Gábor Tóth

From vitamins to peptides – Research topics in Szent-Györgyi's departments

Albert Szent-Györgyi is probably one of the most well-known Hungarian Nobel Laureates, and this is partly due to the fact that he was our only Nobel Laureate who after receiving the Nobel Prize returned to Hungary. However, his works are not very well-known and often it is believed that he received the Nobel Prize for the discovery of vitamin C, which is not entirely accurate. Below you can see the photo of an old article which was published in1932 about Szent-Györgyi's discovery that pepper around Szeged contains a lot of vitamin C.

Szent-Györgyi Albert professzor szenzációs felfedezése a szegedi paprikával kapcsolatban

A szegedi paprika négyszer több C vitamint tartalmaz, mint akár a narancs, akár a citrom — A felfedezéssel kapcsolatban nagy export lehetőségekre van kilátás

Szeged, december 3.

Ebben az évben rengeteg panasz hangzott el a szegedi paprikatermelők részéről. A néhány évvel ezelőtt virágzó paprikaexport az idén az Ausztriával való vámháború miatt teljesen elakadt, s ezzel kiszámíthatatlan kár érte a szegedi termelőket. Pedig Szegeden közel 20 ezer embert érdekel a paprikatermelés, értékesités és kikészítés.

A földmüvelésügyi minisztérium illetékes osztálya megpróbált segíteni a szegedi paprikatermelőkön, sajnos, azonban nem azzal az eredménnyel, amelyet a szegediek reméltek.

A különféle érdekeltségek szintén akcióba léptek a paprikatermelés érdekében, de nem lévén export, a jóindulatu támogatás nem jelentett kiadósabb anyagi hasznot. Allandó volt tehát a panasz a szegedi paprikások részéről. A vámháború és a külföldi lelketlen konkurrencia miatt az árak olyan mélyre estek, amelyek tovább folytatódva, föltétlenül a paprikatermelés válságát idézték volna elő. Hogy ez mit jelentene az ország gazdasági életében, az csak most tünik ki,

Szent-Györgyi Albert professzornak, az egyetemi vegytani intézet igazgatójának szenzációs felfedezéséből,

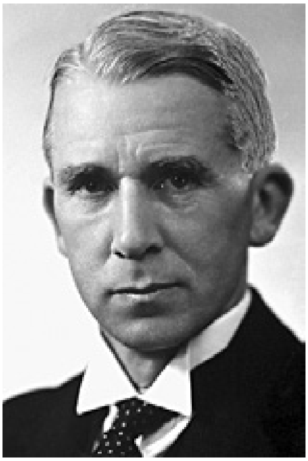
amelyre nemcsak Magyarországon, nemcsak Európában, hanem az egész világon fel fognak figyelni s amelynek üzleti haszna, helyes megalapozással soha nem remélt arányokban bontakozhat ki.

Szent-Györgyi professzor buvárkodásának régebbi eredménye az egészségre annyira fontos C-vitamin. S a kiváló tudós állandóan kutat olyan növények után, amelyekben a C-Vitamin bőségesen fellelhető. Igy kezdett foglalkozni ez év októberében a szegedi termésü paradicsom-paprika vegyi összetételével, s néhány napi laboratóriumi munka után nagyszabásu felfedezésre jutott. Felfedezéséről a következőket mondotta el a **Szegedi Uj Nemzedék** munkatársának:

— Vitaminoknak nevezzük az anyagok egy csoportját, melyek táplálékunkban igen kis mennyiségben vannak jelen, azonban az élethez és egészséghez nélkülözhetetlenül szükségesek. Ezeknek a Vitaminoknak nagy teoretikus jelentőségük mellett igen nagy közegészségügyi és economiai jelentőségük van. A modern ember tápláléka nem felel meg a természetadta tápláléknak és igy könnyen nélkülözi ezeknek az életfontosságu Vitaminoknak egyikét, vagy másikát. Nem kell mást felhoznunk a Vitaminok fontossága bizonyítékául, mint egyes betegségeket, melyek szinte járványszerüen pusztitanak, amelyek lényege nem más, mint egyik vagy másik Vitamin hiánya, amilyen betegségek a beri-beri, az angolkór és scorbut.

Az én szegedi laboratóriumomnak jutott osztályrészül, hogy tisztázhassa egyik legfontosabb vitaminnak, az ugynevezett Vitamin C.-nek a chémiai természetét.

Ez a Vitamin C. csakis friss gyümölcsökben, vagy növényekben található. A Vitamin C. hiánya által okozott betegség vagy gyengeség könnyen sujt olyanokat, Both the article and the discovery are true. However, when Szent-Györgyi received the Nobel Prize in Physiology in 1937, on the same day two other gentlemen, namely Sir Walter Norman Haworth and Paul Karrer, also received the Nobel Prize for research in connection with vitamin C. Furthermore, Haworth received this high-rank award for the investigations he carried out on the structure of carbohydrates and vitamin C.



