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An Escape-Room about Krebs cycle prepared for Chemical Students

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Abstract: Games are excellent teaching tools for self-learning. Students playing a game enjoy themselves and at the same time learn basic and important concepts. Different games including crossword puzzles, word search puzzles, knight's tour games, connecting dots, mazes, matching two sets, amidakuji, and logic games were used for continuous assessment during the confinement due to pandemic. These games were developed as part of the GINDO-UB180 teaching innovation group activities. Since these games were very successful, we decided to combine all of them and construct a new teaching activity, that would help our students to study one essential metabolic pathway: The Krebs cycle. This activity (https://forms.gle/BPvpnLQRNYNPxcLQ7) is an Escape-room, and it was recently tested by our students in a General Biochemistry course. The code of the Escape room after the game is a word: either the longest in the letter soup, an anagram from several boxes from the crossword puzzles, the sentence from the knight's tour game, or a word obtained following a maze. The results obtained after carrying out the Escape room will be compared with those obtained using previous tasks in terms of assessment and acceptance by the students in order to be included in the future as self-evaluation activities.

Keywords: Science education, Biochemistry, Chemistry students, Escape-room, Games

Introduction

Games are often very appreciated by students, and also by general population, because they allow learning without effort and they are very useful for self-learning. Some of the games published in entertaining magazines are thought as solo games, although they can also be played in teams. Those games include mathematics (sudokus, numerical charts, addition or subtraction paths, ...), but these games do not seem to be applicable for the study of Biochemistry. Other games are based on letters, which could be much more useful for teaching, as the words can be enzymes, metabolites or other “specific” biochemical words. The most popular letter games used in teaching are crossword puzzles or self-defined boards, although there are other letter games that include words such as word search puzzles in a board, a knight’s tours in a chessboard with letters, matching two sets (by using arrows or amidakuji), connect the dots puzzles, mazes containing questions and answers, or logic games.

In the GINDO-UB180 teaching innovation group and during the Covid-19 confinement, we created and applied several games for Biochemistry students of the Chemistry Degree, to be solved by them and used for continuous assessment. The games were prepared in Catalan and included crossword puzzles and other letter games and were very successful among the students, who learnt the subject and enjoyed the activities. As these students asked for new games, the objective was to prepare several Escape-rooms in different items for their study. In this paper an Escape-room in English about the Krebs cycle is described. This Escape-room is to be answered by Biochemistry students of the Chemistry Degree for self-learning, and using their own Computer at home.

Method

Escape-Room Software

To perform the Escape-Room, the Google Drive Form online program was used. Google Forms collects information from students via personalized quizzes [Gavin, 2019]. Forms are distributed in Sections and several question types can be used: short answer, paragraph, multiple choice, checkboxes, drop-down, file upload, linear scale, multiple choice grid, checkbox grid, date and time. Questions used as code were ticked as mandatory to be answered by students to move to next section, and usually short answers were used (either numeric or alphanumeric).

The distribution of the Escape-room included a numeric problem or different games in each section in order the students do not get bored if there is a similar game of one performed in another section. For this purpose, we had prepared several games including:

Connect the Dots Puzzles

This kind of puzzle, also called dot to dot or join the dots, is based in a sequence of numbered dots. When the dots are connected following the sequence, the sketch of a design figure or an object is revealed. David Kalvitis is the most famous artist and graphic designer, who published around 20 hand-designed puzzle books, and created The Greatest Dot-to-Dot Books in the World in 2000 [Kalvitis, 2000]. The most used connecting the point puzzles follow a numerical sequence, but also a letter sequence can be used.

Those kinds of puzzles are not very useful for Biochemistry, although in the electronic transport chain an image is developed when the students join the sequence of electron transfer from NADH to oxygen in the respiratory chain. Nevertheless, although the connect-the-dots puzzle allows students to become familiar with the intermediates and the electron transfer, as the sequence has not many intermediates, images obtained seem to be too simple for a University student. On the other hand, to find a key for the Escape-room page, it was better to construct a maze with several labels (with electron transport intermediates) connected with letters. The letters obtained in the way from NADH to oxygen would generate the key code to change section.

Maze with Questions with Answers

This puzzle is a variation of the maze with labels connected by letters, described as a modification of the connect the dots for the electron transfer from NADH to oxygen. In these mazes, one label has the question and is connected through letters to several answers. Students have to choose the correct answer, that has also another question to continue the maze.

