Mapping life's energy cycle

A report on the discovery of the Citric Acid Cycle by Sir Hans Adolf Krebs

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1. Introduction

This report was requested by John O'Neill and Mark Hetherington.
It has been compiled to investigate the work of a prominent scientist and his works subsequent impact on society.
This report will explore the work of Sir Hans Adolf Krebs leading to the discovery of the citric acid cycle. It will show a brief background to research ongoing into the cells respiratory system in the early part of the twentieth century. It will specify the methods and apparatus used by Sir Hans Krebs and his team to gain the data relevant to make his breakthrough discovery, briefly describe the cycle itself and explore the impact on modern society of the discovery of the citric acid cycle.

2. Research methods/Plan

I first identified my topic and hero on 30th September 2008, I created a brief outline plan by 28th October and produced an outline summary by 25th November .The submission date is May 25th 2009.
The information in this report was gathered from the internet and from text books currently in use for the study of GCSE, Higher and Advanced Higher Human Biology. For a list of all sources used please see the bibliography.

3. A Brief history; the life and work of Sir Hans Adolf Krebs

Sir Hans Adolf Krebs led a remarkable academic life: born in Hildesheim, Germany in 1900 he studied medicine at the Universities of Gottengin, Frieburg-im-Breisgau and Berlin. He took his M.D degree at the University of Hamburg after which he studied chemistry in Berlin.

He became assistant to Professor Otto Warburg at Berlin-Dahlem in 1926 after which he returned to hospital work in 1930, working in both the municipal hospital at Altona and then at the medical clinic of the University of Freiburg-im-Breisgau. In 1933 his appointment was terminated when the national socialist government gained power and at the invitation of Sir Frederick Gowland Hopkins he joined the school of biochemistry at Cambridge University escaping Germany before the beginning of the Second World War.

Becoming lecturer of pharmacology at the University of Sheffield in 1935, the Medical Research Council's unit for cell metabolism was established in his department. This moved with him in 1954 when he was appointed Whitley Professor of Biochemistry at the University of Oxford.

During the war he supervised research for the British Medical Council on human nutritional requirements, helping to develop the national wholemeal loaf in Britain that "kept the English people well nourished through the war years despite food shortages".

He published many papers including the survey which revealed his findings regarding the citric acid cycle in 1957, in conjunction with H. L Kornberg. For this work he was awarded the Nobel Prize for Physiology and Medicine in 1953.

He was elected a member of several academic societies including the Royal society of London and was knighted in 1958. He died in 1981.

4. Study of cell respiration; the early twentieth century

Much was known about the respiration of cells before Krebs started his research into cell respiration but a great deal of information was missing. The following are just some of the most significant findings prior to and after the discovery of the citric acid cycle:

- Early 1930s ATP was first isolated by K. Lohmann. Its function was then demonstrated by Albert von Szent – Gyorgyi.
- In 1935 Szent – Gyorgyi explained the sequence of the oxidation of C_4 dicarboxylic acid, showing also that these reactions were in part catalytic.
- Krebs conceived the process of oxidation as a cycle.
- Fritz Lippmann determined the unknown derivative of pyruvic acid to be Acetyl coenzyme A in 1950, showing also that it is related to vitamins B_1 and B_2.
- 1950 Feodor Lynen discovered that Acetyl coenzyme A played a key role in the metabolism of fat.
- 1953 Fritz Lippmann and Hans Adolf Krebs share the Nobel Prize for Physiology and Medicine for their research into the functions of the cell.

5. Cell metabolism and Cell respiration

Metabolism is defined as all the chemical processes occurring in a living organism, in this case a single cell, which keep it alive. Millions of processes happening simultaneously, of which respiration is just one.

Cell respiration: the process by which chemical energy is released from food stuff by oxidation bringing about the regeneration of ATP (the cells high energy compound) (1).

Krebs work centred on the aerobic (needing oxygen) section of cellular respiration, taking place in the matrix of the mitochondria (2), a tiny organelle present in abundance in all living cells. It is here that the citric acid cycle takes place.