Sir Walter Norman Haworth
(1883–1950)

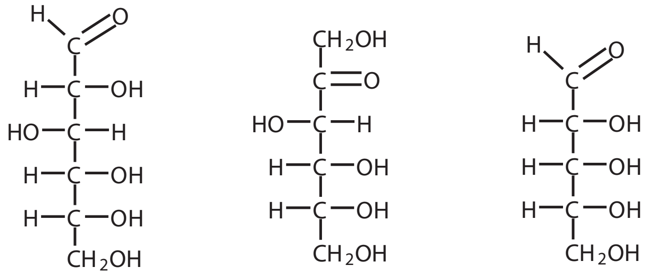
Paul Karrer
(1889–1971)

The components of nutrients

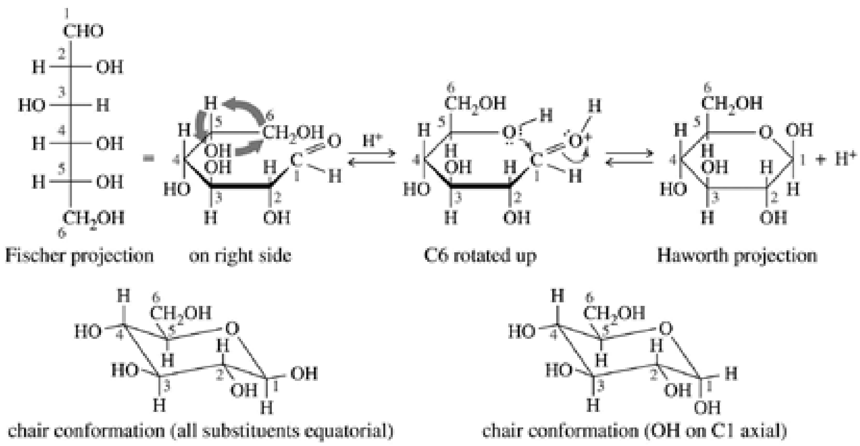
Now let's see what these so-called vitamins are and why they are so interesting that so many Nobel Prizes have been awarded on this topic.

By the end of the 19th and the beginning of the 20th century, chemical structure research was advanced enough to determine what kind of chemicals are in nutrients. During this, 3 main components were distinguished. Nutrients are predominantly made up of carbohydrates, which, according to chemical definitions, are polyhydroxy-oxo compounds, containing an oxo group and some alcoholic hydroxyl groups.

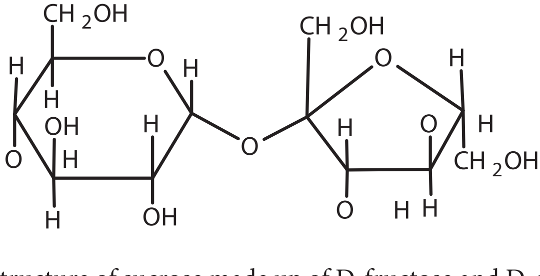
In the following pictures, the formula of some important, well-known and simple carbohydrates can be seen. Carbohydrates have an interesting characteristic, namely, that they are like a snake biting its own tail when they form a cycle between the oxo group and one of the hydroxyl groups. At the end of this cycle the interesting, ring-like structure of the carbohydrates develop, which includes a new hydroxyl group. This has a different reactivity than normal hydroxyl groups because of its surroundings, which makes it possible for carbohydrates to form long chains together. After this process, carbohydrates are able to connect with each other, not just two of them but a lot of molecules and if these molecules are made up of glucose units, we end up with starch, cellulose or glycogen. These are one of the important components of our nutrients.



The structure of D-ribose, D-fructose and D-glucose

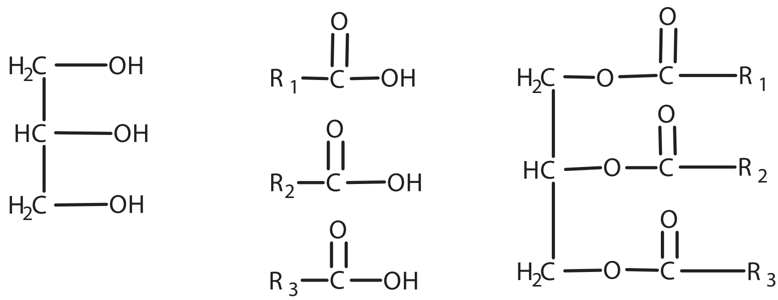


The ring structure of D-glucose and the spatial structure of the ring



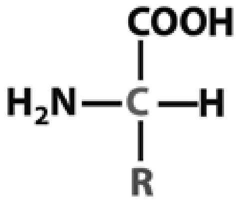
The structure of sucrose made up of D-fructose and D-glucose

The next group is constituted by fats and oils. These are made up of tri-alcohol, glycerol and some fatty acids. This triester is called triglyceride, which is commonly known as vegetable oil or animal fat, depending on the type of fatty acids involved and their level of saturation.



The general structure of triglycerides

Last, but not least, the third group of nutrients is proteins. Proteins consist of amino acids, which are organic compounds having a carboxyl and an amino group with a side-chain attached to them. In case of protein-forming amino acids the amino and the carboxyl groups can be found on the same, alpha-carbon atom. Amino acids are able to combine with the help of the so-called peptide bond between the amino and the carboxyl groups. If 100, 150 or 200 amino acids combine, the resulting molecule is called protein. The importance of proteins is proven by the fact that amino acid or its components constitute approximately half of the dry matter content of living cells.



The general formula of an alpha-amino acid

So these are the components of nutrients. After isolating these substances in a pure form, laboratory animals were given these as nutrients. Within a short period of time, these animals got sick and died. This led to the realization that there are substances which are necessary for a living organism but can be found in nutrients only in a small amount. These substances are called vitamins.

