration in which carbohydrate molecules, such as glucose, obtained from food are processed to generate chemical energy that can be stored in cells until it is required by the organism.

METABOLIC PATHWAYS



01100 5/31/04

Figure 1: High level network of metabolic pathways. From KEGG (Kyoto Encyclopedia of Genes and Genomes) www.genome.jp/kegg



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Figure 2: Carbohydrate metabolism (from KEGG)

Aerobic and anaerobic respiration

Cellular respiration may proceed either aerobically or anaerobically. Aerobic respiration requires oxygen in order to proceed, whereas anaerobic respiration can proceed in the absence of oxygen, for example fermentation.

Organisms are classified as aerobic (or aerobes) if they have an oxygen-based metabolism and anaerobic (anaerobes) if they do not require oxygen. Obligate (strict) aerobes require oxygen to survive whereas facultative aerobes can revert to anaerobic respiration if deprived of oxygen. Obligate anaerobes die in the presence of oxygen whereas facultative anaerobes can switch to aerobic respiration if oxygen is present.

The chief source of energy in all animals, whether they use aerobic or anaerobic respiration is glucose (Glc) ($C_6H_{12}O_6$) a monosaccharide, or simple sugar (Figure 3).

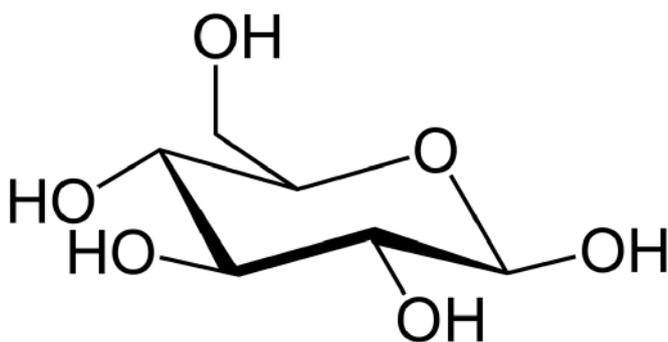


Figure 3: Structure of glucose. From Wikipedia article on Glucose.

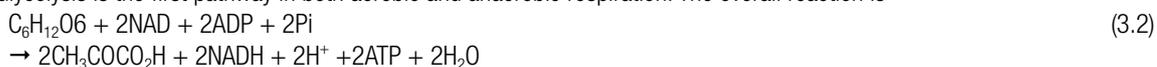
Aerobic respiration in eukaryotes consists of four processes: glycolysis (3.1.2), pyruvate decarboxylation (3.1.3), the citric acid cycle (3.1.4) and finally oxidative phosphorylation (3.1.5) which occurs in the mitochondria of cells (Appendix B.1.3).

An example of anaerobic respiration is fermentation for example that of the yeast fungus, as studied by Pasteur, Buchner and Carlsberg. The product of fermentation depends on the sugar used. Where this is glucose the product is ethanol (C_2H_5OH):

