Histology & Embryology Periodical

Department of Histology and Embryology

Third Faculty of Medicine, Charles University in Prague

Volume 3, Issue 2

November 2015

In this issue:

Dear students….

It has been two months since the new semester began, a time of new topics to study, new people to meet, a time full of stress and enthusiasm, too. One would hope that this time brings more joy than suffering but some hardship is inevitable for those who want to make difference. Such as the Nobel Prize laureates. In this issue we review the work on **DNA repair** of the three laureates of this year's Nobel Prize in Chemistry.

Speaking of hard work, **mitochondria** are also busy organelles. They are, in fact, quite fascinating things given their origin and involvement in the essential processes of the human body. The Q&A of the *Repetitio mater studiorum* in this issue are based on the statements from the website Curricular DB, therefore they are the foundation for the mitochondria part of the coming, Course 2, test.

We are excited to see the team of our Department expanding! In the last issue we introduced those you may have already met. In "***Who is who?***" in this issue we present profiles of the three new members of our team!

Few months ago, in a small town in Ontario, Canada, a remarkable woman died peacefully at the age of 101

years. Her name was Frances Oldham Kelsey and she worked as a drug regulator for the American Food and Drug Administration. One decision of hers made a huge difference, and saved an entire American generation from a tragedy of thalidomide. Dr. Kelsey's life combines both, the necessity of professional knowledge and the importance of the so called sixth sense. "I had the feeling", wrote Dr. Kelsey about the need to focus on the safety of thalidomide. And there is one more aspect of her story. She showed exceptional courage facing the wrath of a big pharmaceutical company. She resisted. She was not afraid. And her message carries over times and subjects; don't be afraid. Of your own fears, of the future. It is that simple, don't be afraid.

Figure 1 Jean Jullien©

Dear students, I hope you find this issue inspiring and thought provoking.

Klára Matoušková, *editor*

***

Who is who?

At the Department of Histology & Embryology
Part two

MUDr. Milada Halašková

milada.halaskova@lf3.cuni.cz

A research fellow and senior lecturer.

I was accepted to the Department of Histology and Embryology, now, the 3rd Faculty of Medicine, in 1977. At that time students found the histology exam in the end of the 4th semester to be one of the most challenging exams! The challenge was posed mainly due to the leadership of an excellent assisting professor Vaclav Janout, and an invaluable lab technician Svatava Humhalova.

Early in my career I wished to start my PhD, however those were the times of centralized organization of all aspects of society. So even research positions were limited, and in our Department, science yielded to teaching. However, doc. Janout had taught me a lot lab work and even more about teaching. He was truly strict, for example all his assistants had to come to his lectures! Well, it came in handy one day when he became seriously sick and the entire Department depended on me and two other assistants!

In 1990, after the collapse of the Iron curtain and the Velvet revolution in Czechoslovakia, Prof. Richard Jelínek took over the Department. He brought a group of excellent colleagues from his previous assignment at the Czech Academy of Science and the Department started to flourish! We did both, science and teaching at our Faculty as well as in the prestigious Institute of Experimental Medicine.

More about Prof. Jelínek in the October issue of the Periodical.

Then for several years I worked in a team of Dr. Peterka in a group focusing on teeth development.

Due to my involvement this group I started collaborating with a similar scientific group in Strasbourg, France. And from year 2000 I had worked for an organization of clinical research (CRO).

Beginning in October of this year, I have happily returned to where it all started for me, to the Department of Histology and Embryology of the 3rd Faculty of Medicine in Prague!

MUDr. Halašková

MUDr. Lucie Nováková

luci.novakova@gmail.com

A lecturer and research assistant.

MUDr. Dimitra Papadopoulou

dim.dim.pap@gmail.com

A lecturer and research assistant.

Just in this year, 2015, I graduated from the General Medicine Programme in the English speaking curriculum of the 3rd Faculty of Medicine.

I have always been interested in teaching and research. Therefore I have taken the exciting opportunity to become a lecturer and researcher here, at the Department of Histology and Embryology.

As I am yet not sure if later in my career I will pursue a clinical or laboratory specialization, I have this option to teach and research now, and want to make the best of it!

You can find me in my office, room no 310.