Water- and fat-soluble vitamins

The term vitamin was compiled in 1912 by a Polish biochemist, Kazimierz Funk, who put an “amin” suffix after the Latin word “vita”, meaning life, since he thought that vitamins are such amines which are essential to life. Today we know that he was only partially right because there are some vitamins which are not amines, e.g. vitamin C. The proper definition of vitamins is that they are organic compounds essential to life, usually relatively small molecules. From a chemical point of view, they are quite diverse, this is why they are divided into two subcategories, that of water-soluble and fat-soluble vitamins. We don’t know the exact number of vitamins, the literature is inconsistent; however, on this picture 13 can be seen. Later on, depending on the author, other compounds were named as vitamins too; but what we consider as a vitamin also partially depends on the way we consider this term. For example, we call vitamin C a vitamin, since our organism cannot produce it; however, for lions it is not, since they can synthetize vitamin C. So we can say that lions don’t need to consume fresh fruit to avoid scurvy.

Water-soluble vitamins:
vitamin B1 (thiamine, aneurin)
vitamin B2 (riboflavin)
vitamin B3 (nicotinic acid)
vitamin B5 (pantothenic acid)
vitamin B6 (pyridoxine)
vitamin B7 (biotin)
vitamin B9 (folic acid)
vitamin B12 (cobalamin)
vitamin C (ascorbic acid)

Fat-soluble vitamins:
vitamin A (retinol)
vitamin D (calciferol)
vitamin E (tocopherol)
vitamin K (phylloquinone)

Why are vitamins important? Below you can see a table summarizing vitamins. In the first column different water- and fat-soluble vitamins are listed.

We can ask why it is relevant that a vitamin is fat-soluble or water-soluble. The reason is that if a vitamin is water-soluble, it is quickly excreted from the body. On the other hand, if a vitamin is fat-soluble, it can accumulate in the body, thus it is not necessary to intake a fresh vitamin dose daily. In the next column we can see enzyme functions which require the given vitamin for their proper functioning. It provides an explanation for the importance of vitamins. If there is no vitamin, certain enzymes are not able to function properly, thus leading to certain disorders. These vitamin deficiency diseases can be seen in the next column. They include a wide variety of disorders ranging from coagulopathy to haematopoietic disorders, skin diseases, visual complaints, osteogenesis and its disorders, etc. At first sight, it does not seem to be something serious if someone has a skin disease, we are sure that the person is going to be cured, no big deal. However, when it comes to these vitamin deficiency diseases, if they are no treated, most of them are fatal.

Name of the vitamin	Chemical name	Solubility	Deficiency disease	Overdose	Recommended daily intake
Vitamin A	Retinol	fat	Night-blindness, Keratomalacia	25,000 IUs	620μg
Vitamin B1	Thiamine	water	Beriberi	n/d	1mg
Vitamin B2 (G)	Riboflavin	water	Ariboflavinosis	n/d	1.1mg
Vitamin B3 (PP)	Niacin	water	Pellagra	2,500 mg	12mg
Vitamin B5	Pantothenic acid	water	Paraesthesia	n/d	
Vitamin B6	Pyridoxine	water	n/a	400 mg	1.1 mg
Vitamin B7 (H)	Biotin	water	n/a	n/a	30 μg
Vitamin B9 (M)	Folic acid	water	[3]	1,000 μg	320 μg
Vitamin B12	Cyanocobalamin	water	Megaloblastic anaemia	n/d	2 μg
Vitamin C	Ascorbic acid	water	Scurvy	n/d	75 mg
Vitamin D1-D4	Lamisterol, Ergocalciferol, Calciferol, Dihydrotachysterol, 7-ehydrositosterol	fat	Rickets	50,000 IU	2 μg for all Vitamin D
Vitamin E	Tocopherol	fat	n/a	50,000 IU	12 mg
Vitamin K	Naphthoquinon	fat	n/a	n/d	75 μg

The name of vitamins, their deficiency diseases and their recommended daily intake

The classification of enzymes		
Main divisions	*Subclasses*	*Reaction catalysed*
Hydrolases	Lipases Nucleases Proteases	ester hydrolysis phosphate hydrolysis amide hydrolysis
Isomerases	Epimerases	isomerisation of the stereogenic centre
Ligases	Carboxylases Synthetases	addition of CO_2 formation of new bonds
Lyases	Decarboxylases Dehydrases	CO_2 reduction H_2O reduction
Oxidoreductases	Dehydrogenases Oxidases Reductases	formation of double bond by reducing H_2 Oxidation Reduction
Transferases	Kinases Transaminases	phosphate group transfer amino group transfer

Enzyme functions

On the figure above we can see different enzyme groups, their subgroups and what these enzymes can do. We can see that in a living organism there are three groups which can break down different things (e.g. nucleic acids, fats or proteins) with the help of hydrolysis. Other enzymes can change the spatial structure of compounds, form new chemical bonds and break down compounds, in other words, they can remove carbon dioxide or water from molecules, reduce molecules or transfer amino or phosphate groups to molecules. It is clear that in these processes the majority of organic chemistry is present, thus our enzyme system and its functioning is vitally important to maintain our health.

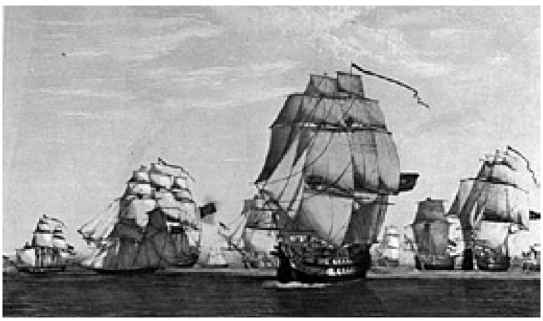
From micrograms to milligrams

How much vitamin is necessary? The amount can range from a couple of micrograms to a couple of milligrams. For example for an adult, 75 milligrams is the daily recommendation from vitamin C, which is needed in the largest amount.

