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Adress:

17/19/28 Kamińskiego st.

90-229 Lodz, Poland

KRS: 0000628361

NIP: 7252139787

REGON: 364954217

e-mail: fundacja@promovendi.pl

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ISBN: 978-83-955366-9-4

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PREDISPOSING FACTORS TO HORSES EXPOSURE TO SELECTED HEAVY METALS

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Abstract:

Cd, Hg and Pb belong to the group absolutely toxic elements about highest accumulation coefficient in plant and animal organisms. Fur and mane hair serve as good indicator of accumulation heavy metals in terms of non-invasive character and often higher concentration this elements in dead tissues than blood serum. The aim of the research had been to measure Hg, Cd, Pb contents in the fur of horses. Materials for research was taken from 17 adult mares and 13 foals kept in two herd. The samples was collected after the grazing seasons: hair from the neck, hair from the mane. Heavy metals content in the fur of examined horses had been low which proves low pollution of the environment. Noticeably higher levels of Hg, Cd, Pb concentration had been observed in maturing mares which could indicate that animals, the menstrual cycles of which are not fully developed, are more prone to accumulation of this elements.

Keywords:

toxicology, heavy metals, horses, hair coat

Introduction

Both human hair as well as animal coat are good materials for assessing the degree of environmental and food contamination by heavy metals. Living organisms are equipped with many protective mechanisms thanks to which they are safeguarded to some extent against the destructive effects of heavy metals. One of them is their deposition in not very active or dead tissues such as skin and its products, for example hair, feathers or horn fragments. The analysis of these tissues offers many advantages compared to the examination of the body fluids like blood, saliva or urine due to the possibility of detecting the presence of certain substances there - including heavy metals - after their absorption or exposure to them and even after death. It is worth noting that hair generally contains a higher level of heavy metals than body fluids themselves because they are metabolized and eliminated from the body fairly quickly.

The impact of individual chemical elements on living organisms occurs with varying intensity. Certain factors like the species specificity, exposure conditions, the efficiency of the body's protective barriers, the type of compound in which the element is absorbed as well as its solubility in the body fluids and lipids, the absorption route and the exposure time play a significant part here. Heavy metals such as mercury, cadmium and lead are particularly dangerous because they are easily absorbed from the air through the digestive tract, they also pass through the placenta, cross the blood-brain barrier and have the ability to damage DNA and RNA. After saturating the tissue threshold, they enter metabolism and cause intoxication of the body.

In the case of mercury, the nature of the compound in which it is absorbed by the body is crucial. Inorganic mercury compounds have caustic effect while mercury vapours cause pneumonia and bronchitis. For organic mercury poisoning, there is a characteristic latency period, after which symptoms, mainly on the part of the nervous system, such as: nerve excitations, movement incoordination, blindness and convulsions can be expected. Cadmium in small quantities causes coughing, shortness of breath and gastrointestinal symptoms, however, in severe cases it can lead to death due to pulmonary oedema. In the case of chronic cadmium poisoning, respiratory symptoms are accompanied by a runny nose and kidney damage, which can be detected in urine tests where protein, sugar, amino acids, as well as increased calcium and phosphorus levels appear. It is not uncommon to experience reproductive problems, greater susceptibility to orthopedic injuries, joint thickening or hair loss. Lead poisoning in horses is usually chronic in its nature and the symptoms are non-specific, which may include weight loss, weakness, diarrhea or swallowing disorders, often resulting in aspiration pneumonia.

Materials and methods

Thirty privately owned horses, all female, were included in the study. The research group consisted of 17 mares aged 3 to 20 years and 13 foals up to 2 years old. The horses came from two separate herds, differing in the way of keeping, maintaining and feeding them. The mares let out to the pasture were of the Małopolska breed and the ones kept in the alcove system came from the Silesian breed. The samples were taken at the end of the pasture period. They consisted of the hair combed from around the animals' necks and the hair cut from their manes.

Hay and oats feed samples from two crop farms – an organic and a commercial one were also analyzed.

Metals such as Hg, Pb, Cd, Zn, Cu, Mn and Zn were analyzed using atomic absorption spectrometry (AAS). In order to remove external contamination from the surface of the hair and fur, a four-step washing process was performed: Triton-X-100 (0.01%), followed by one rinse with deionized water, one successive wash with acetone, and a final rinse with deionized water. The samples were dried at room temperature until constant weight, then kept in polyethylene bags in exicator for further analysis.

The content of mercury was directly determined by the mercury cold vapour technique (MA-2000 NIC mercury analyzer, Japan). Before determination of Pb and other element, ashing procedure for all samples was carried out. In this case, 0.5 g of hair and 0.5 g of fur from each

individual, and 0.5 g of feed were mineralized in an electric stove using the final temperature of 450°C. The obtained ash was dissolved in 1M nitric acid and made up to the mark to 25 mL.

Determination of Pb and Cd was carried out by graphite furnace atomic absorption spectrometer with Zeeman background correction SpectrAA 220Z of the company Varian (Australia). Zn, Cu, Mn and Fe contents were measured using flame atomic absorption spectrometer Avanta PM (GBC, Australia). The methods were controlled by analyzing the series of standard samples from certified reference material DOLT-3 (dogfish liver, National Research Council of Canada, 11 Ottawa, CA). Recoveries in the range of 90 and 110% were accepted to validate of the analytical procedure for studied elements. The obtained limits of detection were as following: 0.001; 0.001; 0.009; 0.008; 0.009 and 0.068 µg/g for Pb, Cd, Zn, Cu, Mn and Fe respectively.

Results and discussion

The analysis was carried out taking into account several criteria. The first one was the comparison between the coat and mane hair metal content.

A statistically significant difference ($p < 0.05$) was found between the mercury, cadmium and lead content in the tested mane hair from horses. In the case of mercury and cadmium, the difference was about twice as large in the coat than in the mane, whereas for lead more than 4 times greater.

The obtained test results are illustrated by the charts below. The averages for mercury (Fig. 1), cadmium (Fig. 2) and lead (Fig. 3) were calculated from 30 fur samples and 30 mane hair samples.

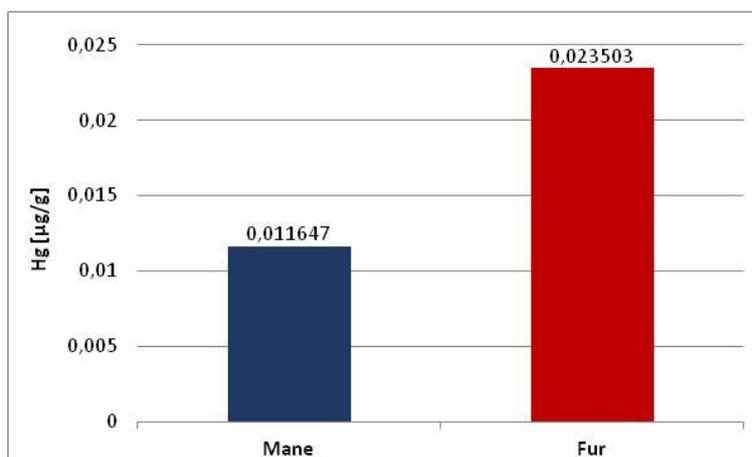


Fig. 1. Average of mercury content in the mane hair and fur of all examined horses
 Source: own calculation

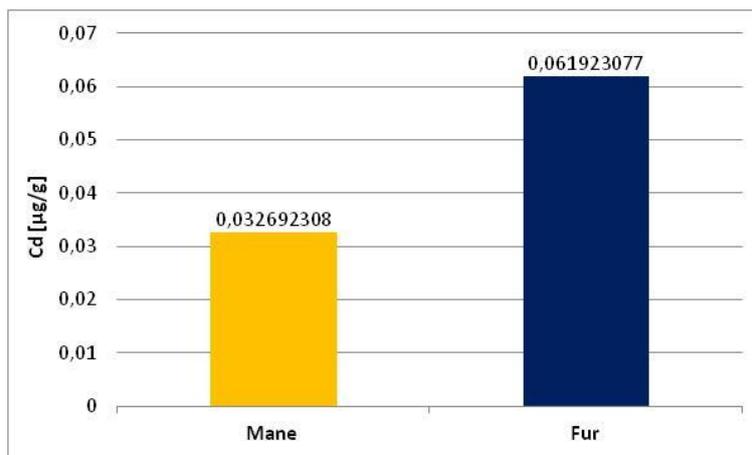


Fig. 2. Average of cadmium content in the mane hair and fur of all examined horses
Source: own calculation

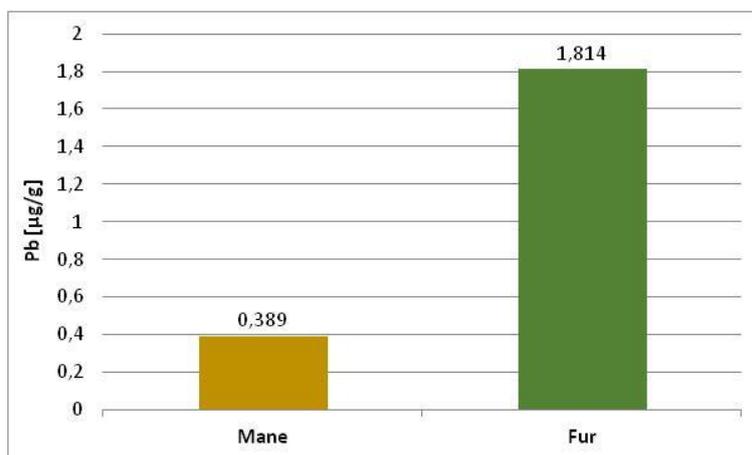


Fig. 3. Average of lead content in the mane hair and fur of all examined horses
Source: own calculation

The differences may result from the fact that the fur is subject to seasonal exchange while the hair is not affected. In relation to the latter, the part of the hair that has been examined also matters because the absorbed metals do not accumulate in a uniform manner all along and it depends on the degree of the body's exposure to a given element in a specific time frame, which, in the case of mane hair, may be different and much longer than for shedding coat.

The next criterion that was taken into consideration was the age of the researched horses.

A statistically significant difference ($p < 0.05$) was found in the content of mercury, cadmium and lead in the hair of foals compared to adult mares.

The average results obtained from 13 foals and 13 mares for mercury (Fig. 4), cadmium (Fig. 5) and lead (Fig. 6) are presented in the charts below.

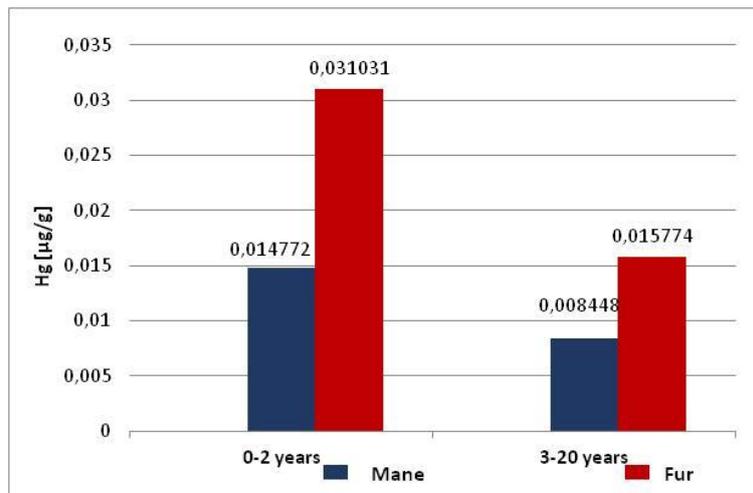


Fig. 4. Mercury content in the horses' mane and fur depending on age
 Source: own calculation

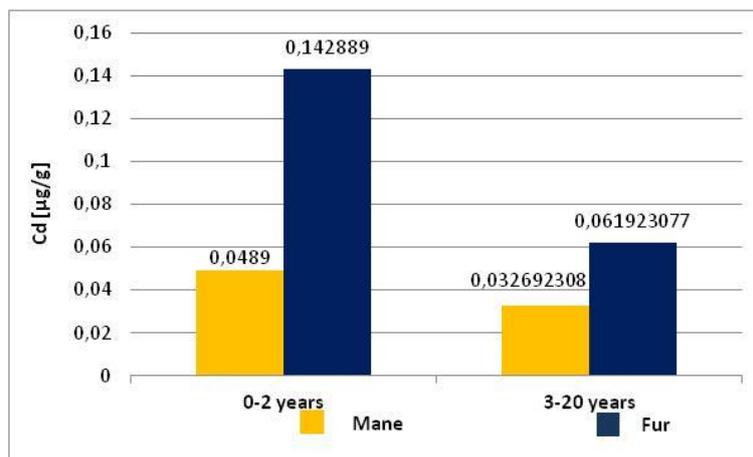


Fig. 5. Cadmium content in the horses' mane and fur depending on age
 Source: own calculation

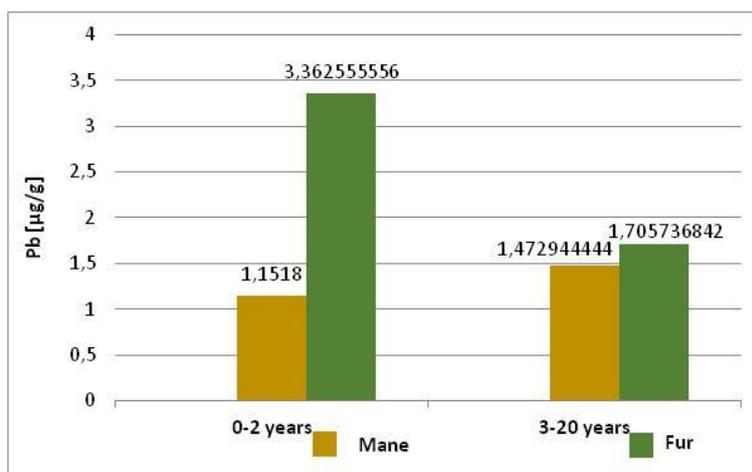


Fig. 6. Lead content in the horses' mane and fur depending on age
Source: own calculation

The juveniles, due to their higher metabolic needs per 1 kg of body weight, may tend to absorb more heavy metals with food. Another aspect that most probably matters is the animals' gender and the hormonal balance associated with it. Only mares within two age group ranges were included in the study as it was assumed that the fillies up to 2 years old did not have a settled oestrus cycle yet. A key role here is played by oestrogens which have the ability to induce a heavy metal binding protein such as metallothionein, which prevents the binding of metals to biologically active proteins and therefore it can be considered as one of the ways to deal with their excess.