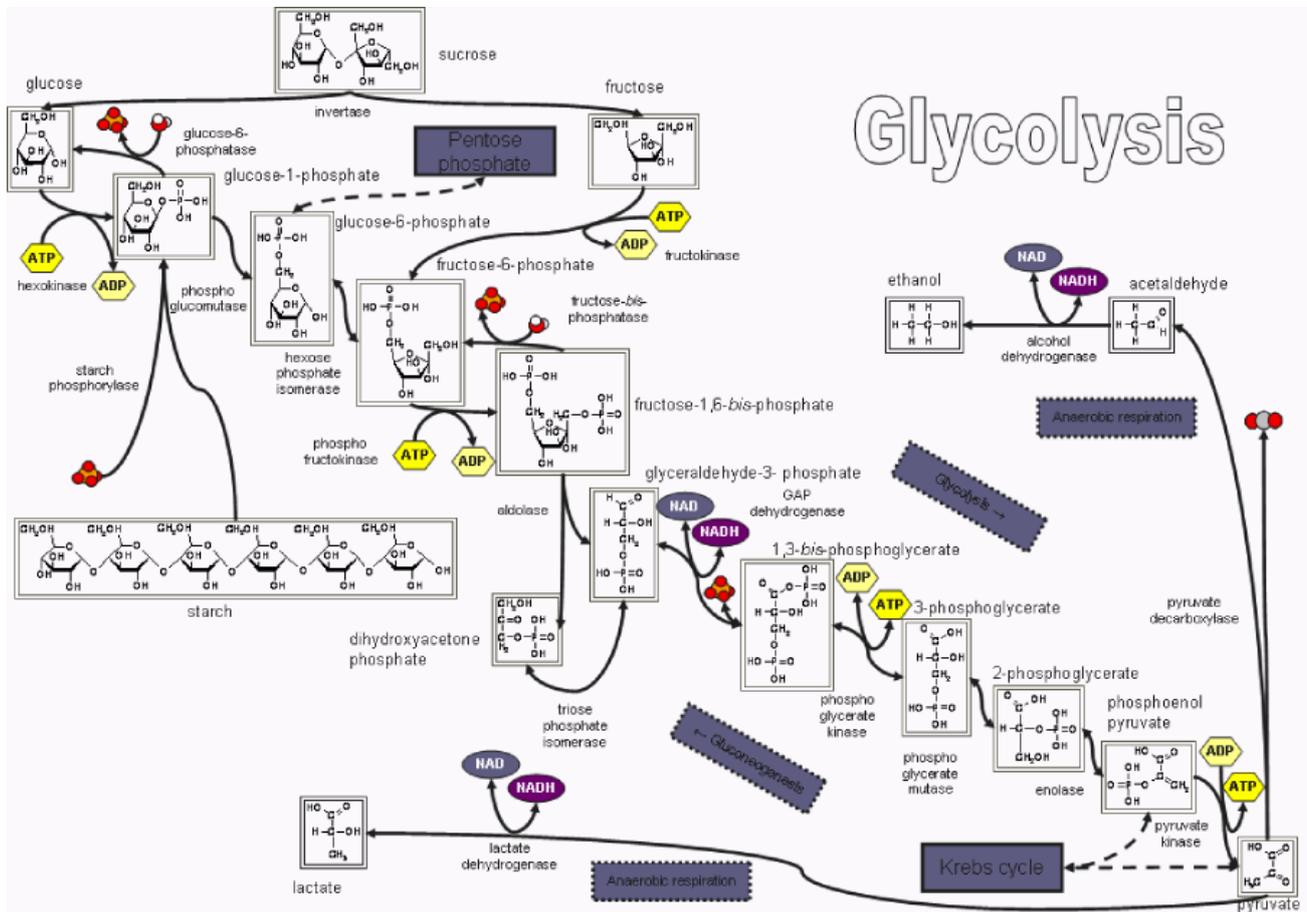


Glycolysis

Glycolysis is the anaerobic metabolic pathway by which a molecule glucose is oxidized to form two molecules pyruvate (pyruvic acid) (CH_3COCO_2H). Glycolysis is the first pathway in both aerobic and anaerobic respiration. The overall reaction is



This reaction takes place in the cytosol of cells. The most common type of glycolysis is the Embden-Meyerhof pathway which consists of a ten step process (Figure 4).



Glycolysis

Figure 4: Glycolysis (from www.steve.gb.com/science/molecules.html)

The glycolytic pathway is regulated at a number of points through various enzymes and transcription factors of which the following have been reviewed as potential points at which glycolysis could be inhibited in cancer cells:

- Hexokinase
- Phosphofruktokinase
- Transcription factor HIF-1

Pyruvate decarboxylation

In aerobic respiration glycolysis is followed by a pyruvate decarboxylation reaction to convert pyruvate to acetyl CoA used in the citric acid cycle.