The key for the Escape-room section will be the letters that connect the way.

Word Search Puzzle

The word search, word find, word seek, word sleuth or mystery word puzzle is known in Spanish as “sopa de letras” (word soup). It consists of the letters of words placed in a grid (or other geometric shape), where the player has to discover several words arranged in the grid vertically, horizontally or diagonally, and in both directions. It was created in a Spanish version around the 60s by Pedro Ocón de Oro (1932-1999), from Madrid, although an English version was published in 1968 by Norman E. Gibat, from Oklahoma [Gamesver Team, 2021]. In 60 years, new variations have been done, including word search without a list, snake words that change directions, and several more possibilities.

To find the key code to change section in the Escape-room, the longest or the shortest word find can be used.

Crossword Puzzles

Crossword puzzle is the most popular and widespread word game in the world. The first crossword puzzle was published in 1913 by Arthur Wynne (1871-1945) from Liverpool. He based his crossword puzzles on a similar game called magic squares from ancient Pompeii and he translated from Latin to English. In the Magic Square, the player has to arrange a list of words on a grid. A crossword puzzle is similar, but the words must be obtained by the player from given clues. In Spain, Pedro Ocón de Oro worked also in these crosswords puzzles.
In order to obtain the key for the Escape-room page, several boxes from the crossword grid can be selected and an anagram related to the item of the crossword puzzle can be used.

Amidakuji

This puzzle has its origin in the Japanese Amida lottery, and it is also named Ghost Leg in China or Sadaritage (ladder climbing) in Korea. It is based in matching two sets containing the same components, for example a set of numbers and a set of letters. Amidakuji is based in vertical lines from one set to the other, but to relate one number to one letter several horizontal lines are drawn between two of the vertical lines. The number of horizontal lines is not important, but at least one line should be between each pair of adjacent vertical lines.
To choose which number matches with a letter, the vertical line is followed since a horizontal line is reached, and then continues with the adjacent vertical line until the next horizontal line or until reaching the end of the vertical line.

To obtain the key in the Escape-room, the match between numbers and letters can be used, or making it a little bit more complicated, a question can be at the end set, with two or more possibilities.

Knight’s Tour Game

This puzzle is based on the movements of the chess horse jumping. The game consists of a grid containing one syllable in each box and the use of the knight’s tour discovers a hidden sentence, that could be the key for the Escape-room.

The problem of the knight’s tour was studied by Leonhard Euler (1707-1785) using a sequence of number, that could generate a semi magic square [Weisstein, 2021].

Logic Puzzles

These problems are based in order and fit several sets by using some postulates. For example, a logic puzzle in amino acids metabolism was prepared for our students using the classification between essential and non-essential amino acids and between glycogenic and ketogenic amino acids. The key for the Escape-room could be the location of a specific amino acid in the logic puzzle grid.

Results and Discussion

Using Google Drive Form, an Escape-Room about Krebs cycle was developed [Gavin, 2019]. This Escape-room allows the students to self-study and learning this metabolism by playing, as they can move only to the following page by setting the key, but they can move to previous pages when studying the pathway. The Escape-room (https://forms.gle/BPvpnLQRNYNPxcLQ7) includes 8 sections.

Section 1

This section introduces the students to the Krebs cycle and shows the sequence of reactions and the classical nomenclature of the enzymes from the cycle. Figure 1 shows the summary of the Krebs cycle included in this section.