6. Methods and apparatus used in the discovery of the citric acid cycle

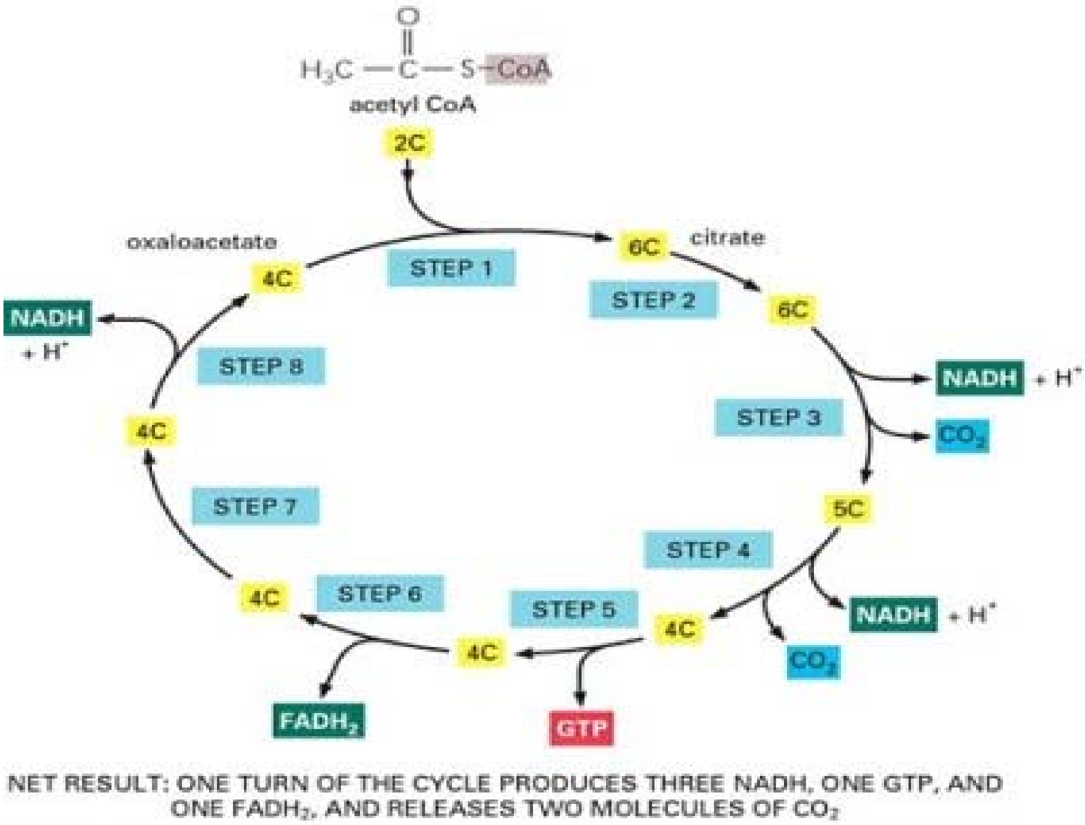
When Krebs began his research into the respiration of cells in the late 1930s methods were primitive and techniques unsophisticated. To understand the machinery of the molecules at work in aerobic respiration Krebs used experimental procedures he was familiar with but modified them to fit the needs of his research.

To study the respiration of a whole organism would be impossible so isolated pieces of tissue were used. Early in his career Krebs devised a new tissue-slice technique, which involved rapidly removing the organs after death and slicing the tissue thinly to be kept fresh in a saline solution. He used this technique successfully in earlier research into the ornithine cycle.

Warburg manometers were to measure the aerobic metabolism (oxygen uptake) of various tissue cells. Material was placed in a flask attached to a manometer, this contained a compartment containing a small piece of filter paper which would absorb any carbon dioxide produced so that a movement of the manometer fluid (held in a u-shaped tube joined to the side of the manometer) represented oxygen uptake. The device was kept at a constant temperature by being continuously shaken in a temperature controlled water bath.
The flask holding the material had side arms from which various reagents and inhibitors could be added during an experiment. This is how different factors influencing the rate of respiration could be studied. By inhibiting an already known metabolic pathway we would expect specific results. Inhibitors would be introduced and the results recorded; if our understanding of the metabolic pathway was correct then inhibition of one part would produce build up of a particular intermediate, or it may produce differing results allowing for further study, advancing our understanding. In this way many metabolic pathways were determined including the citric acid cycle.