The impact of the maintenance systems was analyzed based solely on the results obtained from the adult mares.

The Hg content in the coat and mane of horses from the alcove system was lower compared to horses from the grazing system. The cadmium content in the coat of horses kept in the alcove system was almost 2 times higher compared to horses going out to pasture, and in the case of lead over 4 times higher. No statistically significant differences ($p > 0.05$) were found in the content of cadmium and lead in the mane of the horses tested.

The average results presented in the following charts for mercury (Fig. 7), cadmium (Fig. 8), lead (Fig. 9) were calculated on 8 mares kept in the alcove system (Silesian breed) and 8 mares kept in the pasture system (Małopolska breed).

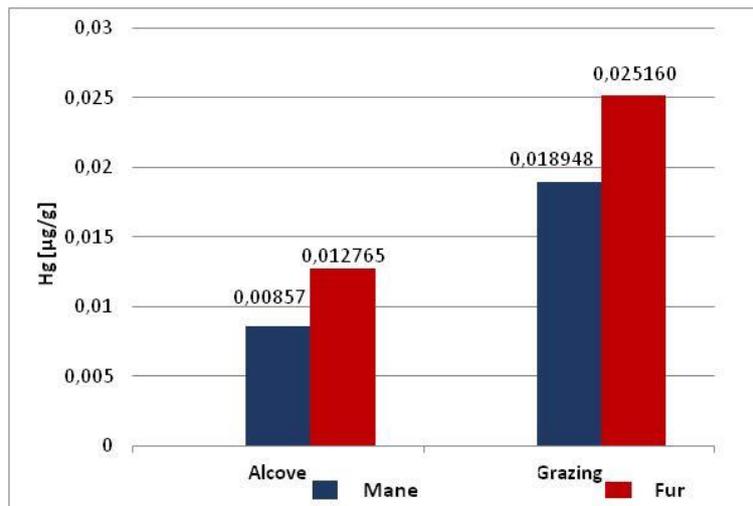


Fig. 7. The impact of the maintenance system on mercury content in adult mares
 Source: own calculation

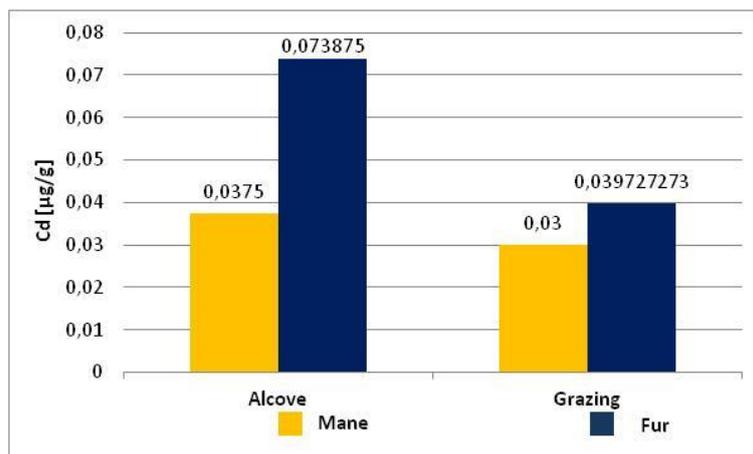


Fig. 8. The impact of the maintenance system on cadmium content in adult mares
 Source: own calculation

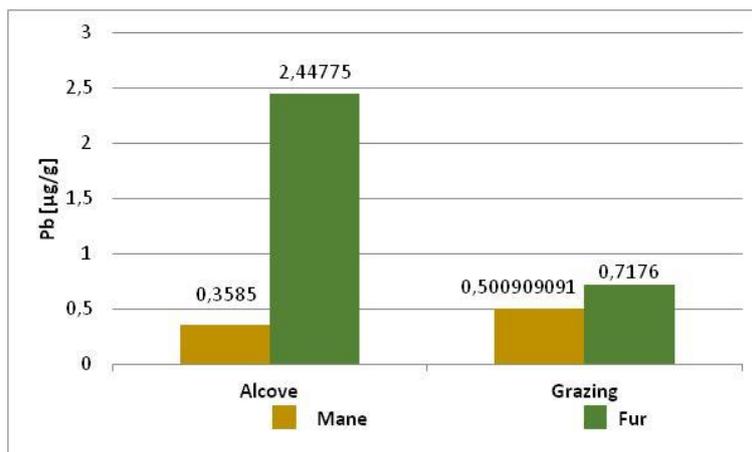


Fig. 9. The impact of the maintenance system on lead content in adult mares
Source: own calculation

The pasture dwelling horses are exposed to a greater deal of various toxic compounds derived from atmospheric air, soil or rainwater. Lead and cadmium belong to a group of chemical elements with a high degree of distribution and are easily dissolved in rainwater, while mercury vapours are very toxic even in small doses and their quantity increases with rising temperatures, hence they reach their maximum in the summer and autumn period. It is worth mentioning that the horses let out to the pasture, received feed from organic farming while the animals reared in the alcove system were fed commercial oats and hay.

The average obtained from measuring the metal content of the feed from three consecutive replications is as follows: commercial hay: Hg 0.014 μg / g, Cd 0.081μg / g; organic hay: Hg 0.007μg / g, Cd 0.055μg / g; commercial oats: Hg 0.002μg / g, Cd 0.104μg / g; organic oats: Hg 0.002μg / g, Cd 0.03μg / g. Pb content in the analyzed samples of hay was below 10 μg/g, which value is define as the natural content of this element in dried grass, originating from unpolluted areas. In turn, the result obtained from the analysis of oats for lead content was below 0.5 μg/g of fresh weight.

Higher cadmium content in the coat and hair of the horses maintained in the alcove system may result from a relatively high amount of this element contained in the commercial feed, which they were given, compared to the horses fed with organic farming feed.

In addition, the horses' breed was taken into account, however, it cannot be explicitly stated that the genetic factor affects the degree of toxic elements accumulation due to breed and upkeep as two overlapping criteria.

In addition, the level of trace metals such as manganese, copper, zinc and iron were also tested. They all play an important role in metabolism therefore both their deficiency as well as their excess adversely affect the functioning of the body. The measurement readings were of an illustrative nature, only referring to whether the studied research group falls within the reference standard and checking the dependence of the absorption abilities of trace metals and those absolutely toxic. The norm's upper limit may result from the supplementation of trace elements since they are added to most commercial feeds.

The table below illustrates the average results for individual elements obtained from the mane hair of 17 tested horses in relation to reference intervals [2].

Tab. 1. Obtained average trace elements compared to reference standards in mane hair

Chemical element	Reference intervals	Results
Mn ($\mu\text{g/g}$)	0.551-12.55	7.53
Cu ($\mu\text{g/g}$)	4.17-6.84	5.18
Zn ($\mu\text{g/g}$)	97.43-167	143.34
Fe ($\mu\text{g/g}$)	10.11-442.2	80.93

Source: own calculation

Summary

Our studies have confirmed that animal hair is a good indicator for assessing environmental exposure to heavy metals. This proves the fact that bodies are equipped with mechanisms that prevent toxic effects of heavy metals. Heavy metals are unevenly distributed in the environment and the degree of their accumulation in the body depends on many variables such as individual factors, age or nutrition. However, taking into account the fact that heavy metals poisoning may have serious effects on bodies, it is worth considering at least a single determination of these elements in animals. Such action can help the breeder make sure that animals are not at risk of excessive exposure to heavy metals. It can also prevent, by leading to introduction of appropriate supplementation, various failures resulting from the deficiency of trace elements which in horses usually give symptoms from the cardiovascular, skeletal, reproductive or cutaneous system.

A useful diagnostic method used increasingly frequently by doctors, veterinarians or crime analysts is the determination of selected heavy metals in hair coat. Many laboratories systematically expand their offer with this type of test as it allows easy collection of material from animals, easy transport and storage of dead tissue, availability of the testing material that is unlimited in time and a relatively high sample reliability as compared to other tissues.

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BOTH SIDES OF THE STORY HOW BACTERIA TRANSFER THROUGH THE BLOOD–BRAIN BARRIER?

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Abstract:

Infections of central nervous system (CNS) still represent an important cause of mortality although globally abundance of antibiotics usage. Sepsis, bacterial invasion and microbial transversal of the Blood–Brain Barrier (BBB) are required to infect CNS. The latest research showed up that bacterial translocation through the BBB includes cytoskeleton rearrangements. Three different mechanism were described: paracellular, transcellular and Trojan-horse mechanism (in phagocytes). The consequences may be dramatic – disruptions of structure and loss of functionality of the BBB causing increased permeability, inflammatory and encephalopathy. Further experimental research should lead us to gain complete understanding of the host-bacteria interaction within microbial transversal of the BBB.

Keywords:

blood–brain barrier, bacterial pathogens, infections, cell junction

The brain-blood barrier

The Blood–Brain Barrier (BBB) is created by the nervous and circulatory systems. It is a structural and functional barrier which performs extremely responsible functions. The BBB regulates molecules' inflow and outflow of the brain (maintaining stable environment) and protects neurons against toxins and pathogens. The vessels inside the brain consist of endothelium and wide range of junctions between cells (especially tight junction). The pinocytosis activity of endothelial cells is considered to be low. Astrocytes and pericytes are located on the outer site of a vessel. They prevent intercellular junction from leak (Fig. 1). This is not all. The structure of the BBB regulates transport of hydrophilic hormones and glucose to the brain. However, on the gases and hydrophobic hormones' transport the limitations are not placed [1].