***

What Happened & What's Coming up

Study Programme: General Medicine
Module: Cellular Basis of Medicine
Course 2: Energy for cells

Week 6 and 7
You have learned about the exocrine and endocrine glands, and the oh-so-important organ of liver and its' hepatocytes. Glad you didn't miss a lecture and practical on mitochondria.

Week 8
Barriers and transports
Week 9
Revision on epithelia

Week 10
Muscle tissue; one of the four basic types of tissue in a human body.

Week 11
Test: Energy for cells
Lecture: Cells producing signaling molecules

*

Study Programme: General Medicine
Module: Structure and Function of the Human Body

Week 8
Head and Neck Development
Development of Gastrointestinal Tube and Glands

Week 9 and 10
Digestive system, part one & two
Histology and Anatomy of Glands

***

Image of the Month

Reckless Growth of Tumor Cells [1]

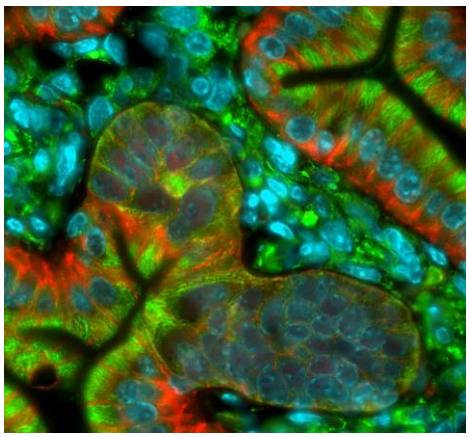


Figure 2
Healthy epithelial cells are well organized. But among the cells of a growing tumor – lower center – the organization is lost.

"The growth of a tumor is characterized by the loss of cellular organization in tissues. In this image of a mouse's intestinal epithelium, well-organized healthy cells at the top right have well-defined cell boundaries labeled in red. In contrast, cells of a growing tumor in the lower center of the image lack cell boundary labeling and are arranged more randomly. Such changes in cellular organization result in a difference in the behavior of the cells as they form the tumor. By using fluorescence microscopy to analyze mouse tissue samples in this way, researchers are able to compare regions of healthy tissue with diseased areas, and improve our understanding of how polyps form in the intestines of humans." [1]

Technical details of the image

Mouse intestinal tissue was dissected, fixed, sectioned and stained with fluorescent dyes for microtubules (green), ß-catenin, which predominantly stains cell boundaries (red), and DNA (blue), and viewed with a widefield microscope.

[1] HHMI BioInteractive. *Image of the Week*, Nov 2, 2015 [online]. [Retrieved Nov 12, 2015]. Available at: https://www.hhmi.org/biointeractive/reckless-growth

Credit

Lauren Zasadil and Beth Weaver PhD,
Department of Cell and Regenerative Biology
University of Wisconsin at Madison, USA

Links:

Web page of the lab of Beth Weaver

TED talks: How do cancer cells behave differently from healthy ones.

Nature Scitable: cell division and cancer

***

Theme:

2015 Nobel Prize in Chemistry

This year's Nobel Prize in Chemistry is shared equally among three recipients:

Tomas Lindahl, a Swedish-born British scientist of the Francis Crick Institute and Clare Hall Laboratory in the UK.

Aziz Sancar, a Turkish scientist of the University of North Carolina at Chapel Hill, USA.

Paul Modrich, an American scientist/biochemist, Howard Hughes Medical Institute (HHMI) investigator at Duke University, North Carolina, USA.

The Nobel Prize in Chemistry 2015 was given for their exceptional participation in **"mechanistic studies of DNA repair"**

*

Each day our DNA is damaged by external attacks, for example free radicals or radiation. Moreover, thousands of spontaneous changes on DNA occur during DNA replications. All living things are subject to dynamic balance, including their DNA.