Vitamin C is important for us because there is a disease, namely scurvy, which caused serious problems to mankind in the past. Back in the age of discoveries, sailors departed on long journeys on their ships without having fresh food. It was quite common to see these sailors go down with scurvy after a couple of days or weeks. At the beginning, scurvy just caused unpleasant symptoms, however later on it often proved to be fatal, thus we can say that scurvy killed ten times more sailors than sea battles. The first major discovery in connection with this disease was made by James Lind, a Scottish physician, who served on a battleship in the 1750s. There was an outbreak of scurvy on his vessel and for the first time in history, he formed a control group in which patients were not treated with anything, while other patients were divided into smaller groups receiving different substances as a treatment. One of the groups was treated with apple cider vinegar, which proved to be mildly effective. However, the patients of another group who were treated with lemon juice showed dramatic improvement. All the other groups showed signs of dramatic deterioration.

James Lind (1716–1794)

At that era it was not easy to introduce new things, so people questioned Lind's methods. However, by the end of the 18^{th} century, it became a common practice required in the British Navy to give sailors on battleships a set amount of lemon juice every day. Their enemies often made fun of the British Navy by calling them lemon juice ships, though this did not prevent England from becoming the ruler of the seas.



18^{th} century sailing vessels

Actually we can say that vitamin C was discovered in certain aspects earlier, however, back then people did not know what was in lemon juice which made it effective. Albert Szent-Györgyi, who for a long timed worked in the building on Dóm Square, joined this research for vitamins many years later.

Szent-Györgyi's departments on Dóm square

Szent-Györgyi and the research for vitamins

Albert Szent-Györgyi was born in 1893 as a member of a landowning family as Albert Szent-Györgyi de Nagyrápolt. From his mother's side, he came from a medical dynasty, that of the Lenhossék family. He completed his high school studies at the Lónyai Street Calvinist High School and received his degree at the Faculty of Medicine of the University of Budapest in 1917. During his studies, he also served in the army and fought on two front lines in the World War. After the war he got a job in Bratislava; the farmer hungarian capital, Porzsony however, soon he was fired because the Czech government did not want to have any Hungarian researchers in a research institute in Bratislava. After this, Szent-Györgyi went on a trip around the world including Prague, Berlin, Leiden, Groningen and Cambridge. He worked for years in Groningen and Cambridge and started to do his researches there for which he later received the Nobel Prize. This is why the University of Groningen considers him as its own Nobel Laureate, since he worked there for years.

In 1928 he accepted the invitation of Kuno Klebelsberg, the Minister of Education, to take up a position and he became a professor at the Department of Medical Chemistry of the University of Szeged, which was called Ferencz József University back then. In 1935, when Tibor Széki moved to Budapest, he became a professor at the Department of Organic and Pharmaceutical Chemistry. He served as the Head of both departments until 1940, when he resigned from one of his positions as he became the Rector of the University. In 1945 he went to Budapest and after two years he emigrated. He died in emigration in Woods Hole, USA in 1986.

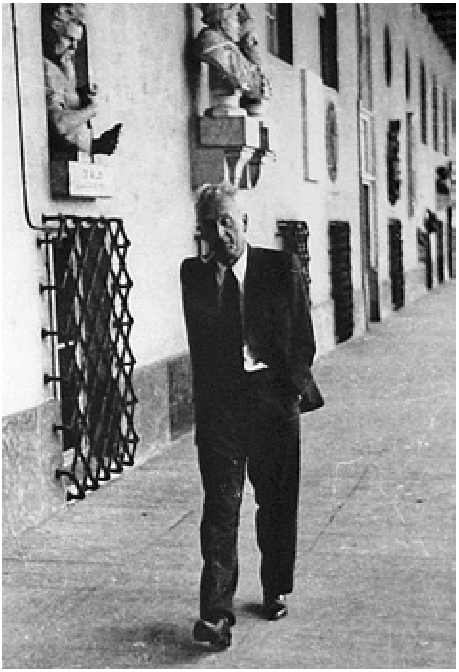
Over the years he received several recognitions. He was awarded the Nobel Prize in 1937. He received the Corvin Wreath and became the Member of the Hungarian Academy of Sciences. He was the Honorary Citizen of Szeged and served as the first Rector of the newly founded Horthy Miklós University in Szeged. After the German occupation he was forced to hide in secret.

Albert Szent-Györgyi

Albert Szent-Györgyi receiving the Nobel Prize. Below you can find the appraisal: "Professor A.E. Lindh of the University of Uppsala addressed the laureate:

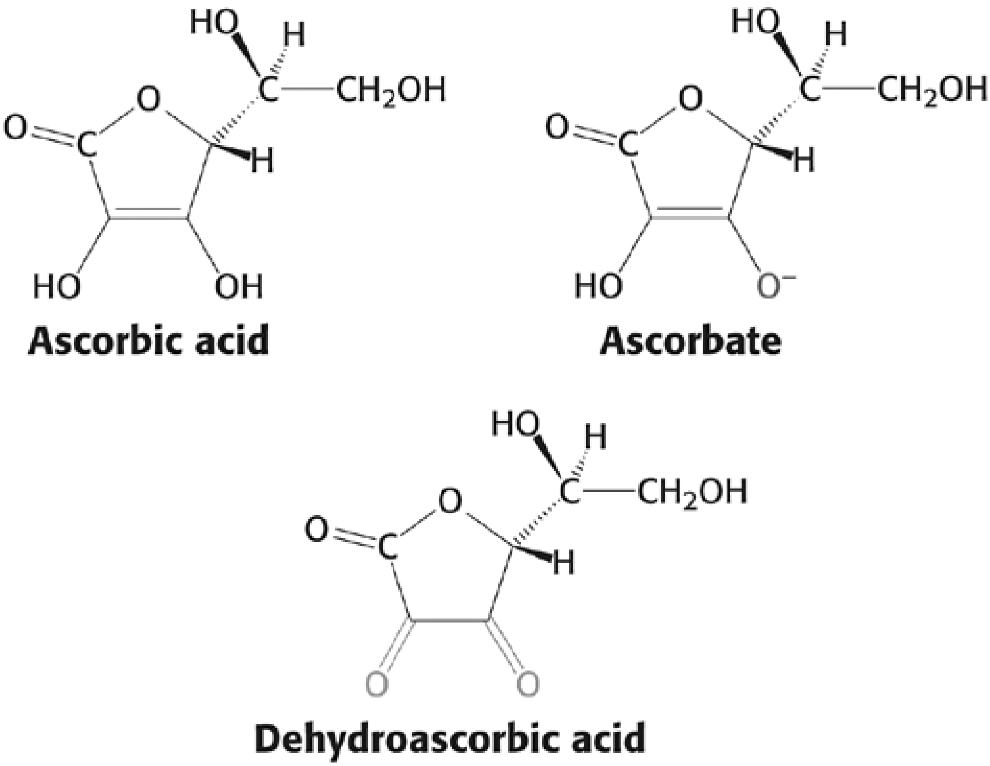
The name of Haworth and Karrer are, via Vitamin C, in close connection with the name of Albert von Szent-György, this year's Nobel Prize laureate in Physiology and Medicine, the ingenious and indefatigable scientist. Kindly accept our greetings, Albert von Szent-György, and at the same time accept our expressions of sincere admiration for the untiring energy you have hitherto shown, and for the extraordinary results you have obtained in your research work despite the difficulties you have, and have had to overcome. Your investigations into oxidation in living cells enabled you to crystallize Vitamin C, a discovery of vital importance to medical science. Your discovery of the Fumaric Acid Catalysis, and your ingenious penetration of its complicated mechanism has opened the way to undreamt of paths within the sphere of medical science and its practical work in the service of suffering humanity. We congratulate you and participate in your happiness in connection with the reward you have gained for your great scientific work, a work of research which has aroused the sincere admiration of the scientific world and in whose continuation we place our highest hopes."

Bálint Hóman giving a speech at the Opening Ceremony of the University in 1940, next to him, Rector Albert Szent-Györgyi.



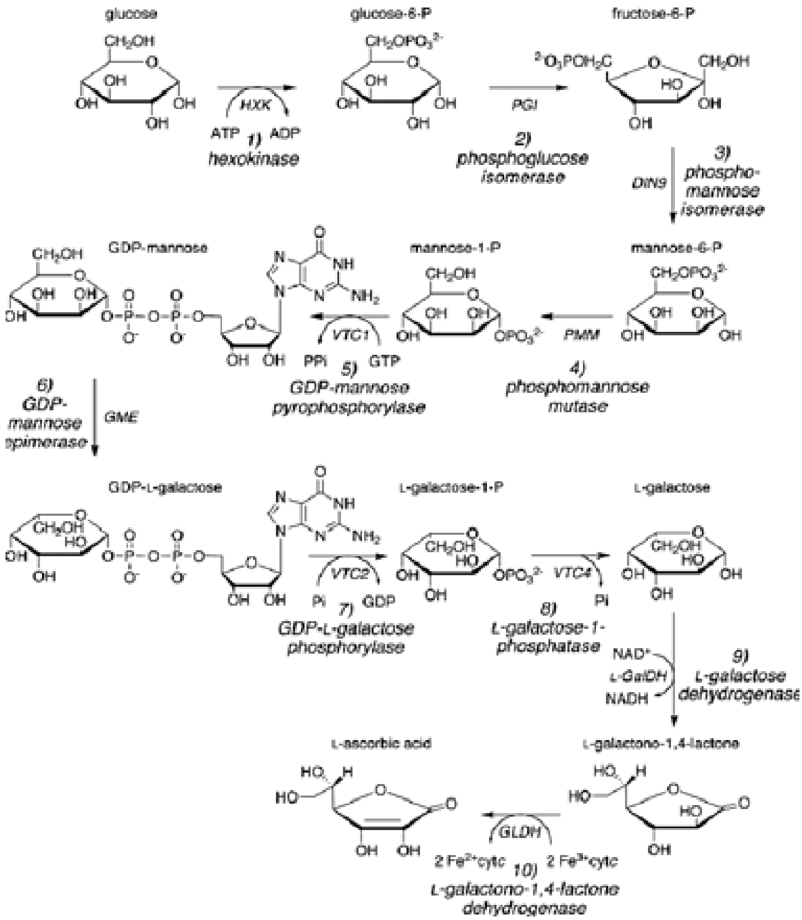
Albert Szent-Györgyi under the arcade in Dóm square and in front of the entrance of his departments

What researches were carried out in these two departments? The most well-known research was concerning vitamin C, the molecule of which can be seen on the following diagram. It also shows its other name, ascorbic acid, and this name originates from the fact that this is the substance which prevents scurvy and has acidic properties.



Ascorbic acid and the results of its two important alterations

For years it was only known that ascorbic acid prevents scurvy and has acidic properties. Then Szent-Györgyi named it hexuronic acid, which is an acid with six carbon atoms. Another important characteristic feature of ascorbic acid is that it easily oxidizes into dehydroascorbic acid. This is a redox process implying that ascorbic acid by itself is a quite good reducing agent. This is a central feature which guarantees that our organism does not get into an overly oxidized state. If we focus on enzyme functions we can state that ascorbic acid is essential for the functioning of certain hydrolase enzymes and collagen synthesis.