Tab. 1. Types of cell junctions with details about structure and function

Type of cell junctions	Structure and function	Literature
Tight Junction (TJ)	<ul style="list-style-type: none"> - fine and tight junctions between cells' membranes - occludins and claudins – homodimeric transmembrane proteins; they brings membranes of two cell closer - ZO-1/2 – proteins binding occludins and claudins with actin cytoskeleton 	[2, 3]
Adherens Junction (AJ)	<ul style="list-style-type: none"> - they provide fine intercellular adhesion, although flow of liquid through AJ is not limited - VE-cadherin – homodimeric proteins - α/β-catenin – responsables for binding cadherins with actin cytoskeleton - catenin p120 – regulates cadherin stability and maintains VE-cadherins surface expression 	[4]
Hemidesmosome	<ul style="list-style-type: none"> - provides attachment between the cell and collagenous basement membrane - integrine $\alpha_6\beta_4$ – laminin-332 receptor; binds cell's surface with basement membranes' protein elements - plectin – intermediates in binding transmembrane hemidesmosome's elements with cytoskeleton 	[5]

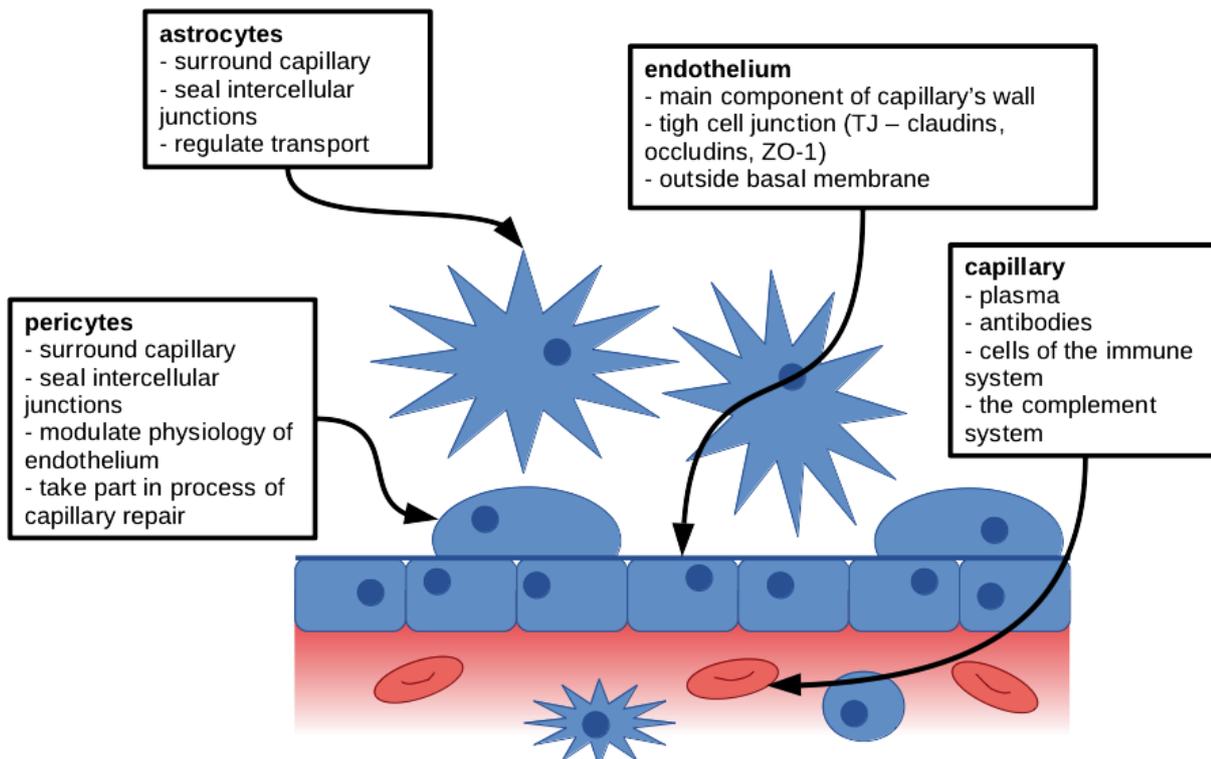


Fig. 1. The Blood–Brain Barrier formed by: endothelial cells, pericytes, and astrocytes

Microbial transversal of the BBB mechanisms

Three different mechanisms of microbial transfer through the BBB were described: paracellular, transcellular and Trojan-horse mechanism (in phagocytes) [6]. For success of paracellular and transcellular mechanisms the most important issue is survival of bacteria in the host’s blood [7]. After sepsis inducing and achievement of threshold bacterial density, pathogens get able to attach inside a brain vessel (because low intensity of this process). In contrast, in Trojan-horse mechanism it is possible to transport bacteria from distal tissues (e.g. small intestine) without sepsis inducing [8].

Tab. 2. Pathogenic bacteria mechanisms of survival inside the host blood

Species	Inside blood survival mechanisms	Literature
<i>Porphyromonas gingivalis</i>	- gingipains – unspecific proteases which degrades complement system elements and extracellular matrix proteins of host’s tissues	[9]
<i>Streptococcus pyogenes</i>	- M protein – block antibodies (binding Fc regions)	[10]
<i>Yersinia enterocolitica</i>	- YadA – unables the complement system to function properly - effectors secreted by T4SS – prevent actin cytoskeleton from rearrangement causing fagocytosis disruptions	[11, 12]
<i>Klebsiella pneumoniae</i>	- polysaccharide layer producing	[13]
<i>Streptococcus pneumoniae</i>	- TatD – DNase enabling NETs degradation (DNA nets connecting with bactericidal activity: LL37, myeloperoxidase, neutrophil elastase)	[14]

Transcellular transversal

Bacteria transverse the BBB indirectly through the endothelial cell. Because of low pinocytosis activity, microbes have to induce it by activating particular receptors. Two types of inducible bacterial internalization may be distinguished: (i) “zipper” – a pathogen adheres to the cell and is slowly surrounded by membrane ruffles of the endothelial cell after particular receptors’ activation [15]; (ii) “trigger” – effectors, which cause endothelial cell’s cytoskeleton rearrangements, are transported indirectly to the cytoplasm through type III secretion system [16]. After successful internalization bacteria has to survive inside the phagosome. The brain-blood barrier is transfer by the phagosome. Endothelial cells are not susceptible to phagocytosis and do not possess any complicated lysosome system. However, the system of NLR proteins (NOD-like receptors) still can be activated. NOD-like receptors play role in immune response against intracellular pathogens [17]. After phagocyte transversion through the BBB, the exocytosis and membrane local disruption may occur [6].

Paracellular transversal

In this case, bacteria induce disruption in intercellular junctions between endothelial cells. Proteases secreted by pathogens as well as boosted immune response activation because of strongly reactive antigens are thought to bring this effect. Some species e.g. *Neisseria meningitidis* activate secretion of host cell' extracellular matrix proteases (MMP-8) [1], what includes dramatic damages of the neural tissue because of leak emergence. An extreme manifestation is complete degradation of the BBB. Bacterial cytolysins lead to disintegration of endothelial cells. The immune system also makes its contribution to pathological aggravation. Proinflammatory cytokines (e.g. TNF- α i IL-17) undermine stability of intercellular junctions (especially TJs) and increase leak through the BBB [18]. All this processes result in proceeding barrier degradation because of a positive feedback mechanism. Within brain vessels activation of the immune system makes the BBB more susceptible to leak. Bacterial replication form a vicious circle and activate secretion of proinflammatory cytokines in further steps of infection [6].

Trojan-horse mechanism

This mechanism is characteristic for bacteria which are able to infect particular type of phagocytes, e.g. macrophages. Pathogens transverse the BBB inside phagocyte which prevent form the immune system activation and provide suitable environment to replicate [19, 20]. Survival in blood is not required. The bacteria may be engulfed by (for instance) a macrophage in different tissues also in regions of primary infection. IL-1 β is one of the cytokines, which induce extravasation (leaving a vessel by a cell of the immune system) [6].

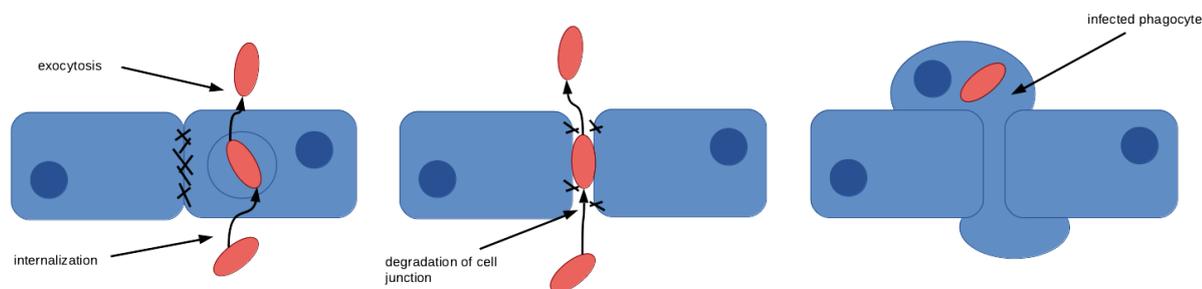


Fig. 2. Three different mechanism transversal the Brain-Blood Barrier were described (sequentially): paracellular, transcellular and Trojan-horse mechanism (in phagocytes)

Examples of bacteria able to transfer through the BBB

Streptococcus pneumoniae

To the present day *Streptococcus pneumoniae* is the main etiological factor in meningitis at the world. The first stage of human infection includes colonization of the nasopharynx. Transmission of the pathogen occurred person to person – via droplets (an airborne pathogen). Human can carry the bacteria without demonstrable symptoms [21]. About 30 per cent of infected persons are estimated to be unaware carriers. From nasopharynx mucous membranes *S. pneumoniae* can be relocated to lungs, tympanum and bloodstream. A well-developed polysaccharide layer

provide protection for bacteria inside blood [22]. Some modifications of a polysaccharide layer occur at the stage of adhesion to the epithelial cells (it pertains to pathogens with a thin or without bacterial capsule). To thick polysaccharide layer enables bacteria to adhere to the surface, because interaction between adhesins and host cell receptors cannot occur. Surface neuraminidase (NanA) and RrgA adhesin belongs to bacterial proteins, which bind to endothelial cells. *S. pneumoniae* produces cytolytic toxin (pneumolysin), which is released as a result of bacteria lysis. Pneumolysin causes indirectly damage of the Brain-Blood Barrier (because of endothelial cell decomposition) [23–25].

Mycobacterium tuberculosis

Extrapulmonary infections of *M. tuberculosis* pertain especially to patients with immunodeficiency – infected with HIV, undernourished and after transplantation. In developing countries children are also susceptible to *M. tuberculosis* infections [26, 27]. This pathogen is highly adapted to persistence inside phagocytes. These cells are thought to be excellent niche inside infected lungs. Bacteria can be transferred inside phagocytes to other host tissues including bones and nervous tissue. The development of infection is relatively slow, what enables microbes to achieve threshold bacterial density in blood necessary to cause sepsis [28]. However, *M. tuberculosis* transverse the BBB using Trojan-horse mechanism, so high bacterial density in blood is not essential [6].

Neisseria meningitidis

Infections of this coccus manifest in life-threatening meningitis. Five main pathogenic serotypes were identified by polysaccharide layer’s chemical composition (called: A, B, C, Y and W135). Vaccines protecting against *N. meningitidis* were based on bacterial capsular antigens. However, a situation is more complicated in case of a serotype B. Surface polysaccharides of *N. meningitidis* serotype B resemble some nervous tissue antigens [29, 30]. The first stage of bacterial invasion of the central nervous system includes tight adhesion to the surface of endothelial cells. A few proteins and structures (type IV pilus, NadA adhesin, Opa and Opc proteins) are responsible for this process. Successful adhesion induce dramatic cytoskeletal rearrangements and decrease in intercellular junction stability [29]. Moreover, *N. meningitidis* activate host cells to secrete unspecified proteases, which start to degrade extracellular matrix elements including adhesins and basement membrane [6].

***Escherichia coli* K1**

Tight adhesion of bacteria to the endothelium is crucial factor the successful invasion to nervous tissue. The main virulence factors at this stage is two elements: type 1 fimbriae and surface protein called OmpA. Fim H (terminal adhesin of the fimbriae type 1) interacts with CD48. OmpA protein has affinity with GlcNAc glycoproteins on the endothelium (e.g. gp96) [31, 32]. Surprisingly, this fact may be used in clinical practice in foreseeable future. Bacterial invasion on Central Nervous System can be blocked in a result of binding oligomers GlcNAc- β 1 and 4-GlcNAc, which are soluble in blood. They interact with OmpA proteins, unable bacteria to adhere to the endothelium and further infection development is impossible. CNF1 and IbeA are additional

proteins which play key role in second stage of the brain invasion. They activate receptors and signaling pathways, what leads to cytoskeleton rearrangement (Fig. 3) [6].