TCA (citric acid or Krebs) cycle

The citric acid cycle, or tricarboxylic acid cycle (TCA) or Krebs cycle, is a ten step cycle in which one molecule of GTP, three molecules of NADH, one molecule of FADH₂ (Figure 5). The full reaction is

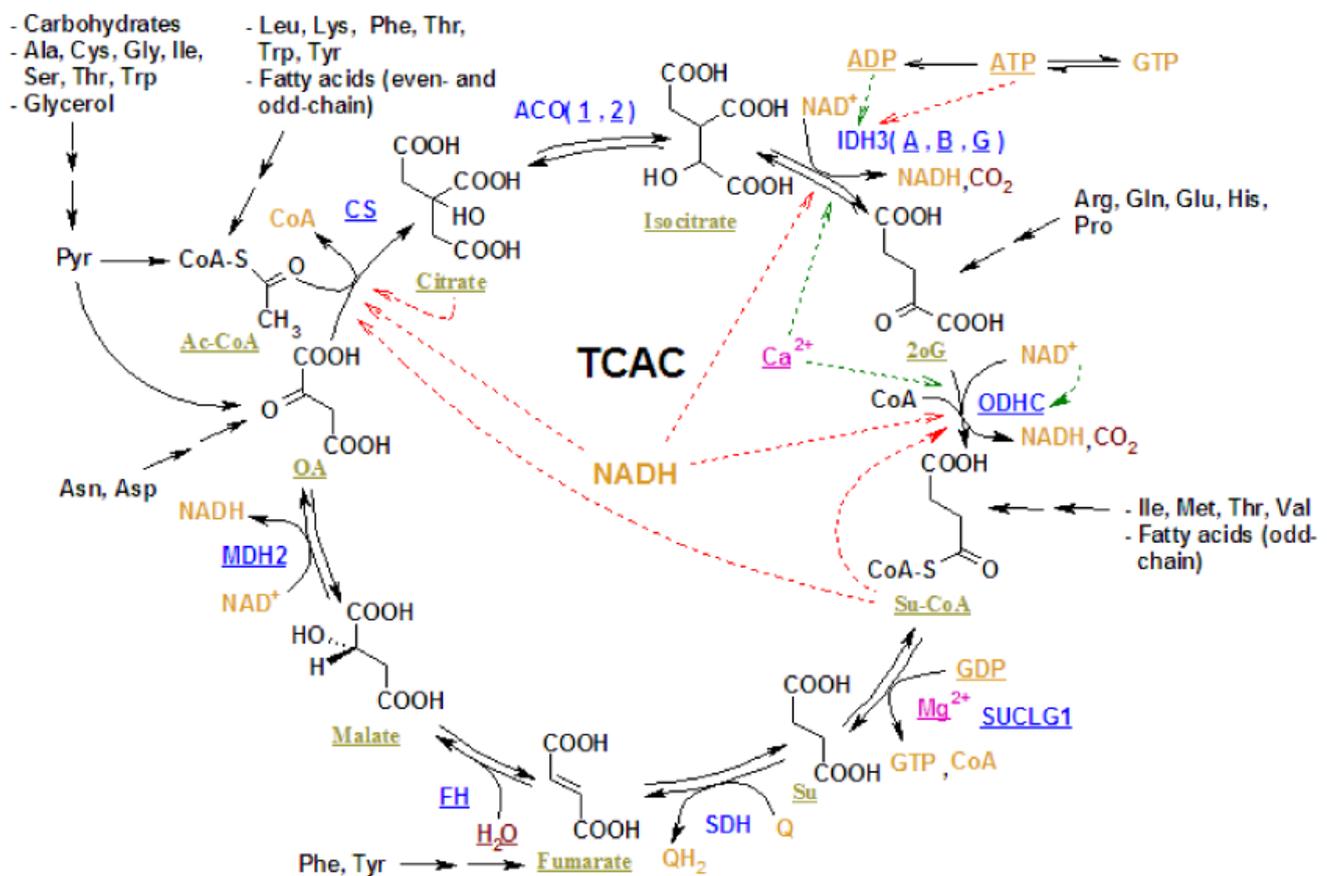
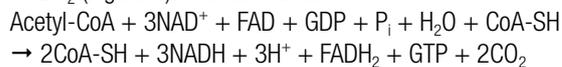
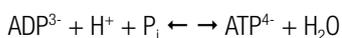


Figure 5: Krebs cycle. From Wikipedia article on Citric acid cycle

Oxidative phosphorylation

Oxidative phosphorylation is the final step in aerobic respiration, one that occurs in the mitochondria (Wikipedia):