No life without a second chance

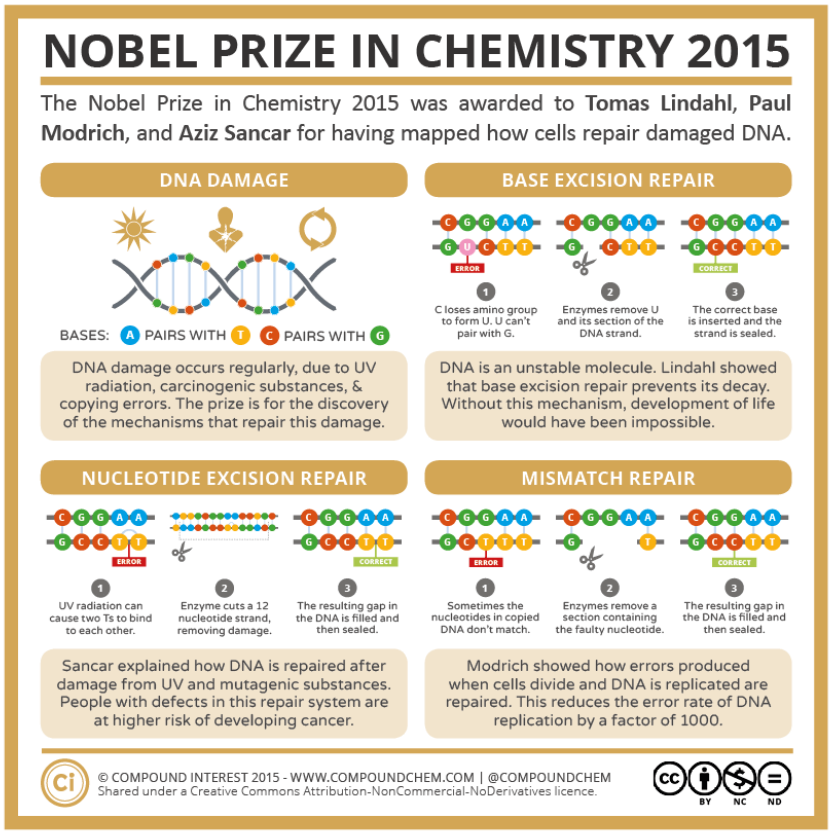
Dr. Lindahl found that DNA is an inherently unstable molecule and that it faces a constant stream of chemical and mechanical attacks. He pondered upon the rate of damage of DNA and figured out that multicellular life ought to be impossible: the DNA in its cells would simply crumble away too quickly.[2] Since multicellular life clearly is possible, he came to the conclusion that there must be some sort of repair mechanism at work! [3]

Figure 3
Dr. Tomas Robert Lindahl

Dr. Lindahl worked on bacteria and discovered two proteins designed to fix the damaged DNA. He was the first to isolate **DNA ligase**, and to describe a totally unanticipated novel group of **DNA glycosylases** as mediators of DNA excision repair.[4]

He also discovered a unique class of enzymes in mammalian cells, namely the **methyltransferases**, which mediate the adaptive response to alkylation of DNA.

Cells nurse their DNA with a variety of means of repair. There are a great number of proteins now known to be used by the cells for "**base-excision repair**" that are capable of fixing more than 100 different types of DNA damage. Dr. Lindahl was awarded the Nobel Prize not only for discovering the first several of such repair proteins, but also for his profound insights into the nature of the DNA repair process.



[2] The Economist, Wisdom, ancient and modern. The Economist, 2015. **417** (8959), pp. 80-81.

[3] LINDAHL, T. Instability and decay of the primary structure of DNA. *Nature,* 1993. **362**, pp.709-715.

[4] THE ROYAL SOCIETY [online]. *Tomas Lindahl.* [Retrieved Nov 8, 2015]. Available from: https://collections.royalsociety.org/DServe.exe?dsqIni=Dserve.ini&dsqApp=Archive&dsqDb=Catalog&dsqCmd=show.tcl&dsqSearch=(RefNo==%27EC%2F1988%2F20%27)

Sleep well - stay well

The research of Dr. Sancar literally shed light, a UV light, on the chemical mechanism called "**nucleotide-excision repair**". Aziz Sancar focused his research at Duke University on cell repair of damage caused by exposure to ultraviolet radiation. UV light causes adjacent nucleobases to form bonds with each other instead of pairing with their counterparts on the other side of the double helix.