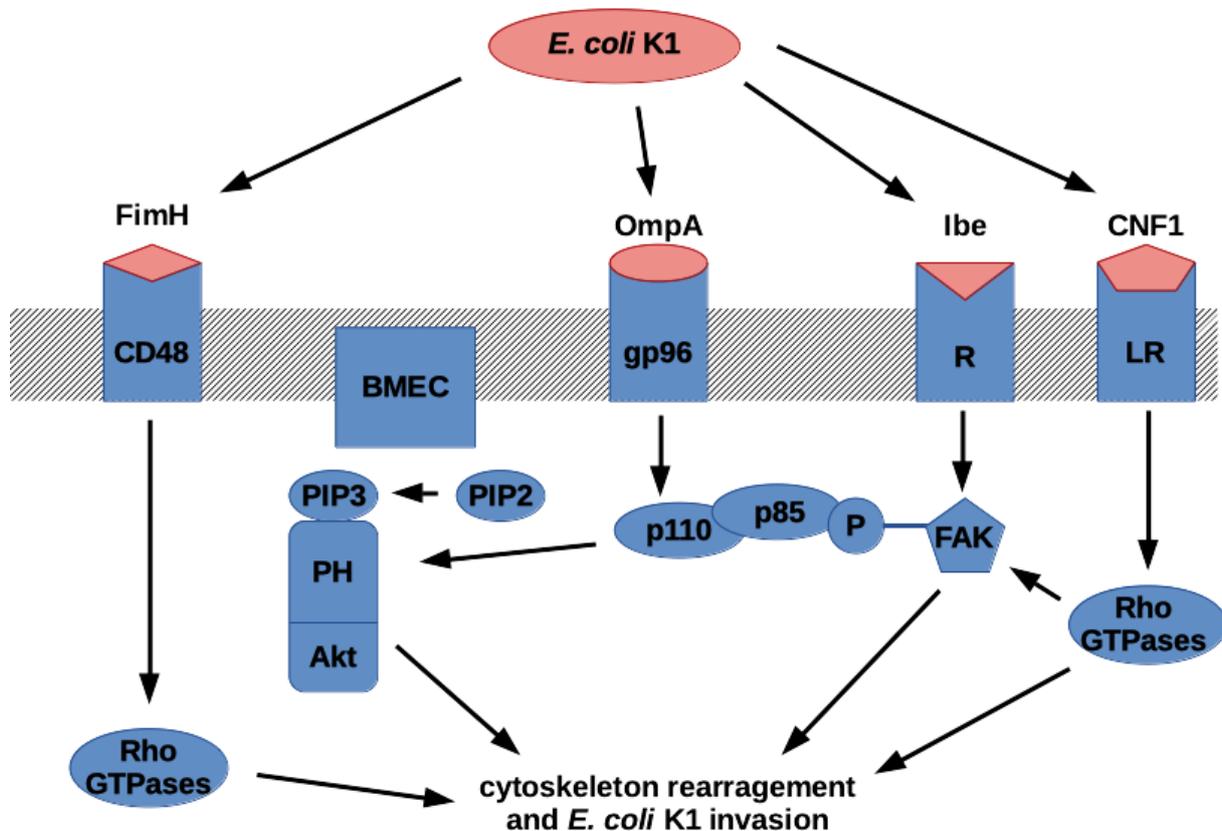


Fig. 3. Signalling mechanisms involved in actin cytoskeleton rearrangements mediated by *Escherichia coli* K1

Listeria monocytogenes

L. monocytogenes Gram-positive pathogen causing gastroenteritis – listeriosis. Patients after chemotherapy treatment and these diagnosed with AIDS are extremely susceptible to get *L. monocytogenes* infection. This pathogen is highly dangerous for pregnant women, because infection may increase risk of miscarriage. *L. monocytogenes* similarly to pathogens mentioned above is also able to cause sepsis and brain infections. The most important molecular hallmark of listeriosis is persistence and replication inside phagocytes and the other host cells. At least 3 hydrolases are necessary for bacteria to leave the early phagosome: listeriolysin O (LLO), cholesterol-dependent cytolysin (CDC) and phospholipase C (PLC) [33, 34]. *In vitro* in stressing conditions *L. monocytogenes* produces intensively membrane vesicles (MVs) containing mainly LLO. Many other compounds were detected inside MVs, e.g. internalin B (InlB), catalase, p60 antigen, Lmo 1028 protein. Moreover, vesicles are able to transverse the Brain-Blood Barrier in regions where intercellular junctions are not leaktight [35]. *L. Monocytogenes* is thought to use three different mechanisms of bacterial translocation through the BBB: (I) paracellular, (II) Trojan-

horse mechanism and (III) retrograde transfer inside cranial nerves axons (mainly vagus nerve) [6, 36, 37].

Conclusions

Microorganisms infecting the Central Nervous System cause serious damages in the brain structure. Effect of this may be more killing than the bacterial infection. Our knowledge about structure and physiology of vessels inside the brain and the brain-blood barrier is not complete. As a result progression of the preventing form infectious diseases of the central nervous system. *In vivo* (on model animals) and *in vitro* research leads us to partial understanding of interaction between pathogen and their host. To develop effective treatment, inhibitors of infection and fast diagnosis tools detailed description of pathogenesis mechanisms is necessary.

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SLASH. THE PRELIMINARY ANALYSIS OF THE MEDIA IMAGE

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Abstract:

Guns N'Roses' Slash is an interesting person to carry out scientific research. He uses social media effectively in order to create his own image while at the same time he stays in frequent contact with fans. He has comic scenic appearance and a very interesting media image, which has changed during last 30 years. He even has his own place in the pop culture, for instance as the main character of the Internet memes. Not everyone knows pseudonym, behind which the guitarists of Guns N' Roses is hiding. It is enough to mention about curly hairs, sunglasses and a top hat and it all explains itself. The aim of this article is to analyze the media and stage image of a legendary guitarist on the basis of theoretical background and examples. Following text was published in Polish in 2017 in a student scientific journal "Consensus" (in a more extensive version), including Slash's Twitter entries, other musicians' opinions about the man himself, charts and my private correspondence with him.

Keywords:

Slash, media image, scenic image, Guns N'Roses, analysis

Introduction

Slash is the leading guitarist to the American hard-rock band Gun's N' Roses, with whom he attained global success in the late 1980s and early 1990s. He has received critical acclaim and is considered one of the greatest guitarists in rock history. When in 1996 he left the Guns N' Roses group, Slash has founded the Velvet Revolver Supergroup, which in the mid to early 2000s re-established him as a legend. He has released four solo albums: *Slash* (2010), featuring an array of guest musicians, *Appocalyptic Love* (2012), *World on Fire* (2014) and *Living the Dream* (2018) with his band: Slash featuring Myles Kennedy and the Conspirators. In 2016, Slash came back to Guns N'Roses. I decided to dedicate this article to the analysis of Slash's scenic, media and social image. For that purpose I needed theoretical considerations on image as well as examples of other musicians who thrown themselves on the cards of history with their appearance.

Music industry

The fame of the artists is inflamed by mass-media, music industry and music charts presented on broadcasting stations. Everyone is recording their songs with the assumption to reach the largest possible number of recipients [2].

Slash, present at music scene since over 30 years, have no mercy in evaluating the industry these days. He claims that the commercialization took place. It is the artists who must adapt, not the industry. In the past days you used to be musician just to be musician, today the most important thing is to step out. Slash believes that it is partly the fault of the Internet. „The record labels still did not find out how to earn on it, while the artists adapt their requirements in order to have any profits and so is everyone cooperating” [3]. In the same interview Slash said that „nowadays too many bands forget about strength of the live sound which is why whole bunch of CD’s is artificial, contrived and sometimes simply boring. Maybe this is where the rock crisis, as you called it, come from. Today, unlike in 90’s, radio stations don’t play guitar music, it is not in the MTV, so kids do not really have the opportunity to listen to the new rock bands. Neither websites have as much meaning as many people think. All of this aspects contribute to the fact that rock is a niche. It was replaced by this, what is promoted by media: pop, r’n’b, etc” [4].

It should be remember that „Guns” started their carrier before the Internet period. If the bands wanted to break through then it had to have talent and good managers. Music industry was controlled by record labels and the artists had to play thousands of free concert, hoping that someone from the industry will appear on one of the concert and offer them conclusion of the contract. The fees for recording the CD’s had to be returned, in addition young musicians were often forced to waive copyrights [5].

In case of Slash and his colleagues from the band, the path to musical career began, according to what I wrote above, with free concerts and handing out leaflets. In February 1986 Axl Rose and Slash’s friend - Vicky Hamilton - spent 25 thousand dollars to purchase the clothes and music equipment for the members of Guns N’Roses. Rose and Izzy Stradlin promoted their band by writing letters to important personalities of the music industry. „In February 1986” – as Steven Davis writes – „the veterans of 60’s managed large record companies in Los Angeles were made aware by their own spoiled teenagers, that they will total nerds if they don’t sign a contract with Guns N’Roses. Suddenly the employers began to threaten their employees that their job depends on the whether they will manage to get signatures of Guns on dishonest, even offensive record contracts” [6].

Guns N’Roses was found at this time and they initially played glam rock. „Metallica managed to get through and they do what they want all the time – stayed as they were. We are yet another band which works this way and after us there will be another – Slash said at the beginning of his career. – One day I saw a bunch of kids who in future will be even more tough and hard-minded than anyone expected. Many people will be surprised then. We really try not to change the world. We only want to make people aware of what is going on around them” [6].

One of GN’R lawyers – Jeff Fenster – said later that „at that time there were 5 cool bands working in Los Angeles - Guns N’Roses, Faster Pussycat, L.A. Guns, Poison and Jet Boy. Poison had a record deal but they were like a “joke”. But I remember that one night in Whisky I saw bands’

battle – there were maybe 5 bands altogether. While Axl stood out like that, he was like the leader of the pack. He looked like a bad boy, but on the other side like someone very sensitive, he moved in a very feminine way. He was the embodiment of L.A. scene of that time. Apart from him there was Slash, presented as the rock’n’roll archetype, but also the best guitarist in town” [6].

„As for specific hard rock – says Slash – Aerosmith came back with *Permanent Vacation*. But besides *Rag Doll* and *Dude (Looks Like a Lady)* most of songs played in the radio were miserable. We had *Here I Go Again* performed by Whitesnake, *Alone Heart*, *Once Bitten* of Great White and cover of *Mony Mony* in Billy Idol’s version. We were somewhere between those worlds there: neither Guns N’Roses nor *Appetite For Destruction* fitted anywhere in the music landscape of 1987” [7].

The rock music scene of the end of 80’s needed such revelation like „Guns”. There was no energy in American music anymore, everything seemed plastic and predictable and hard rock music began to eat its own tail. Steven Davis in the book *Watch You Bleed. The Saga of Guns N’Roses* emphasize that „Guns N’Roses had to cope with strong competition. Motley Crue still ruled at strip and Poison won a record deal and started to prepare album. New bands such as Jet Boy, Faster Pussycat or Shark Island were pushing and shoving at the scene on the same time when Guns N’Roses fought for their own place – everyone competed for place and concerts in the same clubs. There was even a nagging question within the music industry whether a hard rock band, no matter how talented, could survive in a climate where the Big Four of Thrash - Metallica, Megadeth, Antrax and Slayer – were among the biggest” [6].

Guns N’Roses redefined rock world. They were rebellious and vulgar. At the same time they were mainstream and they despised it. They were like the breath of fresh air. There were no dungeons, dragons, fairytale love in their songs. Instead there were prostitutes, porn stars and thieves. They stepped out in the period when most of the bands repeated the same image, compositions, producers and video clips [8].

Few years ago Slash said that Guns N’Roses were „genetic freaks”. They took pattern after hard rock, they wanted to play hard and strong. They were young, they adapted to the prevailing style, but the spirit of 80’s was completely out of them. The members of musical environment were close to each other, today it is not the same anymore [4].

Slash also put attention to the fact that „what happened in the rock music at the beginning of 90’s may be compared only to the musical boom of the middle 60’s and there is no exaggeration. Thanks to guys like Kurt Cobain or Eddie Vedder rock had very charismatic faces, they were musicians who had something to say and they got into heads of teenagers. Today I’m saying with full awareness: rock world is devoid of face, personalities like Cobain or Mike Patton of Faith No More” [4].

Slash. The preliminary analysis of the media image

(Pop) culture visibility

I would like to start this chapter with considerations on definition of „image”, which is needed for the subsequent part of this work. The theoretic analysis of this category will help to verify examples of this form as stage „image” between the musicians, Slash in particular, and image, as

the form of self-presentation used by musicians of Guns N’Roses and Slash via press and/or Internet.

Visual culture is significantly important today. These “are aspects of the social world seen generally as a whole, objectified in part in the form of pictures and principles defining their production, analysis, interpretation and assessment”. It includes drawings, paintings, images of people and surroundings of civilization. What may be seen via visual culture are images put by the creator intentionally in some kind of medium, the purpose of which is to: transmit a message, send some content, make impression or influence the audience [9].

Before moving on to any considerations regarding image of Slash it is necessary to get familiar with the explanation of image, defined in literature already multiple times. There are other terms used instead: reputation, figure, picture, repute. This notion is defined as reception of particular firm, entity by different recipients, built on the basis of its overall activities, which may be the name, logo, undertaken activities, expressed identity or recognized values. It is usual that the image is created in the mind of individual recipient [10].

In this part of thesis media image will be the most important aspect for me. It is “the way of perceiving and picturing the organization or entity created in mass media”. Within this context some people are said to be more or less media, *loved by the camera* or that they cannot behave in easy-going way or do not distance themselves. Therefore those who have media personality “can present themselves in public in positive way, attract attention and arouse interest with their messages. At the same time they stay natural and easy-going. The most important elements of positive media image is enthusiasm and passion (manifesting itself in dynamic behavior) as well as charisma”[11.]

Communities and new media – building presence and interactions

Today media are perfect way to send information to wide audience, especially if Internet or new media are used for that purpose. It may be characterized by interactivity (ability to communicate between two or more people, not limited with time, under users’ control). Most frequently they are referred to as interactive or digital media [12].

Between new medias and their users there is an interaction which is diversified by the environment: media, political and cultural. Internet user may create own communicational environment, easily contact with other users who have identical interests. In new medias, the senders and recipients of the message have equal rights. From the critical perspective, the activity of Internet users is interpreted in following way: “users are systematically used by media enterprises, private activity of users is integrated with operational business processes, users are constant source of creating added value, consumable activity of users in private sphere is subject to new economization, users become employees when they use the resources of media enterprises” [13].