During oxidative phosphorylation electrons are transferred from NADH or FADH₂, created in glycolysis, fatty acid metabolism and the Krebs cycle, to molecular oxygen via a series of protein complexes located in the inner mitochondrial membrane. Protons are pumped from the mitochondrial matrix into the intermembrane space as a result of this flow of electrons. This generates a pH gradient and a transmembrane electrical potential across the membrane. This is a form of potential energy which is referred to as a proton-motive force. The protons flow back into the mitochondrial matrix via a large protein complex ATP synthase. This is how most of the ATP is created in a eukaryotic cell. This is the reaction that ATP synthase catalyses:



The synthase functions almost as a mechanical motor, with each NADH molecule contributing enough proton motive force to generate 2.5 ATP. Each FADH₂ molecule is worth 1.5 ATP. All together, the 8 NADH and 2 FADH₂ molecules contributed through oxidation of glucose (glycolysis, conversion of pyruvate to acetyl-CoA, and the Krebs cycle) account for 23 of the 30 total ATP energy carrier molecules. It is worth noting that these ATP values are maximum values and in reality protons leak across the membrane causing somewhat lower values.

Cori cycle

The Cori cycle refers to the process whereby lactate produced by the muscles is carried to the liver, converted back to glucose by gluconeogenesis and returned to the muscles from where it can be used to provide energy to cells:

The Cori cycle refers to the recycling of lactate or lactic acid produced by muscle during anaerobic metabolism. The lactate is converted to glucose by the liver. When the ATP needs of a cell outpace its oxygen supply is limited, muscle cells produce ATP through lactic acid fermentation. This permits the regeneration of NAD⁺ so glycolysis can continue. The lactate diffuses into the blood and is taken up by the liver, which oxidise it back to pyruvate. This reaction is catalysed by the enzyme lactate dehydrogenase. Most of the pyruvate is then converted to glucose (via gluconeogenesis). This glucose circulates in the blood, where it can be used by muscles if needed or stored as glycogen. This cycle allows the body to continue focusing exclusively on producing ATP while another organ, the liver, handles the lactate produced.

The cycle prevents lactate acidosis by removing lactate from the blood. Otherwise pH would fall as the buffering capacity of blood is exceeded.

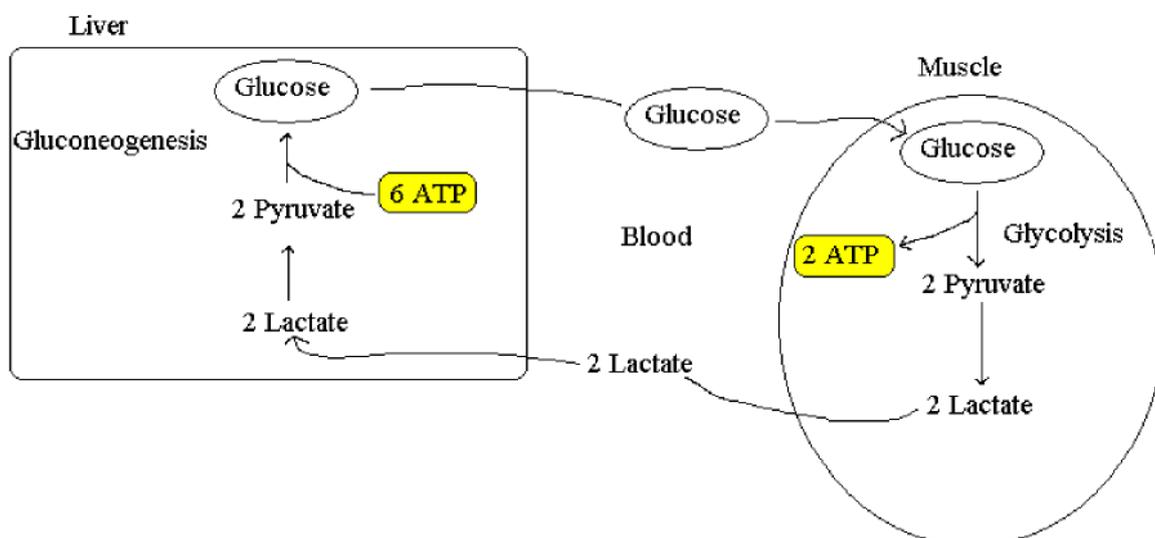


Figure 6: Cori cycle. From Wikipedia article on Cori cycle.