Nucleotide-excision is a general DNA repair system that fixes all base lesions including the carcinogenic lesions induced by the main environmental carcinogens, sunlight and cigarette smoke[5]. This type of repair uses enzymes called **excision nucleases**. The enzymes remove a whole damaged chunk of DNA and replaces it with a piece that is functioning correctly.

Recently, Dr. Sancar has focused on circadian rhythm (an oscillation of more or less 24 hour periodicity by solar cycle of light) of organisms. He discovered **cryptochrome**, a protein that regulates the mammalian circadian clock that is also closely related to **photolyase**, a light-dependent DNA repair enzyme. Dr. Sancar is currently investigating the cryptochrome's mechanisms of action because there is a possibility that disruption of the circadian cycle can cause disruptions in DNA repairs and therefore susceptibility to cancer.[6]

Figure 4 Dr. Aziz Sancar

My dad told me;

"You should learn about this DNA stuff"[7]

Dr. Modrich grew up in northern New Mexico, in a landscape of great diversity which he has ever found "thought provoking". In 1963 his father, a local high school biology teacher, told him "you should learn about this DNA stuff" and the future Nobel Prize laureate decided of his professional path.

Since his early career Dr. Modrich studied how organisms prevent mutations in their DNA. He has identified a key proofreading mechanism used by cells to get rid of errors that occur during chromosome replication. The proofreader is called the **mismatch repair system**.

Figure 5 Paul Modrich

Defects in mismatch repair cause the most common kind of hereditary colon cancer and play a role in the development of a number of neurodegenerative diseases.

The DNA molecule of a zygote is copied and recopied trillions of times over the course of an organism's life, but the process in not perfect. Mistakes that occur during formation of sperms or egg cells may cause mutations that are inherited by the following generations. DNA defects that happen during adult life cause cancer.

"In human cells, mismatch repair reduces the error rate by a factor of a thousand", explains Dr. Modrich[6]. In healthy cells, about one mutation occurs per one

[5] UNIVERSITY OF NORTH CAROLINA [online]. *Aziz Sancar*. [Retrieved Nov 11, 2015]. Available from: http://www.med.unc.edu/biochem/people/faculty/primary/asancar

[6] SANCAR, A. et al. Circadian clock, cancer, and chemotherapy. *Biochemistry*, 2015. **54** (2), pp. 110-123.

[7] HHMI News [online] *Paul Modrich awarded 2015 Nobel Prize in Chemistry*. Release date Oct 7, 2015. [Retrieved Nov 5, 2015].

cell division. Without mismatch repair the number would increase to 1000 or more.

Dr. Modrich began his research on bacteria E.coli and currently further investigates the role of mismatch repair in resistance of some cancer to chemotherapy. In science, he enjoys small steps and emphasizes the eagerness to understand; ”Curiosity-based research is so important. You never know where it is going to lead“ [6]

*

Vocabulary:

Nucleobases

- ✓ *organic compounds containing a nitrogen*
- ✓ *like to link to a sugar to form a nucleoside*

The five primary nucleobases either possess a double ring of purines (adenine and guanine) or a pyrimidine single ring (cytosine, thymine and uracil)

Nucleoside

= nucleobase + sugar (a ribose or 2-deoxyribose)

Nucleotide

= phosphorylated nucleoside

Nucleotide is an organic molecule, a subunit of nucleic acid such as DNA or RNA.

Nucleotide is made of

1. *a nucleobase (A,T,C,G or U)*
2. *sugar (a ribose or 2-deoxyribose)*
3. *phosphate group (one, two or three, depending on which textbook you read)*

*

Wonder about the 2014 Nobel Prize in Chemistry? See the last November issue.

***

Available from: https://www.hhmi.org/news/paul-modrich-awarded-2015-nobel-prize-chemistry

Repetitio mater studiorum…

Mitochondria

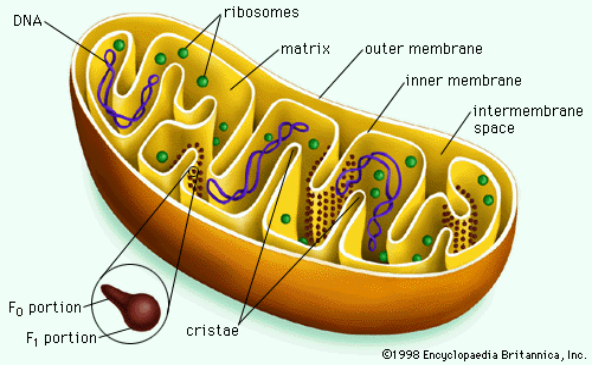


Figure 6 Mitochondria, Encyclopedia Britannica, Inc.