Activity in the Internet may be based on openness, partnership, sharing the resources and activities on global scale, exchanging ideas and solutions to the problems, wisdoms of the crowd (assumption: group is smarter than the smartest person in group), creating cultural and informational contents [14].

Within the meaning of image creation it is worth to mention about the relation student-master, visible in social networks in particular.

Master is present both in pop and high culture. In case of the first one, we deal with master – idol. According to the literature definition: a will to imitate, adopt idol’s style of being, clothing, living, acquisition of certain behaviors and habits. Idol lives as long as his followers want it - by sustaining the worship of their favorite by: photos, biographies, fan wars. Idol provides the knowledge about the world with his creativity, but also with his private life. There is a certain type of trust and partnership relation [15].

Slash on the Internet

The musician has many profiles in social networks. He may be observed on Facebook, Instagram, Twitter and, unknown in Poland, WhoSay.com. He also has his own channel on YouTube. What is worth nothing, he manages all accounts on his own and he is very open for interaction with fans. Below I will analyze Slash’s activity in the Internet.

When Slash created his accounts on Facebook and Twitter ten years ago, he was totally different man. I will remind you that in 2009 the musician went on rehab. So until 2010 we may find relations from numerous parties.

During first two years of existence in the Web, Slash got into interactions with fans more frequently, by answering on comments, asking questions or encouraging for discussion. He also shared his private life more often, describing for example what movie he is watching, what his wife currently does, what he plays with children. Famous emoticon iii|’ :) pictures smiling Slash in top hat, while frequently appearing „RNFNR(FR)” means: „Rock aNd FuckiNg Roll(For eveR)!”

What hasn’t changed until today are his feelings after concert tours, pictures from rehearsals, recording sessions, wishes on different occasions like his friends’ birthday. During the premier of *Nothing Left to Fear* and after watching first season of *The Walking Dead* Slash started to publish on his official profiles pictures from horrors or porno horrors and so he does until today – since last year even more often. It is standard now that Slash congratulates his fans on anniversaries of starting websites related to his person. When observing Slash profiles one can observe that he also has sense of humor. When in 2009 in the episode *Is Slash Real?* he was one of the *South Park* character. Afterwards he wrote on his Facebook: “I am... not real”.

He was also happy to learn about producing *Angry Birds* mascots with long hair and top hat. Another thing are typos in his posts. Thanks to them he even got pseudonym: „Mr. Typo”.

Slash like to posting memes [16], also about himself. Looking through them, I observed different meme categories:

1. Conflict Axl Rose – Slash and reunion Guns N’Roses – today a little bit outdated: *Slash mentions Axl once, everyone loses their minds!, I’ll just wait here until Slash and Axl reunite*
2. Concerning legendary video clip of *November Rain: Fuck your wedding, Axl, I’m gonna make an epic solo, I do not require electricity to wall*
3. Laughing of Slash’s social networks posts: *Confirm Facebook Account Deactivation: I have another Facebook account, My account was hacked, Slash is posting weird shit again, Where’s that fucker with: Maa, Slash is posting weird shit again?*
4. Expressing general sympathy towards Slash: *I never trust a man who doesn’t like Slash, We are what we eat. Slash must have eaten a fucking legend*

5. Memes with his pseudonym linguistic plays: „Slash” which means” „fast”, „separatix”, „scratch”: *Woke up this morning and someone Slashed my tire*, two pictures of Slash preceding „http:”
6. Memes relating to his scenic image: his picture from 80’s with face covered with hair and tag: *I know that my head is in here somewhere*, picture of the guitar and top hat with comment: *There are things that will always assigned to one person*
7. Memes sacralizing Slash (one of my favourites): Jesus with *face palm* saying: *I gave them Slash and they still listen to other guitarists, And on the 8th day, God created Slash, Jesus may have walked on water but did he do it playing a guitar solo?*
8. Memes commenting current situation – when the information regarding official performance of Guns N’Roses on Coachella Festival was confirmed there appeared memes relating to scene in ‘*Star Wars*’, when Lord Vader tells Luke that he is his father.
9. Memes regarding Slash are created by fans. Each person creating meme knows the topic it concerns. Their correct interpretation is dependent on the recipient – if we don’t know linguistic games we wouldn’t laugh at meme concerning slashing the tire; if we don’t know ‘*Star Wars*’ we would not understand the meaning of picture *Come with me to Coachella*, or in *Slash killed that Solo*.

Conclusion

For over thirty years, Slash is active on world’s music scene. He sold one hundred million CDs with Guns N’Roses. He is guitarist with titles (e.g. Riff Lord of Metal Hammer magazine, best guitarist in the world according to Esquire magazine). He also has his own character in computer game - Guitar Hero III: Legends of Rock, he is talented drawer (he designed logo of Guns N’Roses and cover to his first solo album), from time to time he produces his own films or plays in them. As a musician he reached a peak of popularity. – “He is the last one from the great guitarists – writes about him ‘Team Rock’. - He provides everything in package: appearance, behavior, great songs and sound of guitar. He doesn’t have to smash anything with his play. His solos can be played, but never the same as Slash. Sometimes he plays hard, sometimes sweet, but it always influences recipient”.

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THE ROLE AND IMPORTANCE OF SELECTED TLR'S IN THE DEVELOPMENT OF BRAIN GLIOMA

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Abstract:

Cancer is one of the major challenges facing modern medicine today. One of the cancers that has an extremely short survival time since diagnosis is a brain tumor called glioma. Research and therapies focus on radio-, chemotherapy and surgical procedures for removing changed tissues. To increase patient prognosis, scientists began to focus their attention on the tumor microenvironment in which toll-like receptors (TLRs) contribute. TLR receptors are involved in the process of initiating the synthesis of numerous pro-inflammatory factors. They are also an important factor affecting the tumor microenvironment, and thus its development. Literature data suggest that they may be an important therapeutic goal enabling treatment and increasing the prognosis of patients with glioma. This review of the literature focuses on the role of the three TLR2, TLR4 and TLR9 receptors in the tumor formation process and their role in the development of glioma.

Keywords:

glioma, innate immunity, toll-like receptor, TLR2, TLR4, TLR9

Introduction

Glioblastoma is the most common primary malignant brain tumor in adult patients. The prognosis of the sick is extremely low, because the median survival is only about 14 months, with standard treatment, which is surgical removal of so-called burrs, external beam radiotherapy with simultaneous temozolomide (TMZ) chemotherapy and TMZ adjuvant chemotherapy. In recent years, numerous clinical studies focusing on studies of targeted therapies for this cancer have not shown a significant increase in patients' lifespan. That is why it is so important to look for new innovative treatment methods. Recent reports focus on modulating strategies of immune responses to cancer cells and the surrounding tumor microenvironment. The recognition and elimination of malignant cells through immune surveillance over tumor associated antigens (TAA) is a key function of the immune system. TAA's typically represent peptides that are present in cancer cells but not found in surrounding normal tissue. In glioma, these antigens are most often divided into three main classes: (I) abnormally expressed non-mutated self antigens, (II) mutated self antigens, and (III) unique antigens or neo-antigens (new peptide sequences that are the result of somatic

mutations in the cancer genome) [1]. In glioma, there are also typical stem cells that play an important role in growth, invasion, angiogenesis and the avoidance of an immune response. They are also identified as the main cellular unit for conferring chemo- and radio-resistance, which is why they have become a new therapeutic target. A functional feature of glioblastoma cells (GSC) is their ability to form neurospheres in the culture medium (containing B27 supplement) as well as growth factors such as Epidermal Growth Factor (EGF) and primary fibroblast growth factor (EGF) bFGF, basic Fibroblast Growth Factor). These factors affect the development of cells responsible for the production of inflammatory mediators. Inflammatory mediators and inflammatory cells are essential components of the tumor microenvironment. One of the most involved in the above processes and changes in the brain tumor microenvironment are microglia cells, which are responsible for the immune response in brain tissue [2].

Microglia and TLRs

Microglia is a type of tissue macrophage of myeloid origin, which performs brain-specific immune surveillance, aimed at maintaining homeostasis in the brain's microenvironment. These glial cells strongly infiltrate gliomas and can constitute up to 30% of the normal population of infiltrating cells forming the so-called foundations. Local microglia density can be up to 20 times higher in glioma than in normal brain tissue [3]. On their surface, microglia cells have specific receptors responsible for recognizing typical molecular patterns that trigger an innate immune response. These receptors are Toll-like receptors (TLRs). They belong to the family of transmembrane receptors that act as essential elements of cellular defense. TLRs are Pattern Recognition Receptors (PRRs), which include Damage Associated Molecular Patterns (DAMPs) and Pathogen Associated Molecular Patterns (PAMPs) [4]. Microglia is thought to be the predominant type of cell expressing TLR in the normal central nervous system (CNS) as well as in glioma tissue. TLR2, TLR3, TLR4 and TLR9 receptors have also been shown to be strongly expressed on both human microglia cells in normal brain parenchyma and tumor infiltrating microglia. However, despite similar levels in TLR expression, the function of infiltrating microglia is highly dependent on the tumor microenvironment [3]. In addition, microglia / brain macrophages freshly isolated from human glioma tissue showing high levels of TLR2, TLR3, and TLR4, which correlates with the secretion of interleukin 6 (IL-6), which is the cytokine secreted after TLR activation, and its expression correlates with the invasion of glioma.

TLR 2 receptor in glioma

TLRs play an important role in regulating microglia activity during the tumor formation process. Studies indicate that TLR2 expression levels are elevated in glioma biopsies and inversely correlate with patient survival. In the case of TLR2, its expression in microglia is necessary for the activation of Membrane-Type 1 Matrix Metalloproteinase (MT1-MMP) by neighboring glioma cells, and the process is dependent on MyD88. MT1-MMP is a molecule in microglia that controls the response to certain growth factors in angiogenesis, while the involvement of this molecule is one way in which microglia positively contributes to the growth of glioma [5, 6] Based on these

results and the fact that this molecule is highly expressed in human gliomas, TLR2 has been studied as a potential biomarker and its expression has been found to be inversely correlated with patient survival [7, 8]. Increased TLR2 expression was found (by molecular methods) in the U87 human glioma cell line, and was then associated with a positive effect on tumor cell proliferation. It was also found that cells treated with the TLR2-peptidoglycan (PGN) TLR2 agonist initiated signaling by activating NF- κ B (nuclear factor kappa-light-chain-enhancer of activated B cells), which eventually led to an increase in tumor cell growth [3]. Another situation in which the TLR2 receptor is involved is studies in which it has been shown that its action reduces the expression of MHC class II molecules in microglia in an orthotopic model of mouse glioma. TLR2-induced microglia impairment hindered the proliferation and activation of CD4⁺ T cells, which facilitated the avoidance of the immune response by glioma. TLR2-induced downregulation of MHC class II molecules was caused by suppression of the main transcription regulator of MHC class II molecules (CIITA). Activation of TLR2 induced further MAPK (mitogen-activated protein kinases) / ERK1 β 2 (Extracellular signal-regulated kinase) signaling and loss of histone H3 acetylation on CIITA promoters, which in turn inhibited its expression. In glioma tissues, various endogenous TLR2 ligands, including heat shock proteins, which are endogenous ligands of this receptor, were upregulated and the immune response correlated with inhibition of CIITA. Thus, TLR2 supports the avoidance of the immune system by glioma. In a tumor microenvironment, activation of TLR2 microglia induces a decrease in MHC class II microglia expression. In contrast, the change and deregulation of MHC class II expression limits T-cell dependent tumor resistance [9].

TLR 4 receptor in glioma

Literature data estimate that TLR4 protein expression in primary biopsies from patients with glioma is increased relative to adjacent non-cancerous tissue. In addition, there have been reports of in vitro studies that investigated various tumor-promoting activities where an increased amount of TLR4 receptor was observed when the cells were exposed to a TLR4 agonist lipopolysaccharide (LPS). Expression of RNA or TLR4 protein was detected in glioblastoma cell lines U118, U87, A172 and [10,11]. For U118 and U87 cell lines, cell proliferation and invasiveness for U87 cell lines were enhanced. In addition, the expression of the metalloproteinase, MMP-9, which is necessary for increased cell invasion, was elevated in the U87 line in response to stimulation by LPS. Signal transduction via TLR4 has been found to be involved in mechanisms regulating cell survival, migration and avoidance of resistance as well as resistance to treatment directed against TNF- α (tumor necrosis factor - α). In each of the aforementioned cases, TLR4 was either directly involved in these processes or influenced their course [3]. Another way to involve the TLR4 receptor (which has increased tumor invasion) may be a mechanism that involves binding extracellular heat shock protein 90 (HSP90) to TLR4. HSP90 is the main protein produced in response to cancer cell stress and occurs at elevated extracellular levels in the cancer environment. In this mechanism, HSP90 binds to TLR4, which leads to EGFR activation and an increase in intracellular calcium levels necessary to promote tumor cell migration [3, 12].