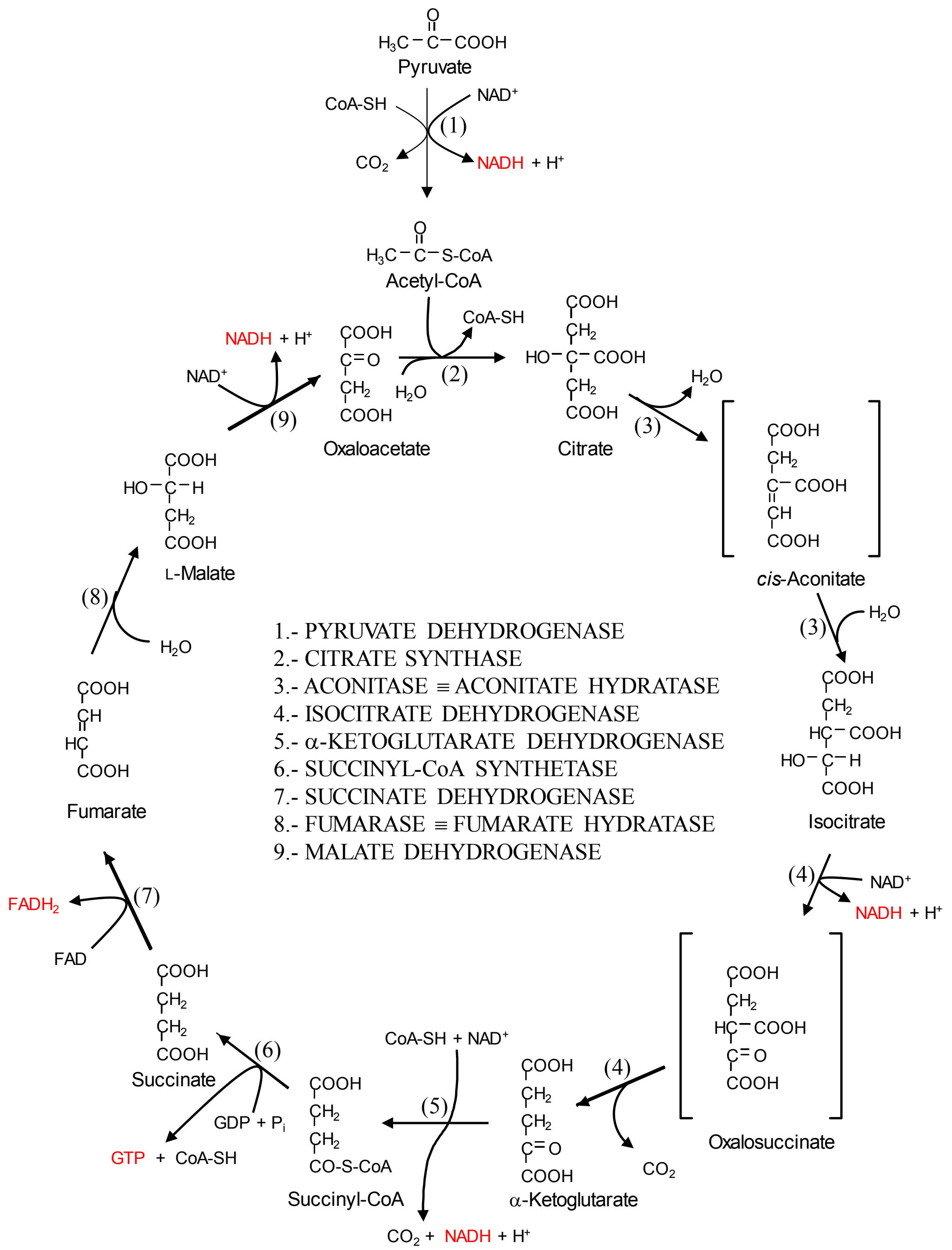


Figure 1. Krebs Cycle Summary

This section contains a video about the Krebs cycle. The student has to watch the video and indicate at what time (in seconds) begins the explanation of aconitase reaction. The key code is between 110 and 130 seconds.

Section 2

This section is based in the E.C. nomenclature of Krebs cycle enzymes. The Escape-room gives a summary of the first number of enzyme nomenclature in order that students can choose which are the numbers of the 9 enzymes that catalyze the reactions:

E.C. 1 Oxidoreductases
E.C. 2 Transferases
E.C. 3 Hydrolases
E.C. 4 Lyases
E.C. 5 Isomerases
E.C. 6 Ligases
E.C. 7 Translocases

Students have to look for the enzyme code of each enzyme and has to introduce these numbers as answer. The key code to change this page is: 124116141

Section 3

This section contains the complete E.C. numbers of Krebs enzymes and their Gibbs free energy under standard conditions (ΔG'º):

1) Pyruvate dehydrogenase: (E.C.1.2.4.1.)	ΔG'º = – 33.4 kJ/mol
2) Citrate sinthase (E.C. 2.3.3.1.)	ΔG'º = – 32.2 kJ/mol
3) Aconitase or Aconitate hydratase (E.C.4.2.1.3.)	ΔG'º = +13.3 kJ/mol
4) Isocitrate dehydrogenase (E.C.1.1.1.41.)	ΔG'º = – 8.4 kJ/mol
5) α-Ketoglutarate dehydrogenase (E.C.1.2.4.2.)	ΔG'º = – 33.5 kJ/mol
6) Succinyl-CoA synthetase or Succinate-CoA ligase (E.C.6.2.1.4.)	ΔG'º = – 2.9 kJ/mol
7) Succinate dehydrogenase (E.C.1.3.5.1.)	ΔG'º = 0 kJ/mol
8) Fumarase or Fumarate hydratase (E.C.4.2.1.2.)	ΔG'º = – 3.8 kJ/mol
9) Malate dehydrogenase (E.C.1.1.1.37)	ΔG'º = +29.7 kJ/mol

After some considerations regarding the standard and real Gibbs free energy, a numeric problem is proposed and the key code is the result obtained.