Mitochondria have been known and observed as early as the first microscope imagining. Given its appearance the organelle was called many names such as chondriozome or chondrioplast. Hence the part of “chondria” or “**chondrion**”, Greek for granule. Later, as the microscope images improved and cristae were observed, “**mitos**” Greek for thread was added to “chondria”, thus mitochondria. The early cytologists stained mitochondria supravitally with **Janus green B.**

Questions:

Mitochondria ….. can/cannot (1) be seen in a scale of light microscope. If present in large numbers mitochondria contribute to …..philia (2) of cytoplasm. Mitochondria is about the size of an average bacteria. True/False (3)

The most accepted hypothesis of how mitochondria ended up in eukaryotic cells is called the …. (4).

Unlike endoplasmic reticulum, Golgi apparatus or lysosomes, mitochondria have a … (5) layer membrane.

The outer membrane of mitochondrion is porous, and …… (6a) by small molecules, and contains so

called … (6b) complexes for protein transfer. Outer membrane protein-to-lipid ratio is about …(7).

Mitochondrial proteins synthetized in cytoplasm are bound to chaperons type **HSP70** before they enter mitochondria.

The inner membrane of mitochondrion:

- Forms cristae and ……(8 a,b,c)
- Protein-to-lipid ratio is about…. (9)
- Is freely permeable only to … (10 a,b,c)
- Contains(11a)… receptors, (11b) synthase, or (11c)… genin in brown adipocytes, and has several antiport systems allowing exchange of anions between the cytosol and the mitochondrial matrix

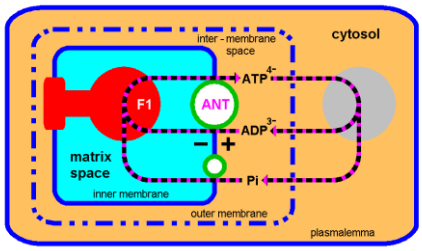


Figure 7 ATP synthase are on the *inside* of the mitochondrial inner membrane, but most of the cellular ATP is required *outside* the mitochondria, in the cytosol. The inner membrane contains a special protein called the **adenine nucleotide translocator** (ANT) that is responsible for ATP transport. This transport protein is the most active enzyme in animal cells. As well as exporting ATP it simultaneously imports ADP for recycling by the mitochondria. This swapping system is a very common arrangement for transmembrane proteins, and is called an ***antiporter***. [8]

Ions transporting cells have mitochondria localized close to a membrane with …. (12).

Insufficient mitochondrial function and deficiency of ATP causes impairment of Na+ and K+ transport through membrane and therefore it causes cell edema (swelling).

Intermembrane space contains… (13).

Besides (13), mitochondria may contain cytochrome …. (14)

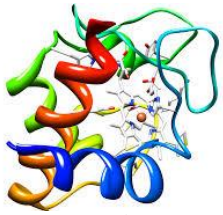


Figure 8 Cytochrome c

Cytochrome c *(cytochrome complex) is a hemeprotein located in the inner mitochondria membrane. It is an essential component of the respiratory chain where it carries one electron!*

The cytochrome c oxidase reaction accounts for about 90% of total oxygen uptake in most cells. This protein complex is therefore crucial for all aerobic life!

Mitochondrial matrix

- contains the enzymes of the … (15) cycle

Mitochondrial elementary particles

- Are/are not (16) electro-microscopic equivalent of ATP synthases.
- Are/are not (17) mitochondria ribosomes

Mitochondria are a **semi-autonomic organelle**, e.g. the divisions of mitochondria are not synchronized with the cell cycle.