The release of specific cytokines that protect cells against apoptosis or lead to the total avoidance of the detection of cancer cells by the immune system may also result from the expression of TLR4 on

glioblastoma cells. The resistance of gliomas to apoptosis, induced in one scenario by TNF- α , is probably due to the release of IL-6, IL-8 and MCP-1 (Monocyte chemoattractant protein 1) cytokines. In fact, responses to TNF- α and IL-8 treatment are associated with hypoxia-induced glioma progression, and MCP-1 is responsible for microglial activity to promote tumor development. In cancer cells, TLR4 is activated by TNF- α , via the interferon- β -mediated pathway (TRIF, TIR-domain-containing adapter-inducing interferon- β) containing the TIR domain (Toll / interleukin-1 receptor), creating a TNF- α feedback loop that triggers the release of these cytokines [12].

TLR 9 receptor in glioma

TLR9, which is a member of the TLR family, is an evolutionarily well-preserved transmembrane protein that recognizes molecular patterns derived from microbes. TLR9 is found in the endoplasmic reticulum, and its activation causes an increase in the production of inflammatory mediators. In addition to expression in plasmacytoid dendritic (DC) cells and B lymphocytes, TLR9 is also expressed in breast, stomach, lung and prostate cancer cells. Literature data indicate that CpG ODN (oligodeoxynucleotides CpG), which are TLR9 agonists, may promote tumor development and metastasis. Researchers suggest that the TLR9 signaling pathway may also be important in the development and progression of glioma [13]. Clinically increased TLR9 expression was associated with higher tumor development and worse patient prognosis. Studies have also shown that TLR9 mediates the properties of increased invasion and proliferation of cancer cells. The TLR9 agonist CpG dinucleotide has also been shown to enhance cell invasion in vitro via TLR9. In addition, there is a correlation between TLR9 and MMP2 and MMP9 metalloproteinases in clinical samples. Activated TLR9 also promoted the growth of glioma stem cells by activating STAT3 signaling (Signal Transducer and Activator of Transcription 3) in vitro, which is a pathway involved in numerous cancer promoting activities. In addition, CpG increases the level of nitric oxide (NO) in U87 cells via TLR9, thereby enhancing the sensitivity to cell radiation in vitro [3]. The TLR9 signaling pathway seems to be an important aspect of glioma development, but further research is needed to determine the pathophysiological role of TLR9, which may in the future be a useful prognostic biomarker [14].

The importance of TLR in the treatment of glioma

TLR signaling activates innate immunity and helps shape adaptive immunity. Therefore, TLR ligands may be relevant when used in immunotherapy and are primarily used as adjuvants for specific triggering of humoral and / or cellular responses. They can also increase the immune response to some weakly antigenic targets. The number of trials involving TLR ligands as adjuvants (64%) is twice as high as when TLR ligands are treated as drugs (35%). This highlights the role of TLR in immunotherapy in various diseases and their potential use or further research into immunomodulatory therapy. In addition, TLR activation may also alter other signaling pathways and it is desirable to target them for better treatment efficacy. There are TLR ligands approved by the Food and Drug Administration, such as MPLA80 (TLR4 agonist) and imiquimod81 (TLR7

agonist), which can act as an adjuvant or drug [14]. TLR agonists have been identified as therapeutic targets for the treatment of various cancers. Many synthetic ligands are being tested for use in immunotherapy. ODNs are the most commonly used TLR agonists in therapy; are potent activators of both innate and adaptive immunity, and are therefore capable of inducing cytokine production and activating NK, dendritic cells, monocytes and anti-tumor T cell responses [15]. TLRs mediated T cell responses and programmed cell death against the tumor environment. After TLR binding to PAMP or DAMP, pro-inflammatory response and other programmed cell death are induced. On the one hand, various antigen presenting cells (APCs) are activated and programmed maturation is initiated to induce APC migration into the lymph node. Various TLRs are activated by MYD88 and TRIF to induce a further pathway, leading to phosphorylation of NF- κ B or IRF and consequent transcription of various genes encoding the pro-inflammatory cytokine. In addition, virgin T cells in the lymph node are activated. Differentiated T cells migrate through the blood to the tissue or tumor environment and are further activated by another factor (Fig. 1). On the other hand, programmed cell death associated with the tumor gene is triggered by activation of the TLR signaling pathway. Apoptosis, autophagy and necrosis are independent, but have some connections with each other that have the same signaling pathways or a lower-acting adapter to induce cancer cell death [16].

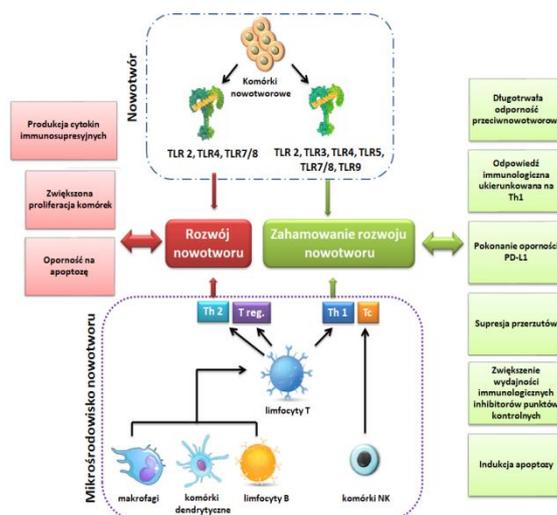


Fig. 1. The effect of TLRs on tumor development as well as inhibition of tumor cell proliferation (based on [17])

Conclusion

The response induced by activation of TLRs leads to anti-cancer or promoting proliferation and development of cancer cells. Factors determining the type of response depend on the agonists used, type of cancer, TLR expression level and tumor microenvironment. The molecular mechanisms by which TLRs modulate tumor initiation, development and progression are not fully understood, but evidence suggests their involvement in processes such as apoptosis, angiogenesis and tumor cell proliferation. It has been suggested that the treatment of some cancers by combining TLR agonists with radiation and chemotherapy is effective. It can be considered so that the effect of TLR signaling and microglia are tools that can be manipulated for therapeutic purposes, especially in

light of the fact that these cells represent a significant part of human gliomas. Although the involvement of the TLR3 and TLR4 pathways alone may not be sufficient to eliminate tumors, targeting these receptors can be considered a complement to therapy until the mechanisms of microglia glioma cell suppression are better understood. The therapeutic use of TLR agonists in the treatment of CNS tumors is a challenge for modern science. The research should be aimed at understanding the immunobiology of various CNS-derived malignancies and determining the efficacy and safety of immunotherapy based on TLR activation, which leads to the establishment of therapeutic alternatives in cancer treatment [15,18].

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MG-BASED MATERIALS FOR HYDROGEN STORAGE APPLICATION – PROBLEMS

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Abstract:

Magnesium hydride has for a long time appeared to be one of the best candidates for the solid state hydrogen storage material. It is mainly associated with its wide availability, low costs, fairly good capacity and reversibility of the absorption and desorption process. However, Mg-based materials also have properties that can be a barrier to their commercial use. High enthalpy of decomposition requires the use of high temperatures and very low hydrogenation kinetics are serious problems for large-scale implementation of this material. This problem will be discussed in this work.

Keywords:

Mg-based materials, hydrogen storage, solid-state hydrogen storage, magnesium hydride

Introduction

The demand for energy is still growing and currently the main energy supply is based on sources of fossil fuels, which are limited. An additional problem is CO₂ emissions and the burning of these fuels, which contributes to global warming. There are alternative energy sources, however, each of them needs a carrier in which it can be stored. Hydrogen is a promising energy carrier. It is the lightest element and offers the highest energy to weight ratio of any fuel. It is also the most numerous element. However, it has several disadvantages, mainly that it is a very light gas with very low density and it is difficult to store it in the amount required for commercial application so that it does not take up too much space. That is why finding a way of to develop a safe and cost-effective method of storing it is a key problem in starting the hydrogen economy [1].

Storing hydrogen in the form of compressed gas is a fairly common method. However, in order to achieve the highest density of stored hydrogen, it is necessary to maintain high pressures, however, to obtain high pressure of the tank it is necessary to use very high energy, the safety theme is also important - hydrogen can penetrate into the material of the tank and lead to the brittleness, so it is important to choose a suitable material [2, 3].

Another way of storing hydrogen is to convert it to a liquid form by liquefaction, but the problem with this method is the need to use very low temperatures as the boiling point of hydrogen is 20.3 K (-252.88°C) and its critical temperature, i.e. above which it can only exist as a gas, it is

32.9 K (-240.25 °C). In addition, the storage of hydrogen in this way requires the use of effective insulation, so as to maintain the temperature and reduce losses associated with evaporation [4-7].

However storing hydrogen in a solid state has become a very attractive research direction, because this type of storage provides a much higher density of hydrogen in a small volume than in the case of storage in tanks storing hydrogen in the gas phase under pressure or storing hydrogen in cryogenic tanks in the liquid state [8].

Magnesium hydride

Magnesium is one of the most attractive mediums that can be used to store hydrogen, mainly due to its low cost and high hydrogen storage capacity (7.6 wt. %). Comparison of hydrogen storage options for magnesium to other pure hydrogen gas and liquid hydrogen as well as to other hydrides is given in Tab. 1.

Tab. 1. Properties of Some Representative Hydrogen Storage Systems

Storage Media	Hydrogen Storage Capacity	
	By weight (%)	By volume (g/mL)
Gaseous hydrogen	100	0.008
Liquid hydrogen	100	0.070
MgH ₂	7.6	0.101
Mg ₂ NiH ₄	3.3	0.081
VH ₂	3.8	0.095
FeTiH ₂	1.9	0.960
LaNi ₅ H ₆	1.4	0.089

Source: [9]

Hydride created on the basis of magnesium may be used as a semiconductor material for hydrogen storage in cars with a fuel cell, it can be used for heat storage or to compensate for irregular heat supplies and electric energy for fuel cells in various energy sectors [10, 11]. However, there are a number of problems associated with the commercial use of magnesium.

Problems

The first problem that needs to be overcome if magnesium hydride is considered as a material for hydrogen storage is kinetics. Kinetics determine the rate of reaction for the formation and decomposition of hydrogen absorption and desorption kinetics, at a given temperature. This is quite an important parameter affecting the rate of hydrogen replenishment as well as the output power.

For magnesium-based alloys, the reaction is very slow and in case of pure magnesium it requires a temperature about 350-400°C. However, the process takes many hours. The equilibrium pressure is not too high and is 1 bar at 280°C, therefore from a thermodynamic point of view the

hydride should easily form at room temperature but, this never happens because of kinetic limits. There are several factors that affect such a low reaction kinetics [12].

First is oxidation of magnesium surface by exposing the magnesium surface to air, magnesium oxide is very easily formed the presence of an oxide film hinders the penetration of hydrogen molecules into the magnesium particle. To start the absorption of hydrogen it is necessary to pass through the oxide layer, then the speed depends on the rate of growth of the surface hydride phase. The way to get through the oxide film is to break it. This layer on the magnesium surface breaks when the annealing temperature exceeds 400°C, however annealing at a temperature higher than 350°C causes the decomposition of magnesium hydride. That is why it is important to use activation processes, which usually consist of continuous work at high temperatures in a vacuum and under hydrogen pressure. Another method that reduces the need for activation is ball milling of pure magnesium powder, which leads to breaking oxide layers and discovering new magnesium surface layers [12-14].

Second factor with affect to kinetics reactions are particle size and their surface. After defeating the oxygen layer formed on the surface, a continuous layer of hydride is formed, the hydrogenation rate drops significantly and with an oxide film thickness is more than 30-50 μm, the reaction stops. This process will take place differently when we reduce the size of the particles. By reducing the size of particles, increase in the surface to volume ratio and a reduction in the diffusion path occurs. For example, nanoparticles can be used to increase the reaction kinetics, the absorption properties also change as shown in Fig. 1 [14, 15].

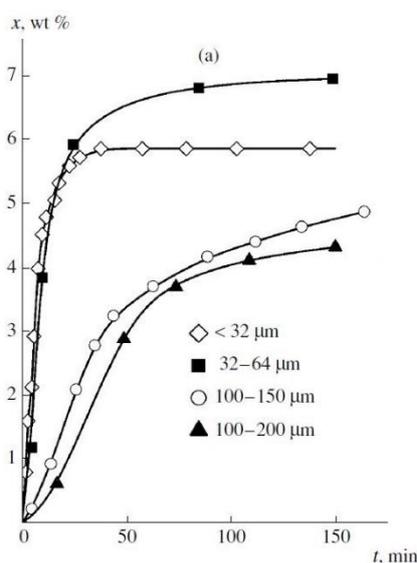


Fig. 1. Hydrogen-absorption curves for different fractions magnesium powders at 400°C
Source: [14]

Other studies also found that the method of obtaining powders also affects the kinetics of the reaction. Powders produced by mechanical milling due to their imperfect, irregular surface with cracks are characterized by a better hydrogenation reaction than powders deposited by gas-phase synthesis which characterized by smooth surface [15].