Problem: Standard and physiological Gibbs free energy (ΔG'º) is defined at pH 7.0 (physiological). But pH inside the mitochondria is more basic (pH = 7.7) [Santo-Domingo and Demaurex, 2021]. Under standard conditions, the Gibbs free energy (ΔGº) of the malate dehydrogenase reaction (Malate + NAD^+ → Oxaloacetate + NADH + H^+) depends on pH and it is ΔGº = +68.4 kJ/mol. Inside the mitochondria, the ratio NAD^+/NADH can be around 100 to 1000. From the variation of standard Gibbs free energy (ΔGº) calculate the equilibrium constant of malate dehydrogenase reaction: Keq, and from this constant calculate the ratio Malate/Oxaloacetate if we consider the ratio NAD^+/NADH as 500 and pH inside mitochondria equal to 7.7. Which will be the ratio Malate/Oxaloacetate when this reaction is in equilibrium?
ΔGº = – RT ln Keq; R = 8.314 J/K·mol.

The result obtained from this calculation yields a Keq = $3.015 \cdot 10^{-12}$, and when NAD/NADH = 500 and $[H^+] = 10^{-7.7}$, the ratio Malate/Oxaloacetate is 13.2. Thus, the key code to change this page is: 13.2 (a value between 8 and 20 is also accepted).

The student has to realize that this reaction will be in equilibrium when ratio Malate/Oxaloacetate is 13.2. But according to the thermodynamic equation:
ΔG = ΔGº + RT ln Q
if the ratio Malate/Oxaloacetate is higher than 13.2, the real Gibbs free energy ΔG would be negative and then the reaction catalyzed by malate dehydrogenase will be spontaneous in the direction malate to oxaloacetate. Fortunately, the concentration of oxaloacetate inside mitochondria is very low, even lower than the malate

concentration (as it synthetizes citrate with a very negative Gibbs free energy), thus making that real Gibbs free energy of malate dehydrogenase is negative, although the standard Gibbs free energy is positive. Thanks to this, the cycle can continue working.

Section 4

In this section, a maze with questions and answers is used (see Figure 2). Questions were regarding the pairs amino acid / keto acid, and intermediates and enzymes related with the cycle.