Brown adipocytes

- produce heat by cleavage of ATP, which is produced in abundant mitochondria
- produce heat instead of … (18)
- produce heat instead of ATP synthesis (hydrogen protons don't transfer through ATP synthases instead, they make their way through via transmembrane UCP (uncoupling proteins) and

[8] Illingworth J. *Supercharged cells*. [online]. University of Leeds, UK. [Retrieved 11/10 2015]. Available at: http://www.bmb.leeds.ac.uk/illingworth/6form/

the energy will turn into warmth instead of storing the energy in a form of ATP molecule.

Neurons

Energy metabolism of neurons naturally does require mitochondria.

Mitochondrial DNA is .....(19), i.e. has no end, and there are multiple - often more than ten copies of mitochondrial DNA in matrix of single mitochondria. In contrast, **nuclear DNA** of eukaryotes is linear, i.e. has two ends. Nuclear DNA codes about 600–1000 mitochondrial proteins, in contrast in mitochondia itself is located a few dozens of genes. [9]

Apoptosis

Mitochondria are/are not (20) involved in starting cell death.

> ***Cardiolipin*** *is an important component of the inner mitochondrial membrane*
>
> ***Cytochrome c*** *binds to cardiolipin in the inner mitochondria membrane. Thus anchoring its presence and keeping it from releasing out of the mitochondria and initiating apoptosis.*

A typical mammalian **sperm** midpiece contains ... (21) mitochondria, one copy of mtDNA each. In contrast, the mammalian **oocyte** contains around 100 000 – 100 000 000 mitochondria, particularly the human oocyte is estimated to contain about ... (22) copies of mtDNA. [10]

Oocyte (or embryo) **is** able to eliminate damaged mitochondria

In healthy development, mitochondria are practically exclusively **inherited** from .... (23).

Mitochondrial impairment affects predominantly long-living cells such as (24).

*

Answers:

1. Mitochondrion can be seen in a light microscope. However they are invisible, unless stained specifically.
2. Acidophilia, because of the large amount of membrane they contain.
3. Given the endosymbiotic hypothesis of their origin, mitochondria are about the size of an average bacteria, indeed. Mitochondria are about 05-1μm by 3-5μm.

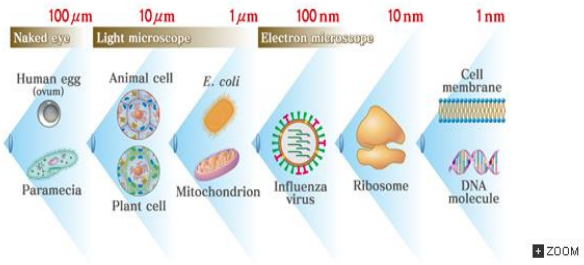


Figure 9 Size chart of biological entities, Division of Life Sciences, University of Tokyo

4. Endosymbiotic theory; mitochondria were originally prokaryotic cells that somehow became endosymbionts living inside the eukaryote. In other words, as a Curricular DB statement states, mitochondria have their origin in living bacteria.
5. Two.
6. a) permeable, b) TOM complexes, or translocase of the outer mitochondrial membrane

[9] POLLARD, Thomas D; EARNSHAW, William C. *Cell Biology*. 2. vyd. [s.l.] : Saunders, 2007. ISBN 1416022554. S. 928.

[10] ANKEL-SIMMONS F., CUMMINS, J.,M. Misconceptions about mitochondria and mammalian fertilization: Implication for theories on human evolution.*Proc.Natl. Acad. Science,* 1996. **93**, pp. 13859-13863.

7. 50:50
8. tubules, vesicles (saccules) and prisms
9. 80:20
10. oxygen, carbon dioxide, and water
11. Contains a) respiratory chain receptors, b) ATP synthase, c) thermogenin
12. Ion pumps
13. Cytochrome c

> Cyanide and azide are extremely toxic because they bind to the heme iron atoms in cytochrome c oxidase much more tightly than does O2, thereby greatly reducing ATP production.

14. Cytochrome P450
15. citric acid (Krebs)
16. are
17. are not
18. energy
19. circular
20. are
21. 50-75
22. 100 000
23. Mother
24. cardiomyocytes and neurons

***

Figure 10: B. Reynek, St. Martin, graphics

Poem of the Month

Shadows

By Bohuslav Reynek

Cobwebs wound round the empty swallows' nests
(the morning's redness taken by their wings).