A factor that also strongly affects the kinetics of hydrogenation is also the limited rate of hydrogen dissociation on the metal surface. The surface of pure magnesium is not active. The dissociation barrier can be reduced by using a catalytic metal coating most commonly such as nickel or palladium. P. Hjort et. al [16] they examined the influence of oxygen on the absorption properties of magnesium with a Pd layer. What is more, with a small amount of oxygen, the dissociation of hydrogen is better than in the case of pure Pd-Mg samples. However, a larger amount of oxygen, thicker oxide layers cause a monotonous decrease in the rate of hydration and thus magnesium oxide plays a dual role in magnesium hydration. It should be mentioned, however, that even a very long exposure of the material to oxygen cannot completely prevent the absorption of hydrogen. Therefore MgO is only a partial barrier for diffusion.

The speed of the hydrogenation process also depends on the hydrogen pressure. At higher pressure, the effect of increasing the speed of the hydrogenation process is associated with a greater driving force of the reaction. However, at pressures greater than 30 bar, the rate decreases due to the very rapid formation of a magnesium hydride coating, which blocks all hydrogen absorption [12].

A way to achieve faster sorption kinetics and an easier activation process can be the creation of two or multi-component magnesium-based systems. Such additions can be divided into 6 groups [17]:

1. Nickel, it forms Mg_2Ni [18, 19].
2. Elements that can be used as hydrogen pumps such as Ce, Nb, Ti.
3. Catalysis that do not form hydrides Co, Fe, Cr.
4. Metal oxides.
5. Elements which form covalent bonds with magnesium.
6. Other metal hydrides such as $LaNi_5$ or $FeTi$ [20].

Another problem in magnesium-based materials is the reaction thermodynamics. In order to be able to use them commercially, the operating temperature should be in the range of $-40^{\circ}C$ - $85^{\circ}C$, taking into account the enthalpy of desorption (-74.4 kJ/mol H_2) and entropy (-135 kJ/mol H_2), the temperature at 0.1 MPa, is $287^{\circ}C$. Therefore, it is too high, the thermodynamics of MgH_2 desorption should be changed [13].

To change the thermodynamic properties, it is necessary to add a second phase (Z) to the hydride (YH_2), which reacts with hydrogen to release hydrogen and produce a new alloy or compound (YZ). The reduction of enthalpy for dehydrogenation is determined by the enthalpy of formation for the alloy or compound formed (YZ). As can be seen from Fig. 2 [11].

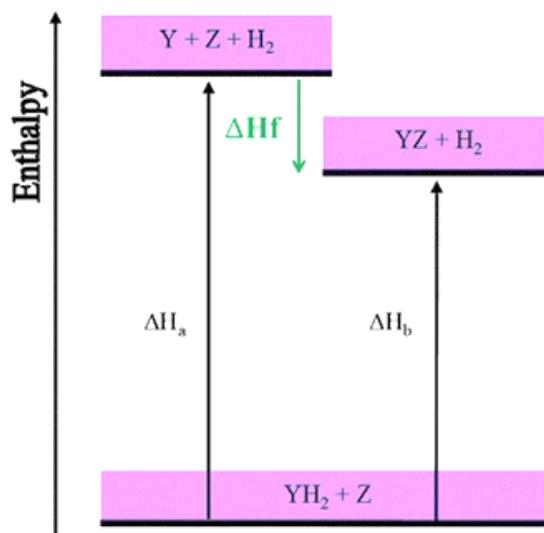


Fig. 2. Thermodynamic changes for multicomponent systems.
Source: [11]

An important issue in this case is also to keep as much the largest as possible capacity at the highest temperature reduction. Some of the materials introduced into the hydride allow the temperature to decrease, however, it leads to a reduction in the possibility of hydrogen storage. An example is nickel which allows the desorption temperature to be lowered to 250°C at a pressure of 0.1 MPa, however, it reduces the capacity to 3.6 wt.% of hydrogen.

Another way to change the thermodynamics of magnesium without changing the capacity is to reduce the particle size, part of the study confirmed a decrease in enthalpy when using small MgH₂ particles [21].

Summary

Magnesium hydride is so far very intensively tested due to the prospective use of them in hydrogen storage due to properties such as wide availability or low price. Unfortunately, the use of pure magnesium is very limited due to a number of problems such as slow kinetics or high desorption temperatures. Many attempts have been made to overcome these limitations, in particular by introducing various additives that will affect the kinetics of the reaction and improve the thermodynamics in order to reduce the dehydrogenation temperature.

Finding the right additives or introducing appropriate modifications can significantly affect the kinetics or thermodynamic properties of magnesium-based alloys, which allows for their effective commercial use.

Acknowledgments

This work was financially supported by The National Centre (NCN) in Poland, No. 2018/29/N/ST8/01417

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MAIN PROBLEMS IN HYDROGEN ENERGY

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Abstract:

Hydrogen is one of the alternative energy sources. Its use and application is increasing every year. Researchers, politicians and environmental activists have many hopes for this green source of energy. It is said that in a few decades, hydrogen can replace oil and other fossil fuels. Before this happens, however, many serious problems need to be solved related to the use of hydrogen as an ecological energy source - not only for mobile or home applications, but also for large-scale power applications (power stations). About what problems engineers, society and government have to face are described in this paper.

Keywords:

hydrogen, hydrogen energy, hydrogen storage, transport of hydrogen, H₂ production

Introduction

The "Green New Deal" policy, which is increasingly appearing in many industries, calls for investment in renewable and alternative energy sources. According to this policy should be counter climate change or oil production. One of the purposes of this policy is to make the economy independent of fossil fuels [1]. Therefore, hydrogen is becoming a response to these changes in Europe and around the world. There are more and more opportunities to use this green energy in place of fossil fuels. Theoretically, hydrogen solves all ecological problems and, in addition, has a number of beneficial properties. H₂ is a potentially excellent energy storage, it can be used in both energy and transport, as well as in gas transmission and distribution systems [2-5].

Its unique properties include the amount of energy released during the combustion process (120 MJ/kg) in the absence of any contaminants - the only product of combustion reaction is H₂O [4-8]. Which in the current world, with a constantly polluted environment, is a very big advantage. H₂ also has a predisposition to directly convert energy from the reaction of oxygen with hydrogen into electricity in fuel cells. Hydrogen storage capacities are much larger than the electric current. The possibilities of producing hydrogen by zero-emission and low-emission methods are also being developed [2, 5, 8].

So where are the problems of the hydrogen energy? In theory, hydrogen is an ideal source of energy. Problems begin in practice. For example in hydrogen properties, hydrogen storage, H₂ production (ecological production), production energy from hydrogen, hydrogen in a gas pipeline, hydrogen in transport application [9,10].

Hydrogen properties – first problem

Hydrogen is the simplest and most common element in the Universe - it constitutes up to 75% of its mass. It is also the lightest chemical element - consists of a single proton and electron (atomic mass = 1.00794 u) [11,12]. Density of hydrogen in the gas state is 0.082 kg/m³ (273 K, 1013 hPa) in the liquid state is 70.8 kg/m³ and in the solid state is 88 kg/m³. These values are the smallest with respect to other chemical substances. H₂ at room temperature is in gaseous form - the boiling point of this substance is - 252.8 °C, and the melting: -259.2 °C. Hydrogen is also characterized by very good energy properties as shown in Tab. 1 [3,7,11,13]. In comparison with other energy sources (such as propane, gasoline or methane), hydrogen has a much higher heat of combustion, calorific value or auto-ignition temperature. Additionally the octane number of hydrogen is 130 - this value is also much higher than for typically used fuel substances. For comparison, octane number of gasoline is 87-98, diesel: 30 and methane: 125. The higher the octane number, the higher the fuel value. The octane number is also intended to determine the fuel quality for spark-ignition or turbine-ignition engines. Unfortunately, despite these many advantages hydrogen is characterized by a small degree of energy packing - about 9 MJ/l so in order to obtain energy from hydrogen it is very often necessary to compress it or liquefy it under high pressure (from about 15 to even 80 MPa). Which very often involves additional costs [13-16].

Tab. 1. Selected energy parameters of hydrogen and other energy raw materials

Parameter	Hydrogen	Propane	Gasoline	Methane
Heat of combustion [MJ/kg]	141.9	48.9	44.4	55.5
Heat of evaporation [kJ/kg]	444	388	302	577
Calorific value [MJ/kg]	120.0	46.4	47,0	50.0
Ignition energy [mJ]	0.02	0.25	0.25	0.30
Auto-ignition temperature [°C]	585	466	222	534

Source: [3, 7, 11, 13]

H₂ combined with oxygen in the form of water, is not flammable, but present in hydrocarbons, it can react violently with oxygen, producing heat and water vapour. In the presence of oxygen, hydrogen gas burns with an almost colorless light-blue flame. The mixture of hydrogen and air may ignite spontaneously - but it depends on the concentration of hydrogen. If the volume hydrogen concentration is between 4 % and 75% at 293 K, the self-ignition probability of this mixture is very high [11,14,17]. Therefore, the use of hydrogen in the gaseous state is very dangerous and requires caution. The use of liquid hydrogen creates an additional, stronger threat - there is then the possibility of liquid explosive hydrogen mixtures with solidified oxygen or air enriched with O₂. Here comes the next problem: hydrogen is still for the human society one of the most dangerous

substances used as fuel - although contrary to appearances, it is less flammable than fossil fuels or gasoline. Safety for people is very important - as long as perception of hydrogen as a dangerous substance no changes, it will be very difficult for people to invest in hydrogen energy [5, 13, 17-19].

It should be also regard to the that hydrogen is a very small molecule that is able to diffuse through porous materials, rubber, and at elevated temperatures even through steel. It easily leaks through even minimal gaps or leaks and cracks. As mentioned earlier, hydrogen is very light so its diffusion in the air is very fast, much faster than natural gas or gasoline. Therefore, proper hydrogen storage is very important [5, 7, 12, 20].

Hydrogen storage – second problem

Currently, three ways of storing hydrogen are known: in the gas phase (compressed hydrogen), in the liquid phase (condensed hydrogen, additionally stored at very low temperatures around 20 K) and in the solid phase (adsorption or absorption of hydrogen in solids, chemical reactions) [2-5, 10, 19, 20]. These methods differ from each other in many respects (pressure, temperature, size and quality of tanks). There is no easy way to say which technique is the best, the cheapest or the simplest, but certainly it is possible to point many examples of how to improve the efficiency of storage. Efficiency depends on many factors, including material, economic and technological aspects [19-21].

The main problem with storing hydrogen in the gas and liquid phase is the very high pressure at which H₂ should be stored (up to 800 bar). This is dangerous because it can be explosive, it also generates high costs, especially for the liquid phase method, because the H₂ must be kept constantly at the temperature of liquid nitrogen. An alternative to these two methods is solid phase storage. It is the latest and constantly developed technique. When H₂ is stored, much lower pressures and temperatures are used. Hydrogen ab- or adsorbs on the surface of solids and the next one, thanks to the fuel cells, gets energy from it. Materials of special importance for this technique are for example magnesium hydrides, because thanks to them you can store large amounts of H₂ which results into a greater amount of energy. The use of solid materials in hydrogen storage tanks contributes to the safety of this process. Hydrogen is so strongly associated with the solid material that even during a leak, the gas slows down considerably, eliminating the risk of explosion. Currently, hydrogen storage technologies are developing rapidly, however, these technologies are still quite expensive, which is why every detail that can reduce the cost of hydrogen storage and increase the safety of using such tanks is important [4, 9, 14, 15, 20, 21].

Hydrogen production – third problem

Hydrogen on the globe exists in a bound form, among others in: rocks (e.g. the percentage of hydrogen in granite is: 61.68% by mass, in gneiss: 61.93%, and in basalt: 36.15%). It is also present in natural gas (methane), in oil or coal and water. In its free form, it occurs in volcanic gases and in the upper atmosphere. There are many methods for hydrogen production, the best known are water electrolysis, coal gasification, steam gas reforming and liquid petroleum reforming. Currently, the most hydrogen is produced using natural gas reforming - 47%. The next 30% is hydrogen produced

from crude oil, about 18% comes from coal, and only 4% is water electrolysis, the remaining 1% are other unconventional methods (for example biomass processing or the use of enzymes and bacteria) – Fig. 1 [2, 10].