The biosynthesis of vitamin C. The last step is blocked in primates.

What contains vitamin C? The popular belief that lemon, as well as pepper, contains lots of vitamin C is true. However, it can also be found in other things like, for example, fresh meat, this is why the Eskimos did not get scurvy though they were eating only small amounts of vegetables for centuries.

Szent-Györgyi realised that it was quite difficult to find vitamin C for researches. They tried to isolate vitamin C from left-overs, adrenal glands from abattoirs. The purity of this was not satisfactory and only a small amount of vitamin C could be gained this way. Szent-Györgyi managed to isolate vitamin C from pepper in great quantities and with the required purity, which was then used for structural analysis and function tests. During these function tests Szent-Györgyi made significant discoveries, for example, how succinic acid is transformed in several steps into malic acid. If we consume the nutrient, after many steps, what happens to it? We live on it, gain energy and build up the molecules of our body. Eventually a significant proportion of nutrients is transformed into carbon dioxide and water. How is carbon dioxide transformed into lard? People did not know the answer for that for many years and Szent-Györgyi managed to find the answer to these questions through his researches.

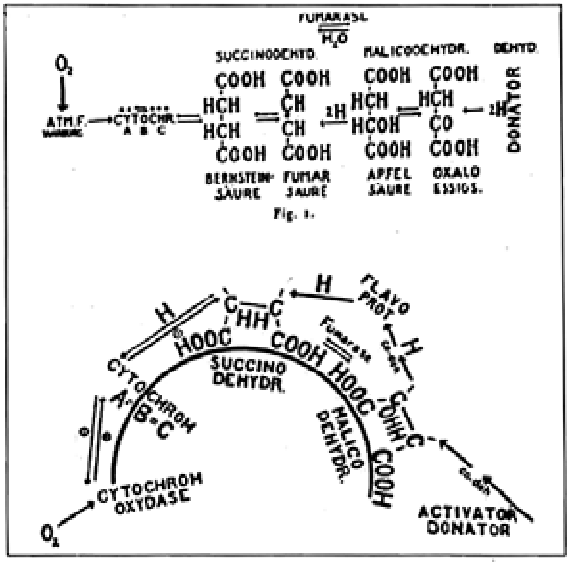
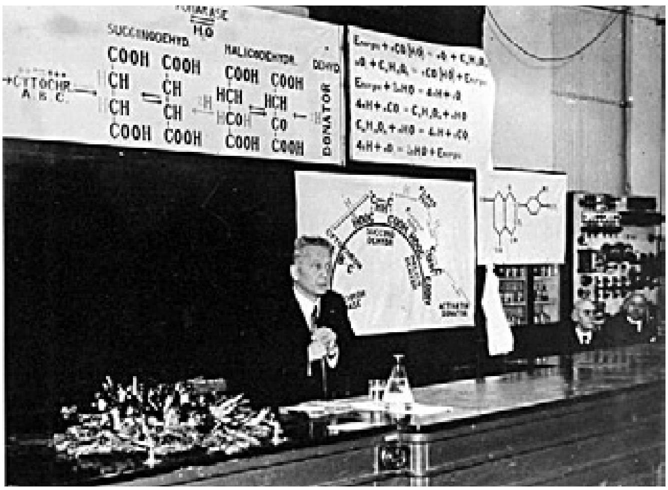
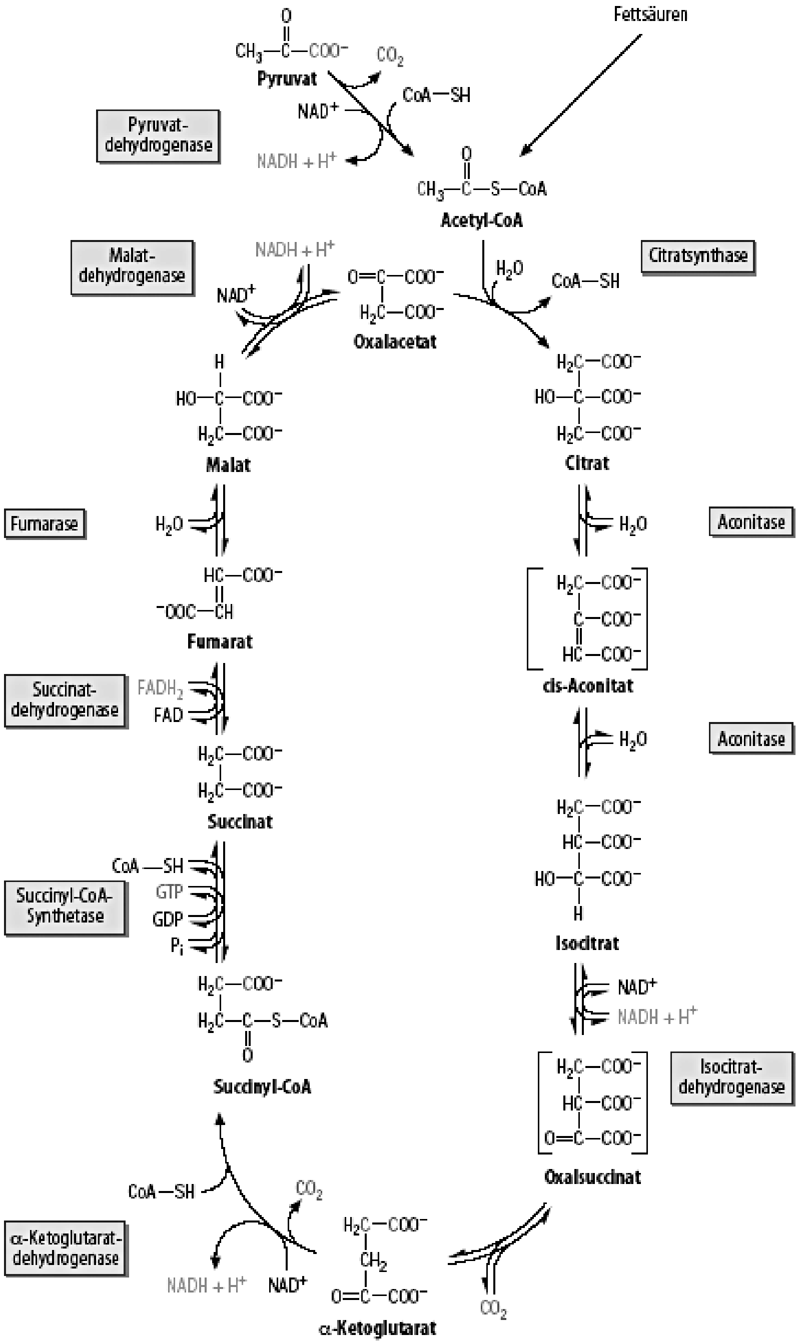