The blackened lamp whispers and attests
to darkness falling and to brightenings.

The byre at morning. Darkness rich with gleam.
A glint of head brass, cribs, some wood beyond.

Shadows and lights cleave to the cattle team.

They are like calves, their bodies dunned and blond.
Beneath black eaves, they lie down in the straw.

They huddle even closer with their mates
to the sound of milk: its froth and bubbles draw
the ginger cat, who licks her fur and waits.

Darkness. A lamp in it. The pillars start
to fade and shadow edges almost glisten.

Mortal eyes can hardly tell apart
what is the earth and what already isn't.

(*Translated, from the Czech, for the New Yorker, by Justin Quinn*)

***

Obituary

Frances Oldham Kelsey

(1914-2015)

A Canadian pharmacologist who saved a generation of Americans from the tragedy of thalidomide.

Figure 11
Dr. Kelsey and President J.F. Kennedy in the White house in 1962

Dr. Kelsey became a 20th century American heroin for her role in the thalidomide case. She died in August 2015, at the age of 101.

In 1960 Frances Kelsey, by then a highly qualified and experienced medical doctor, pharmacist, teacher and mother of two daughters, moved to Washington D.C. Her husband was appointed to a post at the National Institute of Health (NIH) there and she accepted a job for the Food and Drug Administration (FDA) reviewing requests to approve and license new drugs, a low level position for a task that at that time was regarded as pretty much a routine task.

One of the first drug approval requests that landed on her desk was Kevadon, a sedative that had already being sold to patients in Europe, including to pregnant women. The application to the FDA seemed to be a mere formality, ready for the rubber stamp.

Merrell, the company marketing Kevadon in the US made ”glowing” statements on the drug’s safety and effectiveness. It said Kevadon, known better by its’ generic name thalidomide, was an excellent sedative giving prompt, deep, natural sleep without hangover. Moreover, doctors in Europe, Canada, the Middle East and elsewhere had been prescribing it to women in early pregnancy to suppress morning sickness, headache, nausea and insomnia. Dr. Kelsey, along with her chemist and pharmacist at the FDA, found the evidence of the drug’s safety insufficient. She asked Merrell for more data on toxicity, strength and purity of the drug.

The company was not happy about the delay, because tons of the drug were already in warehouses ready for distribution. Expecting an easy approval, a thousand American doctors had been given samples to distribute to their patients.

Dr. Kelsey resisted the pressure from the company and refused to be hurried, insisting on additional safety testing. What followed was a test of wills instead. Merrell supplied more data but also launched a campaign to pressure Dr. Kelsey, calling her a petty, unreasonable bureaucrat. But time played in her favor. Later in 1961 reports from Europe begin piling up indicating that the drug was linked to an epidemic of phocomelia (a rare but terrible malformation of limbs creating flipper-like arms and legs) and other birth defects affecting eyes, ears, genitals, heart, kidneys and digestive tract.

President John F. Kennedy gave Dr. Kelsey the nation’s highest federal civilian service award in 1962 saying that “her exceptional judgement in evaluating a new drug for safety for human use has prevented a major tragedy of birth deformities in the United States”. Seventeen births of babies with deformities attributed to thalidomide were reported in the USA,

according to the FDA. The estimates for the rest of the world goes to tens of thousands.

The thalidomide case helped the Congress to pass stronger rules for drug safety and effectiveness. Moreover, it increased the public awareness of birth defects. And it also shattered the myth of the placental barrier; that is a belief that the placenta acts as an impenetrable shield that protects the developing organism from any harm.

Dr. Kelsey's brilliant analysis, intellect and her strong will to resist Big Pharma carries an inspiring message for generations of doctors and scientists. For the seemingly simple fact that the vigilance and fortitude of Frances Oldham Kelsey saved much of human misery.

***

The Internal Newsletter, Third Faculty of Medicine, Charles University in Prague.
Editorial Board:
MUDr. Klára Matoušková, MPH – editor
klara.matouskova@lf3.cuni.cz
MUDr. Lucie Hubičková-Heringová, Ph.D.
MUDr. Eva Maňáková, Ph.D.

Full text available at:
http://www.lf3.cuni.cz/en/departments/histologie/hep/