7. The citric acid cycle simplified

Using the above mentioned methods Krebs determined the reactions of his original hypotheses taking place as an endless cycle.
Acetyl-Co A transfers its two carbon acetyl group to a four carbon acceptor to form the six carbon compound citrate.
The citrate is chemically transformed, losing one then another carboxyl group as carbon dioxide.
Most of the energy made available by these steps of the cycle is transferred as electrons to NAD^+ (a hydrogen acceptor) forming NADH. For each acetyl group three molecules of NADH are produced.
At the end of the cycle the four carbon compound has been regenerated and the cycle continues. (3)



https:/.../file/view/citric_acid_cycle.jpg

8. Conclusion

Krebs cycle showed the links between all the individual reactions of cellular respiration, through degradation reactions which yield energy and synthesising processes which use energy.
It explained the metabolic pathways followed by carbohydrate, fat and protein, allowing scientists to determine how cells use these compounds for further cellular processes i.e.; protein synthesis, the building of cell membranes.
As it is the intermediate section of all catabolic and anabolic processes it proved how these compounds are stored in the event of excess enabling nutritionists, dieticians and epidemiologists to research weight gain, weight loss and the effects of poor diet across the globe (7).
It is utilised by athletes and their trainers as an aid to adapting and training the machinery of the human body for efficiency and performance (6).
It is an important application in the research and study of all human cells, referenced in the study of cancerous cells and tumour growth (5).
It is also one of the most important factors for medical and pharmacological research, allowing scientists to study the effects of new drugs before they are widely used (4).
It is interesting to note that Sir Hans Adolf Krebs had links with almost all the above mentioned fields of study proving how fundamental he was to

recognising that biochemistry is one of the most important tools in the study of human physiology.
Today the citric acid cycle is one of the most recognised of all cellular biochemical reactions, it is studied the world over by high school and university biology students, its current and future applications in modern biological science are limitless as its uses are essential to the study of so many different fields.

9. Evaluation

This report was difficult to research. The data and documentation regarding results of experiments conducted 80 years ago was difficult to obtain, on the other hand there is so much information about the citric acid cycle as it is so widely used in teaching that it was often difficult to isolate specific applications within the main body of research. I had to cut back enormously on the amount of information I wished to include as I found it all relevant to the subject if not to the hero himself.
I found both structuring and formal writing important to the atmosphere of the report and difficult to use as I write quite informally. I found it difficult to know when to use more scientific language within my report and for this reason left the more difficult scientific terms to the appendix.
It was also difficult to find any specific uses referenced within research papers as most medical and drug studies are patented and almost impossible to access.

10. Bibliography and references (see section 2)

Biology: a functional approach (4th edition) MBV Roberts

Higher human biology – James Torrance

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- www.bookrags.com
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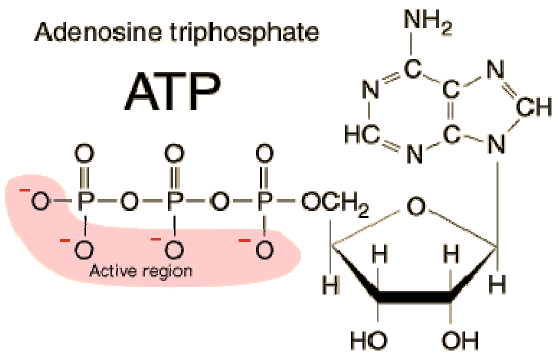
- http://www.garfield.library.upenn.edu/essays/v5p627y1981-82.pdf
- **http://nobelprize.org/nobel_prizes/medicine/laureates/1953/press.html**
- http://hyperphysics.phy-astr.gsu.edu/hbase/Biology/atp.html
- www.cartage.org.lb/.../mitochondria.jpg
- http://student.ccbcmd.edu/~gkaiser/biotutorials/cellresp/fg17.html