Figure 6. Figs. 1 and 2 of Szent-Györgyi's Nobel Lecture.



Szent-Györgyi's lecture in Szeged



The Krebs cycle (also known as Szent-Györgyi – Krebs cycle).
The metabolic pathway of fatty acids.

Sir Hans Adolf Krebs (1900 – 1981)

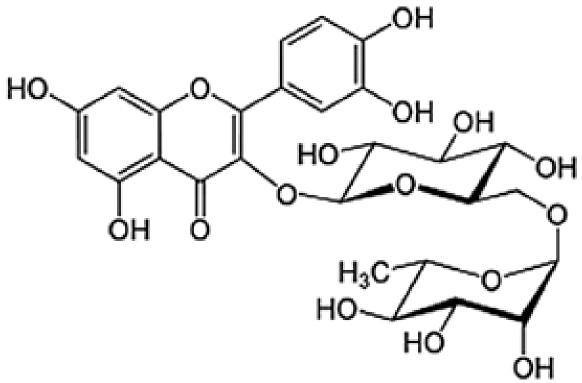
In the picture above we can see how fatty acid enters the cycle and how through several steps this acetic acid part is oxidized by losing two carbon dioxides, thus these carboxylic acids are broken down to their original form. There is a carboxylic acid in our organism containing four carbon atoms which with the help of an acetic acid part containing two carbon atoms is transformed into citric acid containing six carbon atoms. Then in several steps, we regain the original carboxylic acid containing four carbon atoms from the citric acid while two carbon atoms are transformed into carbon dioxide during which process we gain energy. The full cycle was described by Krebs, who was Szent-Györgyi's friend.

Albert Szent-Györgyi received the Nobel Prize for "the results of his research on biological oxidation processes, especially for clarifying the catalysis of fumaric acid and the importance of vitamin C". This was one of the fundamental questions of biochemistry back then, resolving the contradiction between the oxygen activation theory of Warburg and the proton or hydrogen activation theory of Wieland. By setting up the steps of the oxidation process of dicarboxylic acid containing four carbon atoms, one half of the citrate cycle was discovered.

Our title was from vitamins to peptides and so far we covered only one vitamin. Szent-Györgyi discovered with István Rusznyák that with the pure form of vitamin C which he synthetized, he was not able to carry out things which were possible with the less pure form of this compound. He discovered that the less pure form contained something else, a biologically active substance.

Beneficial effects

From a chemical point of view, these substances were polyphenols, belonging to the group of flavones. They can be found in great concentration in citruses, tea and wine. These are not real vitamins but they have significant physiological effects. Szent-Györgyi called it vitamin P; they have many beneficial effects that is why we drink tea, eat fruits and those who like it, drink red wine.



One of the flavonoids, rutin, which Szent-Györgyi called vitamin P

There was one more thing with which Szent-Györgyi dealt with in Szeged, the foundation of muscle biochemistry. Back then it was not well-known how muscles work. It was known that there is a protein complex, the actin-myosin complex, from which the myosin part was already described. The actin part was discovered by the department which Szent-Györgyi led.

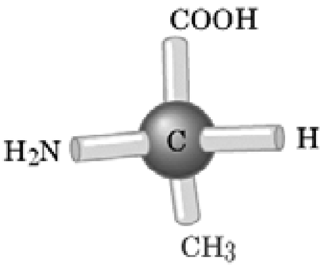
The model of the actin-myosin complex

On Dóm square, Szent-Györgyi has a bust under which there is a bronze plaque, which was put up by the American Chemical Society. They only put up a plaque where a great discovery was made in chemistry. This is an English-Hungarian plaque appraising Szent-Györgyi's discovery, and we have to mention that there are only a few of such plaques outside the USA.