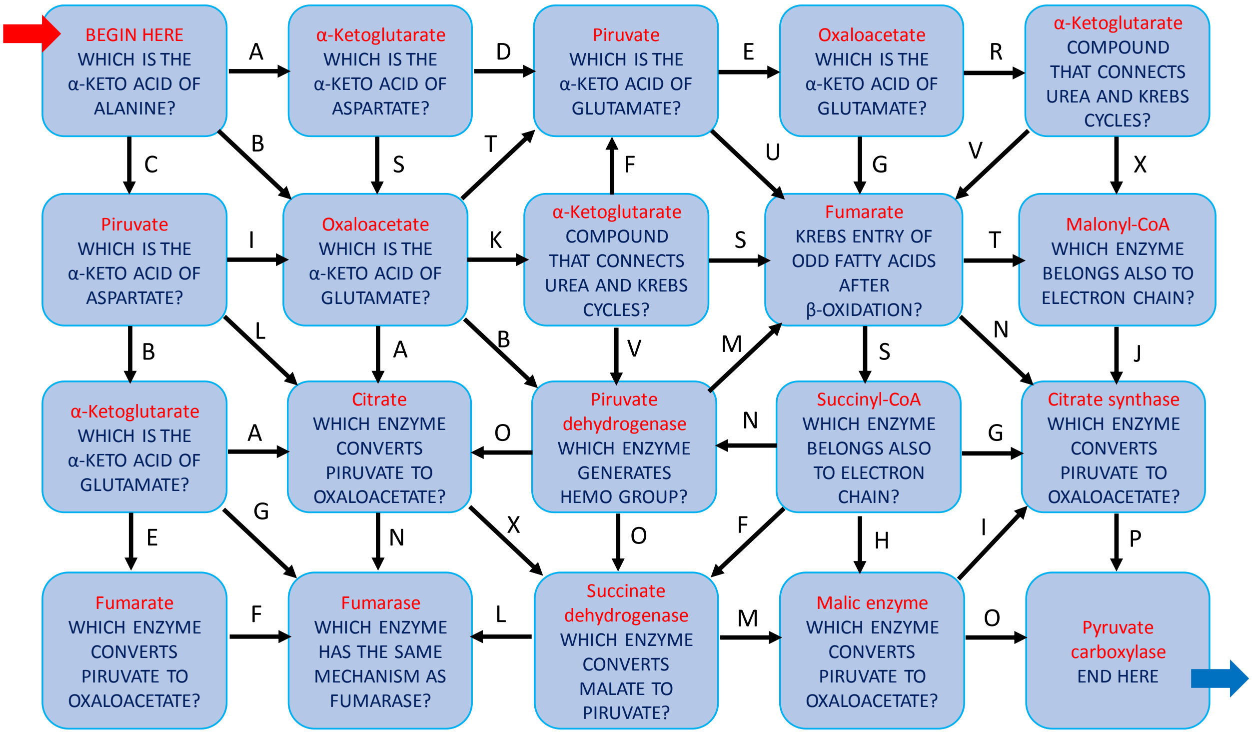


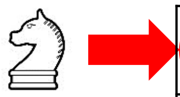
Figure 2. Maze with Questions and Answers from Krebs Cycle

The key code to change page is based in a mnemotechnic sentence to remember the Krebs cycle intermediates (CIKSSFMO). This code is explained in the following section and comes from a question: “Can I keep selling substances for money, officer?” [Quizlet Inc., 2021]. Each letter of this question is related to an intermediate:

Can	Citrate
I	Isocitrate
Keep	α-Ketoglutarate
Selling	Succinyl-CoA
Substances	Succinate (remember succinATE is LATE, comes after succinyl-CoA)
For	Fumarate
Money	Malate
Officer	Oxaloacetate

Section 5

A knight’s tour game was used in this section. Figure 3 shows the 5 x 5 grid presented in this section.



THE	TO	TER	CALLED	AC
RO	ARE	RE	THE	ME
IN	IN	TIC.	TIONS	AN
CLE	PLE	SUP	DI	KREBS
PLY	ATES	CY	A	THAT

Figure 3.Knight's Tour Game from the Krebs Cycle

The key code to change page is the full sentence obtained: "THE REACTIONS THAT SUPPLY INTERMEDIATES INTO THE KREBS CYCLE ARE CALLED ANAPLEROTIC."

Section 6

This section contains several organic chemistry considerations about the chirality of intermediate molecules in the Krebs cycle. When reacting Acetyl-CoA and Oxaloacetate in an Organic Chemistry laboratory, four different compounds are obtained and yield will be very low compared with the enzymatic reaction of citrate synthase (see Figure 4).

Similar considerations can be done for water addition in fumarase reaction (L-malate or D-malate for Organic Chemistry). On the other hand, also for Organic Chemistry water addition in cis-aconitate yields better citrate through the Markovnikov rule than anti-Markovnikov isocitrate, which is the compound obtained by aconitase (see Figure 5).