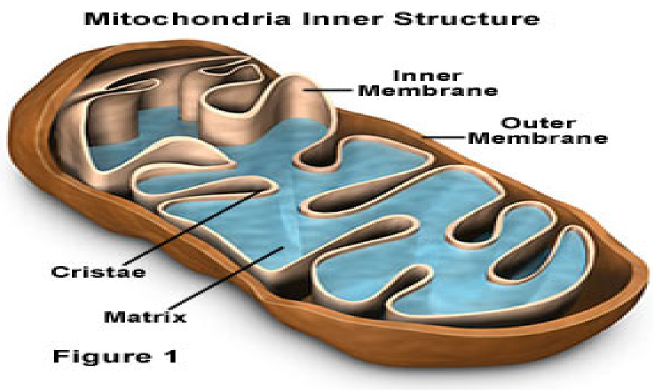
11. Appendices

1. ATP – the cells high energy compound



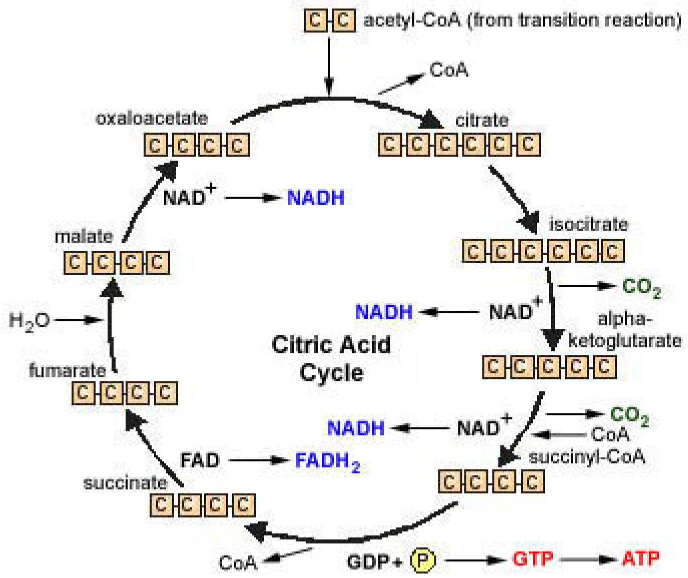
http://hyperphysics.phy-astr.gsu.edu/hbase/Biology/atp.html

2. Mitochondria of the cell the site of cellular respiration



www.cartage.org.lb/.../mitochondria.jpg

3. The citric acid cycle



The citric acid cycle continued:

1. Before the pyruvates from glycolysis can feed into the citric acid cycle, they must undergo a transition reaction. The pyruvate is converted into a 2-carbon acetyl group as the third carbon is lost as CO_2. The acetyl group is attached to coenzyme A to form acetyl-CoA.
2. The 2-carbon acetyl-CoA combines with the 4-carbon oxaloacetate of the citric acid cycle to form 6-carbon citrate.
3. Citrate is converted to isocitrate.
4. The 6-carbon isocitrate is oxidized by NAD^+ to produce reduced NADH and 5-carbon alpha-ketoglutarate. (One carbon is lost as CO_2.)
5. The 5-carbon alpha-ketoglutarate is oxidized by NAD^+ to produce reduced NADH and 4-carbon succinyl-CoA. (One carbon is lost as CO_2.)
6. Oxidation of succinyl-CoA produces succinate and one GTP that is converted to ATP.
7. Oxidation of succinate by FAD produces reduced $FADH_2$ and fumarate.
8. Fumarate is converted into malate.
9. Oxidation of malate by NAD^+ produces reduced NADH and oxaloacetate.

The two molecules of acetyl-CoA from the transition reaction enter the citric acid cycle. This results in the formation of 6 molecules of NADH, two molecules of $FADH_2$, two molecules of ATP, and four molecules of CO_2. The NADH and $FADH_2$ molecules then carry electrons to the electron transport system for further production of ATPs by oxidative phosphorylation.

http://student.ccbcmd.edu/~gkaiser/biotutorials/cellresp/fg17.html

Citric acid cycle uses in research studies:

The below referenced articles, papers and books are examples of the many scientific applications of the citric acid cycle.