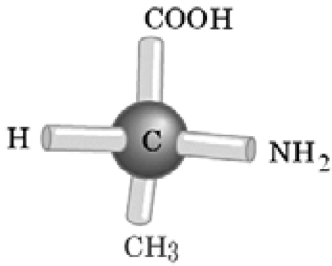
Ivánovics György
(11th June, 1904 –1st September, 1980)

Bruckner Győző
(1st November, 1900 – 8th March, 1980)

Now I would like to talk about Győző Bruckner, who was one of the outstanding and dominant figures of Hungarian organic chemistry. Back then he was Szent-Györgyi's employee and later he became his successor. Bruckner made a great discovery in which Szent-Györgyi was not involved directly; however, this discovery was made in Szent-Györgyi's department. This discovery was on amino acids, more precisely, on proteins. Amino acids always contain at least one carboxyl group and an amino group. In case of amino acids which constitute proteins, both groups can be found on the same carbon atom. If four different substituents are attached to a single carbon atom, the carbon atom has chiral form and the compound has enantiomer forms.

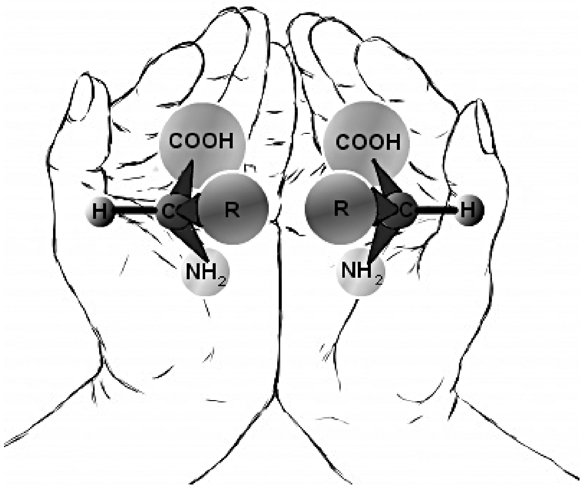


L-Alanine



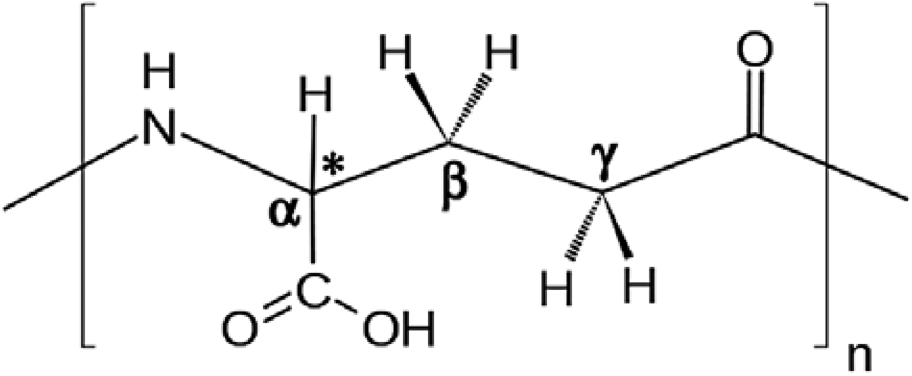
D-Alanine

If four different substituents are attached to a single carbon atom, the carbon atom has chiral form and the compound has enantiomer forms.



The link between the different versions of amino acids and the left and right hand

When they started to examine the structure of proteins it was discovered that different amino acids constitute proteins and almost all of these amino acids are alpha-amino acids. It also turned out that one of them is slightly different than the rest since it contains two hydrogen atoms, thus they are not mirror-image versions of each other. All the other 19 amino acids have two mirror-image versions and proteins are solely built up of one of these versions, the L-alpha-amino acids. This was a scientific doctrine; however, there was an interesting observation which challenged this theory. There is a fatal disease which is caused by the anthrax bacillus. Normally, if an animal got sick and died, its body was buried and underground its proteins decomposed the same way as the pathogens. However, the bodies of animals which died because of anthrax were found to be infectious even after decades. This went against all knowledge of the era.



The polypeptide structure of anthrax

In 1937, Győző Bruckner and György Ivánovics published that the capsular material of anthrax has a very unique structure in many ways. Firstly, a normal protein contains 20 different L-alpha-amino acids, in different sequences. On the other hand, the capsular material of the anthrax pathogen consists of only glutamic acid, so it can be considered as a polyglutamic acid. What is more, it is a polyglutamic acid in which the alpha-amino and the gamma carboxyl groups take part in the peptide bond formation. Thus it is not a substrate of proteolytic enzymes. It also turned out that this is also a chiral, hand-like molecule, just like the constituting amino acids. After this realisation Bruckner and Ivánovics published that the capsular material of anthrax is a poly-gamma-glutamic acid. However, they realised later on that they made a mistake and rectified it by saying that though it is a poly-gamma-glutamic acid, more precisely, it is a poly-gamma D-glutamic acid, explaining why it is so resistant to proteases. This might be the reason why they continued their research on anthrax as they might have suspected its great potential as a biological warfare agent. Anyhow, this discovery, which happened exactly at the time when Szent-Györgyi received the Nobel Prize, can also be considered as the birthday of Hungarian protein research.

To sum up, we can say that Hungarian vitamin and muscle research were founded in Szent-Györgyi's departments. After his departure, these departments started to undertake new projects.

Moreover, at the University of Szeged there are four departments which regard themselves as the successors of Szent-Györgyi, though he was the Head of only two of these departments. The reason for this is that after the war, the Department of Organic and Pharmaceutical Chemistry was broken up into the Department of Pharmaceutical Chemistry and the Department of Organic Chemistry. At the beginning of the 1960s, the Department of Medical Chemistry was separated to the Department of Biochemistry, where muscle biochemical researches are still carried out today. Currently at the Department of Medical Chemistry peptide research is conducted, which cannot be connected to Szent-Györgyi directly, but to his department.