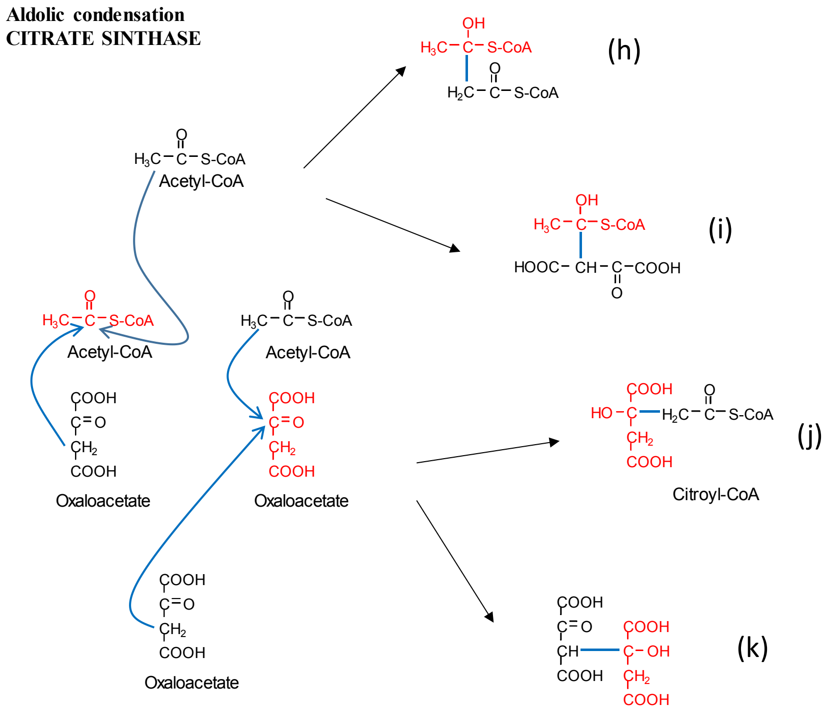


Figure 4. Reactions of Aldolic Condensation between Acetyl-CoA and Oxaloacetate

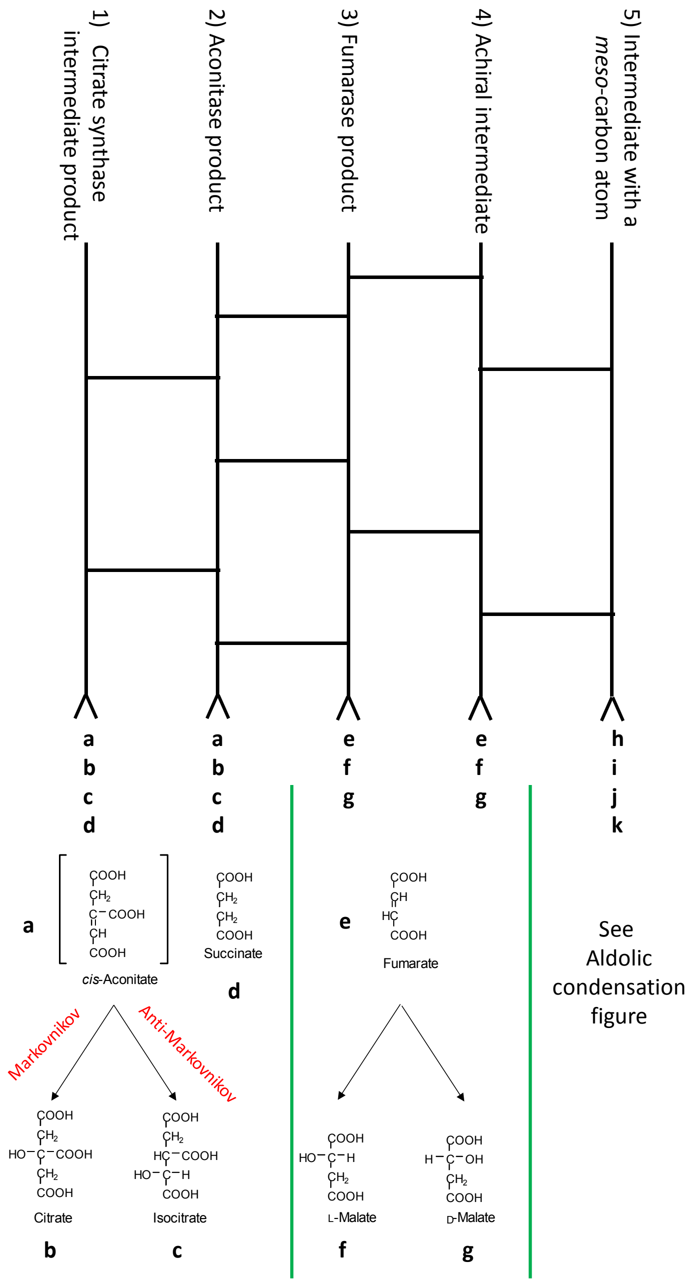


Figure 5. Amidakuji for Organic Chemistry Considerations of Krebs Cycle

In this section, an Amidakuji was used to get the key code (Figure 5).