4. Medical and pharmacological:

The citric acid cycle is central to the regulation of energy homeostasis and cell metabolism. Mutations in enzymes that catalyse steps in the citric acid cycle result in human diseases with various clinical presentations.
http://www.ncbi.nlm.nih.gov/pubmed/15141213

5. Cancer research:

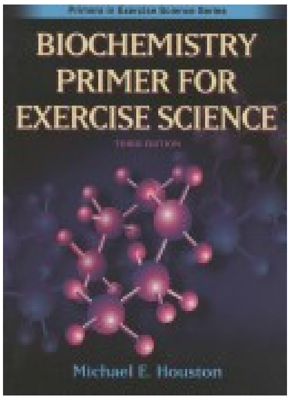
A study of the citric acid cycle in certain tumour tissues

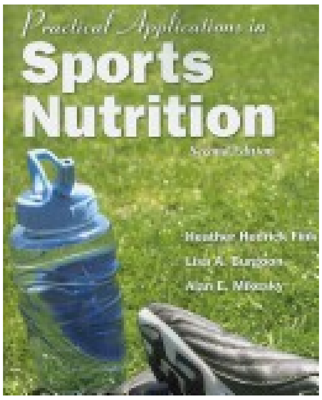
R. M. Dajani, Joyce Danielski, W. Gamble, and J. M. Orten
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1243370

KREBS OR CITRIC ACID CYCLE: In normal cells, after glucose is broken down to pyruvic acid it is then carried into the mitochondria and totally combusted by some twelve enzymatic reactions into carbon dioxide and water. From the breakdown of pyruvic acid and fats through the Krebs cycle, normal cells derive approximately 70 percent of their total energy needs. However, because cancer cells have a defective Krebs cycle, they must derive almost all of their energy needs from Glycolysis. CAAT is designed to cripple the enzymes that function in Glycolysis thereby literally starving cancer cells to death. Normal cells are not affected because they have an intact Krebs cycle to provide them with their daily energy needs. (Source: Review of Physiological Chemistry)
http://www.apjohncancerinstitute.org/physician-2.htm

6. Sports science/ Nutrition:

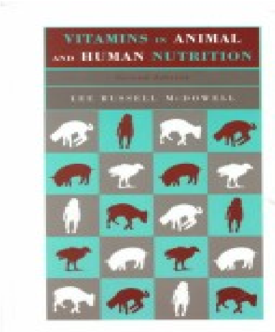
Pyruvate and citric acid cycle carbon requirements in isolated skeletal muscle mitochondria Jeffrey I. Messer, Matthew R. Jackman,Wayne T. Willis **http://ajpcell.physiology.org/cgi/content/full/286/3/C565**





http://books.google.co.uk/books?id=DY3Xjt6ffNEC

7. Nutrition and Dietetics:



http://books.google.co.uk/books?id=dXOPBMYIPcQC

Contribution of various substrates to total citric acid cycle flux and] anaplerosis as determined by^{13}C isotopomer analysis and O_2 consumption in the heart
http://www.springerlink.com/content/w838tm6835231603/

Modifications of citric acid cycle activity and gluconeogenesis in streptozotocin-induced diabetes and effects of metformin
To better define the modifications of liver gluconeogenesis and citric acid cycle, or Krebs' cycle, activity induced by insulin deficiency and the effects of metformin on these abnormalities, we infused livers isolated from postabsorptive or starved normal and streptozotocin-induced diabetic rats with pyruvate and lactate (labeled with [3-13C]lactate) with or without the simultaneous infusion of metformin. Lactate and pyruvate uptake and glucose production were calculated. The 13C-labeling pattern of liver glutamate was used to calculate, according to Magnusson's model, the relative fluxes through Krebs' cycle and gluconeogenesis.
http://diabetes.diabetesjournals.org/cgi/content/abstract/48/6/1251nfusion of metformin.