Metabolic control of cancer cells

Warburg's hypothesis

In 1924 the German physiologist and medical doctor Otto Heinrich Warburg advanced the hypothesis that tumour cells have a fundamentally different metabolism to normal cells, in that they rely to a large extent on the less-energy productive process of anaerobic respiration (fermentation) even when there is sufficient oxygen present for the cells to use rather than utilizing the more efficient process of aerobic respiration (Warburg 1931,1956). In other words, cancer cells tend to behave as partial anaerobes while normal cells function as obligate aerobes.

In 1931 Dr Warburg was awarded the Nobel Prize in Physiology or Medicine for "his discovery of the nature and mode of action of the respiratory enzyme." Dr Warburg's hypothesis was summarized in his book on the causes of cancer (1966).

The by-product of the anaerobic glycolysis is increased levels of lactic acid. The large amount of lactic acid produced by cancer cells is then transported to the liver. This conversion of glucose to lactate creates a lower, more acidic pH in cancerous tissues as well as overall physical fatigue from lactic acid build-up. Therefore, larger tumors tend to exhibit a more acidic pH.

The theories of Dr Warburg have in recent times gained more attention in view of findings linking the impairment of mitochondria with a breakdown in normal cell apoptotic processes and tumour growth (Bonnet 2007 and The Economist 2007). The implication of mitochondrial dysfunction in tumour growth is shown in Figure 7 reproduced from a review by Ristow (2006) which observes that:

The hallmarks of cancer growth, increased glycolysis and lactate production in tumors, have raised attention recently due to novel observations suggesting a wide spectrum of oxidative phosphorylation deficits and decreased availability of ATP associated with malignancies and tumor cell expansion. The most recent findings suggest that forcing cancer cells into mitochondrial metabolism efficiently suppresses cancer growth, and that impaired mitochondrial respiration may even have a role in metastatic processes

Energy deficiency in cancer cells

The Institute has conducted some preliminary literature review into the hypothesis of Dr Warburg. The following is a summary of anaerobic respiration in cancer cells and the potential for inhibiting this process:

'It seems to have been established that malignant cells derive most of their energy for growth and reproduction from anaerobic glycolysis in the cytoplasm where glucose is converted to lactate which in turn is then, via the CORI cycle, reconverted back to glucose in the process of gluconeogenesis in the liver. The number of ATP molecules produced by glycolysis is small (2 per glucose molecule) compared to that produced via the aerobic cycle (38 per glucose molecule) hence the cell is relatively energy deficient.

The reason for the predominance of glycolysis, rather than the more energy efficient process of oxidative phosphorylation within the mitochondria is not clear. There is postulation that the mitochondria are somehow damaged by the process that causes the cell to become malignant and unable to convert pyruvate to ATP. Other research indicates that the enzyme processes that control the first three steps of glycolysis are rate limiting and control both the rate of glycolysis and ATP production. In cancer cells this modulation of the enzymes, hexokinase and phosphofructokinase, is lost, perhaps due to relative hypoxia inside the malignant cell and anaerobic glycolysis becomes the predominant energy producer for the cell. It seems also that glycolysis is made more efficient in hypoxic tumour cells by the action of a transcription factor known as HIF-1'.