1.- Citrate synthase intermediate product line leads to the fifth line and an intermediate metabolite should be selected from the Organic Chemistry products of aldolic condensation of Figure 4 (h, i, j, k).
2.- Aconitase product line leads to the first line and an intermediate should be chosen from a, b, c, d.
3.- Fumarase product line leads to the forth line and an intermediate should be chosen from e, f, g.
4.- Achiral intermediate line leads to the third line and an intermediate should be chosen from e, f, g.
5.- Intermediate with a *meso*-carbon atom line leads to the second line and an intermediate should be chosen from a, b, c, d.
Therefore, the key code to change page is: 1j2c3f4e5b

Section 7

Anaplerotic reactions are presented in this section, and specially the entrance of carbohydrates or lipids (see Figure 6). We ask students to identify which enzyme of the figure catalyses an anaplerotic reaction.

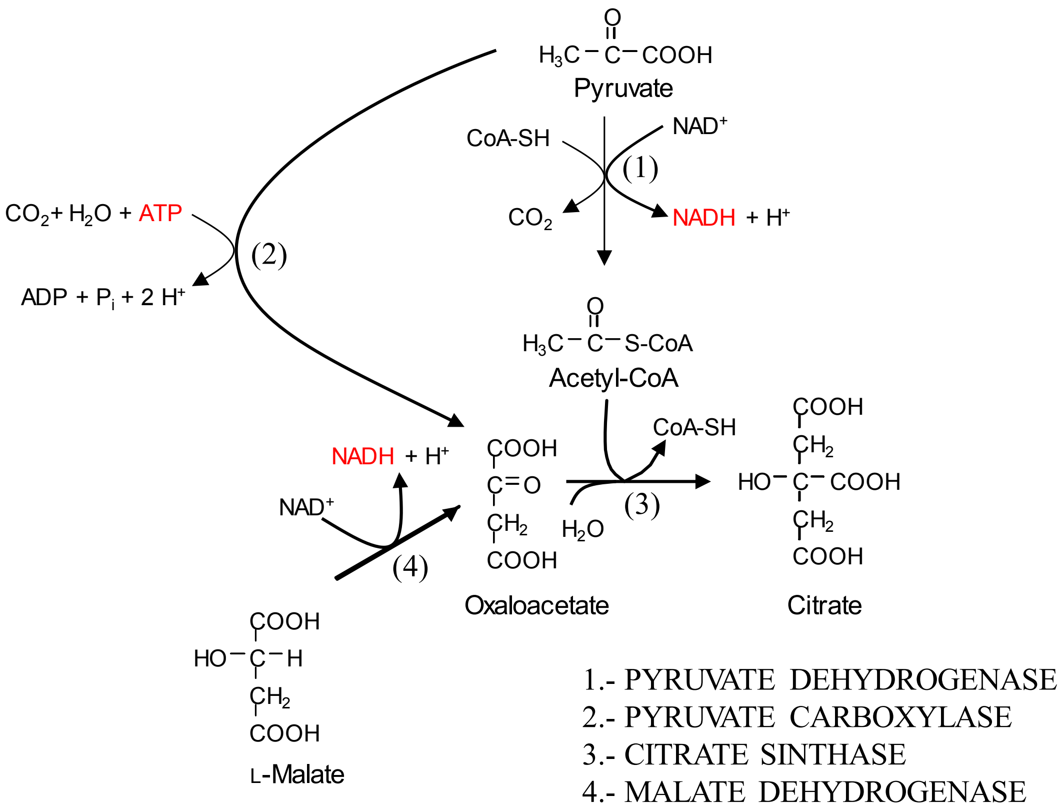


Figure 6. Entrance of Pyruvate to the Krebs Cycle

The key code to change page is the anaplerotic enzyme: PYRUVATE CARBOXYLASE.

Section 8

The last page of the Escape-room congratulates the student for his hard work, and asks the name, e-mail address, time used and other comments that students want to add. Google forms allow teachers to control the answers and to know students' comments.

The Krebs cycle Escape-room has been designed to ease the acquisition of basic skills and competences not only in Krebs cycle but also in enzymes classification, reaction mechanisms and energetics of biochemical reactions. In addition, through the organization of competitions between teams, the students will also acquire basic horizontal skills on efficient organization and communication that will help in their future career to perform their responsibilities in the best way.

Conclusions

Word games are very useful for self-learning Biochemistry, and specially in an Escape-room, where students cannot change page if they do not answer correctly.

Games are preferred by students than typical tests, thus Amidakuji or mazes with questions and answer could substitute the usually tests.

Escape-room should include several games to be more appreciated by students, as some students can prefer a game ad others another and it is good that they can find an appreciated game in the Escape-room.

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