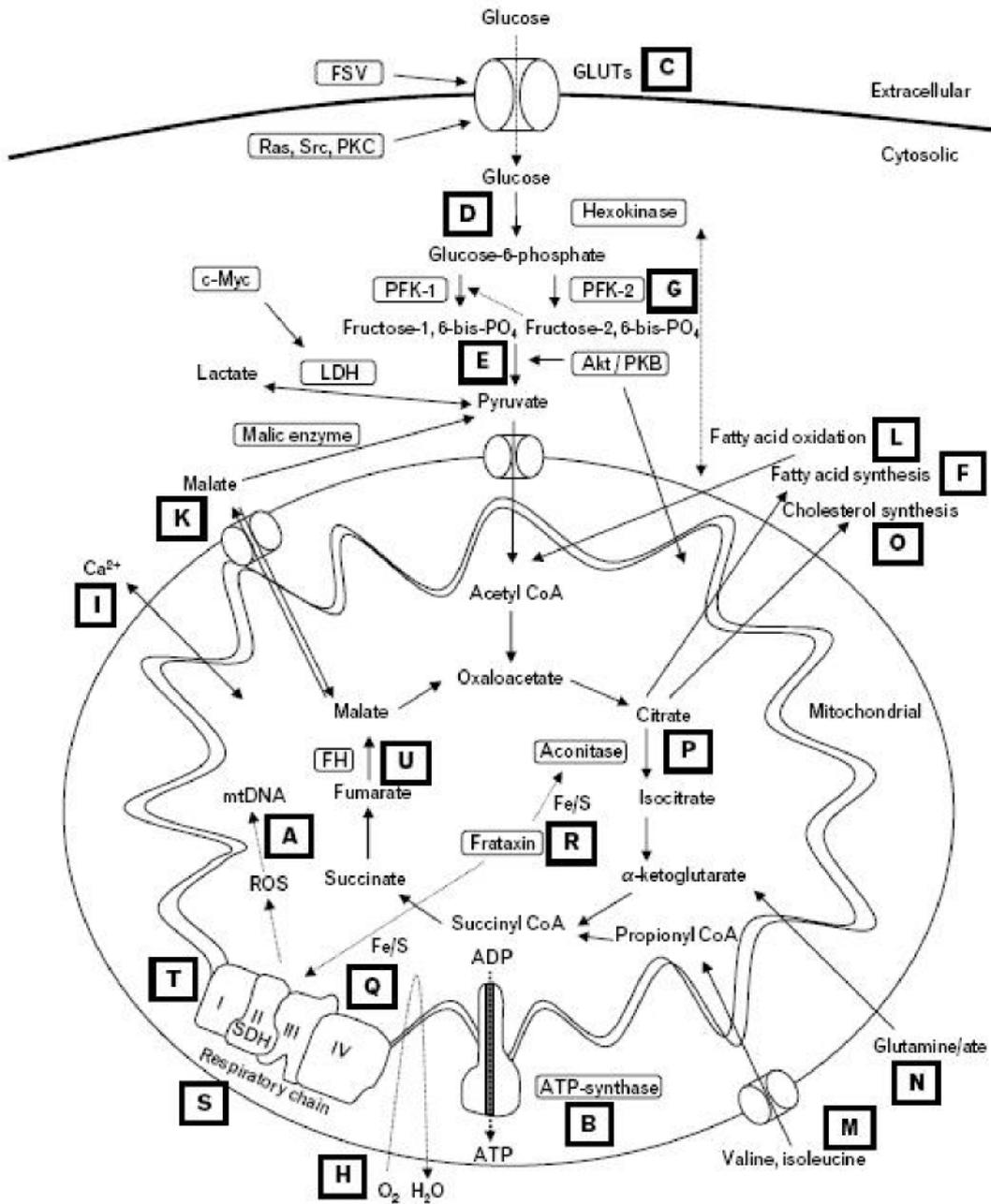


Figure 7: Summary of published interactions of mitochondrial metabolism and cancer growth. From Ristow (2006).

Glutathione in the body

3.3.1 Chemical structure

Glutathione (γ -glutamyl-cysteinyl-glycine) is a tripeptide made up of three non-essential amino acids: cysteine, glutamic acid and glycine. Glutathione comes in two forms: reduced glutathione (GSH) (Figure 8) including a sulfhydryl (SH) and oxidized (GSSG).

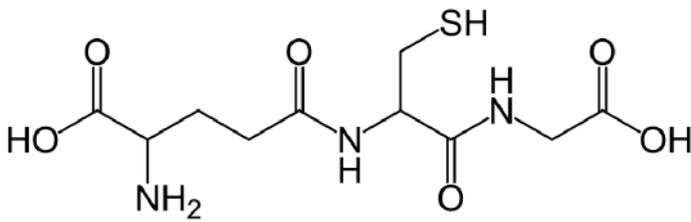


Figure 8: Skeletal formula of reduced glutathione (GSH)

Glutathione in the human body

Glutathione is an anti-oxidant, protecting the body against free radicals, such as those which cause the lipid peroxidation of cell membranes. Glutathione also protects the body against metabolic wastes and environmental poisons.

Lack of glutathione plays a part in many diseases (Wu 2004):

Glutathione deficiency contributes to oxidative stress, which plays a key role in aging and the pathogenesis of many diseases (including kwashiorkor [a protein deficiency in children], seizure, Alzheimer's disease, Parkinson's disease, liver disease, cystic fibrosis, sickle cell anemia, HIV, AIDS, cancer, heart attack, stroke, and diabetes).

3.3.3 Glutathione synthesis in the body

Glutathione occurs naturally in the human body and is manufactured from amino acids (Figure 9).

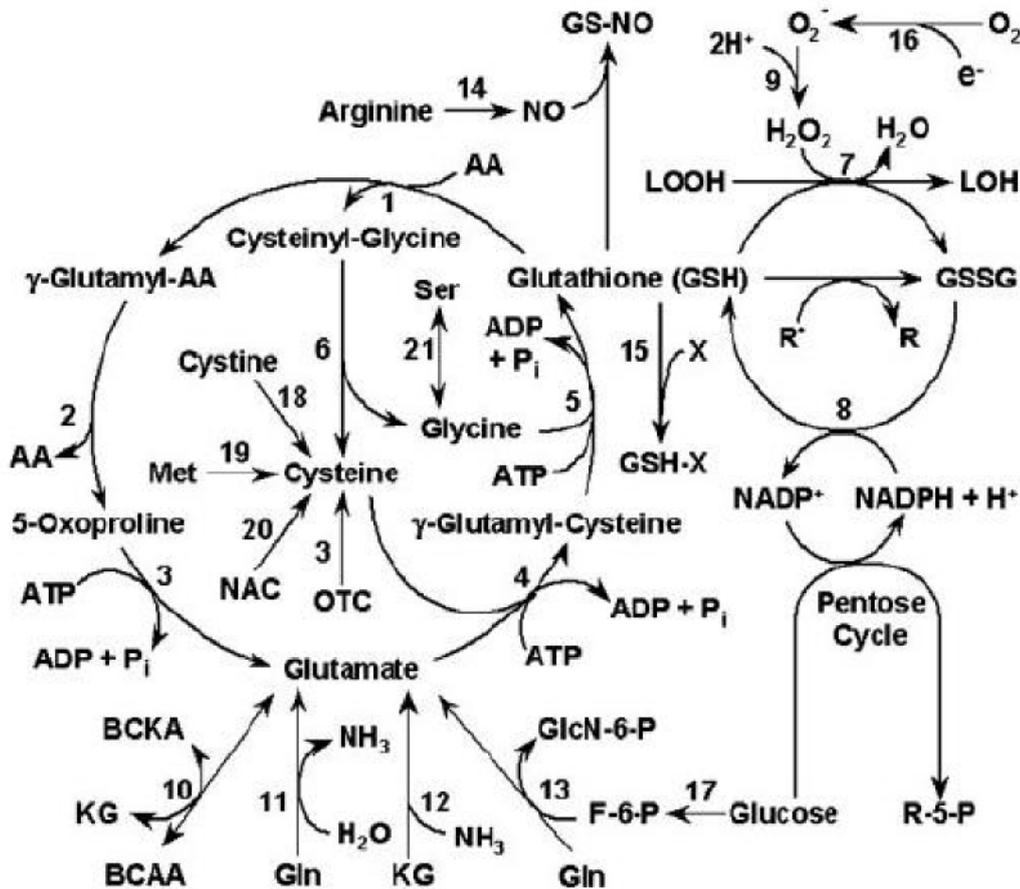


Figure 9: Glutathione synthesis and utilization in animals From Wu et al. (2004).

Glutathione in the diet

Glutathione is found in fresh vegetables, and is particularly rich in the Brassica family – broccoli, spinach, cabbage and cauliflower. Dietary glutathione is, however, broken down into its constituent amino acids during digestion and does not play a role in the GMI effect – this requires glutathione to be injected directly into the bloodstream.