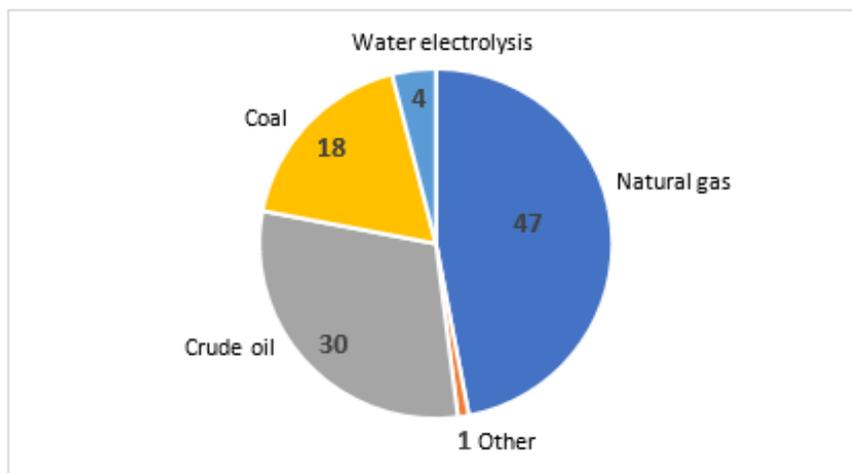


Fig. 1. Hydrogen production in global market at 2018
 Source: [2]

Problem is in that the currently used hydrogen production techniques are either very expensive (electrolysis) or harmful to the environment. For example when reforming methane for 1 ton of hydrogen produced 9 to 12 tons of carbon dioxide and other greenhouse gases are produced. Today it is said that hydrogen production is under control and even Poland is a significant player (at least on the European market). We forget that today’s global hydrogen production is based on methane, and therefore fossil fuel, they are the cheapest technologies – but unfortunately harmful to environment [2, 10, 14, 16].

In addition one of the key technological challenges is the production of hydrogen with a very high purity of 99.999%, accepted by the currently most developed PEM fuel cell designs. Which requires the use of expensive equipment and increased mass production of hydrogen.

Therefore, the priority is to find and develop an efficient, cheap, fast and fully ecological method of obtaining this green energy source. Without solving this problem, unfortunately, hydrogen will never become a fully ecological energy carrier, which in the future will be able to replace oil or natural gas. Fig. 2 summarizes typical methods of producing hydrogen in relation to their production costs. Environmentally friendly methods are marked in green, harmful ones in red [3-6].

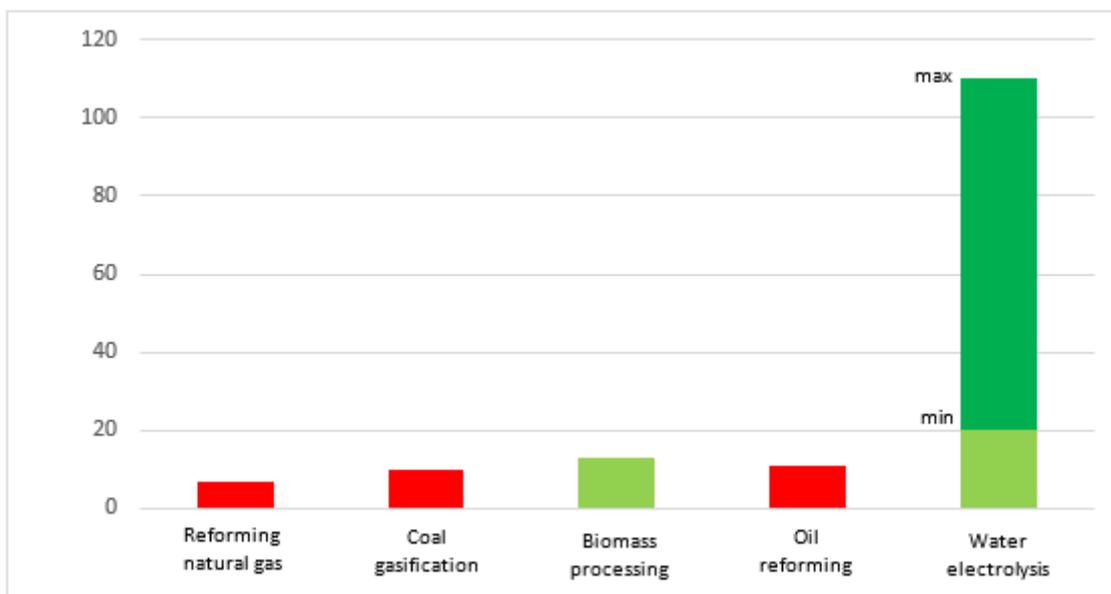


Fig. 2. Graph showing selected methods of obtaining hydrogen to their production costs
 Source: [3-6]

Energy production from hydrogen – fourth problem

The energy from hydrogen can be obtained in two ways: as a result of the combustion reaction (for example in gas turbines that burn hydrogen instead of gas or in internal combustion engines) as well as by using appropriate fuel cells. Fuel cells have been known for almost 200 years, gas turbines and engines for over 200 years, and there are still technical problems and so-called "game changers" - the need to solve key problems [17-21]. Fuel cells are commercially available on a wide scale, but they cover rather power levels of a few or over a dozen kW and cannot really break through to large-scale energy. These solutions are ideal for transport as well as for home energy and heat production, but are still not suitable for large power station (producing energy on the order of MW - competitive in terms of price and technology). In addition, there are problems with many concepts of the fuel cell structure (electrolyser materials, fuel composition), various operating temperatures. In mobile solutions, the weight of the fuel cell is very important, as well as their high efficiency - so they must be as light as possible and allow car to travel as long as possible. These two features are often mutually exclusive and finding the golden mean is very laborious, heavy and expensive. So maybe the answer is hydrogen turbines? In this case, the largest producers have already started intensive work and maybe we will see hydrogen burning in combined heat and power station or in gas (hydrogen) power plants. As of today, leading companies are announcing that it is possible to burn fuel with an admixture of up to 30% hydrogen, and new research projects allow the introduction of 100% turbines for hydrogen by 2030. The problem is not at all trivial, because although in theory the turbine itself looks the same the hydrogen combustion process is different (much faster) and entire burner systems should be redesigned [17-21].

However, currently fuel cells are recognized in the world as the most promising electricity generators for both high-power power plants and small, distributed energy generators, as well as

electricity sources for the propulsion of motor vehicles. It is potentially possible get over twice as much usable energy from a unit of fuel mass in a fuel cell than in a heat engine. The development of fuel cell technology will largely determine the success of the entire 'hydrogen program. The high efficiency of the fuel cell, which works quietly, because it has no moving mechanical parts and produces minimal amounts of pollution, favors this technology for future power sources. The speed of "charging" such a generator (fuel filling) as well as the potential reversibility of this process (storage of temporary excess electricity in the form of chemical energy) are additional advantages [17-21].

Sending hydrogen through a gas pipeline – fifth problem

In order for hydrogen to replace fossil fuels in the future, it must be supplied by gas pipeline to customers. Here in the theory, everything seems simple. On the maps there is a gas line (yellow), we turn green (hydrogen) and it's ready. There is a pipe and gas - there will be a pipe and hydrogen. Problem, as usual, is in the details here in the technical ones. As mentioned before, hydrogen diffuses easily, penetrates through walls and seals and has a very adverse effect on materials (corrosion, strength reduction). The release of 100% hydrogen in current gas pipelines is a fiction, according to various standards and tests from different countries, the limit is today 2-8% (sometimes hypothetically 10%). It does not seem possible to use these pipelines for hydrogen at all once (they are built of different materials and have different characteristics). It will probably be possible to introduce an admixture of hydrogen. The final receivers - devices such as ovens and the like, were never supposed to burn hydrogen - so there are no standards/tests and approvals. In the modern world of predatory lawyers, just wait for some domestic accident with compensation. So hydrogen will not stay in gas pipelines for long, and certainly not in larger quantities, unless new pipelines are built and new devices using it are developed - but this means at least a decade of research and very high costs) [2, 10, 20].

Use hydrogen in transport - sixth problem

The problem here is not mainly related to technology - because technology is already present and constantly improved. The problem is related to the dynamically developing competition of electric cars. Cars with traction batteries are already common, and charging stations are becoming more common, it only remains to shorten the charging time. The use of hydrogen is currently limited only to heavier vehicles (despite the efforts of some manufacturers such as Toyota). The hydrogen used in transport is safe (manufacturers are even oversensitive on this point), but it is not so beautiful in the case of hydrogen stations for example in cities (power supply is 350 or 750 bar). For now, the future is rather a niche in specialized trains, ships or special devices. Everything seems simple in politics and presentations. In technique - engineers are making problems. Research, pilots, approvals and further tests are still needed. And all this goes slowly, and sometimes with an unsatisfactory result [2, 5, 15, 19, 21].

Conclusion

There are certainly many more problems with hydrogen energy. The most obvious and common ones have been described above. When analyzing the above, one should be optimists but also realists. You should expect results, but leave time to refine the technology. We do not count on the impossible and approach it carefully. Emotions associated with hydrogen technologies should be carefully dosed, but one should also believe and support their growth - because so far everything is going in the right direction. The topic of energetic use of hydrogen is becoming crucial for the future of energy in the world and is currently the subject of intensive research and large financial outlays in many countries around the world. Research needs of the broadly defined hydrogen economy can be classified in three areas:

- Development of hydrogen storage methods in the aspect of transport application and creation of a safe hydrogen transmission technology,
- Diversification of hydrogen sources so that the cost of obtained fuel is comparable with the cost of fuels obtained from minerals (crude oil, natural gas, coal),
- Energetic use of hydrogen in fuel cells for highly efficient generation of electricity.

However, it should be remembered that despite many aspects of "hydrogen energy", the key to the success of this idea is to solve fundamental material problems.

Acknowledgments

This work was financially supported by The National Science Centre (NCN) in Poland, No. 2018/29/N/ST8/01417.

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BETWEEN TWO GENDERS: INDIAN THIRD SEX AND ITS NO MAN’S LAND PORTRAYED IN „THE MINISTRY OF UTMOST HAPPINESS“ BY ARUNDHATI ROY

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Abstract:

Anjum, main character of the latest book by Arundhati Roy: *The Ministry of Utmost Happiness* [1] does not make it easy for us to know who (s)he is. Born as a hermaphrodite in a Muslim family and grown up as a boy, is then reborn as a hijra, and only that indeterminate make-up gives him/her the full sense of his/her own being. The hijras, with their indeterminate self-sense which shapes their experience of life, with their vulnerability, marginalization and displacement, living in the midst of society that does not understand them and hardly tolerates them, are a perfect illustration of the existential dimension of precariousness. This paper is an attempt to focus on the literary representation of the hijras in contemporary Indian society, as it is exemplified in the person of Anjum, who lives in a “no man’s land”, the metaphorical space in-between, which is ruled by no one and is in no one’s possession seems to adequately represent Anjum’s existential situation of displacement.

Keywords:

Arundhati Roy, gender, third sex, hijra, precarity

Introduction

We are living in a civilisation, in a society and in a historical moment of a precarious frame of mind. Precariousness is the postmodern declination of existential restlessness. In the history of literature and philosophy the attribute ‘precarious’ is used to define the main structure of the human existence: in fact, it refers to the immanence, the physicality, the temporality and the transience of all things. English economist Guy Standing in his *A Precariat Charter: From Denizens to Citizens* talks about modern denizens who don’t inhabit any more fields nor forests, but who live in a peculiar place which is not theirs [2]. They are foreigners allowed only certain rights in their adopted country or society. Therefore, we have many precarious social groups which differ

essentially the ones from the others. Especially in the city, where strangers live close remaining strangers, being condemned to the chronic and irreducible precariousness [3].

Arundhati Roy [4] is an Indian writer and social activist very well known for her commitment to the defence of the environment as well as the civil rights of the weakest. Being very sensible to the affairs of those who for one reason or another are ostracized, rejected, forced to the fringe of society, she could not remain indifferent to the precarious situation of the people of the third gender. Indian three genders concept differs totally from the Western, binary one [5]. It recognizes three sexes or three gender variants: male, female and neuter, hijra, who is a representative of the third gender (nature) [9]. The third gender which for centuries was inherently inscribed in the landscape of India, nowadays is commonly marginalized in the caste-based social structure. The persons belonging to the third nature are seen by the homophobic society as the non-complete, non-fully human beings [12].

Postcolonial neoliberalism [14] in India has increased precarity of all the social groups, including the one involved in the movement for the recognition of gender equality for all three sexes. Thus, hijras, who were mentioned already in the later Vedic [15] time, that is since 1000 B.C. under the name *Napumsaka*, nowadays live in the no man’s land occupying an indeterminate, undefined place in the society [17]. After immemorial period of being one of the integral elements of Indian culture and social structure, based on specific rights, the hijras became misfits and outcasts without voice. But the claim of Arundhati Roy:” There’s really no such thing as the ‘voiceless’. There are only the deliberately silenced, or the preferably unheard.” [4], resounds very clearly in her writing. She decides to break the silence about people of the third sex who are a neglected part of the community and who, being victims of intolerance, misconceptions, ostracism and different kind of deprivation, struggle for their human rights.

Anjum and The Precarious Life of a Hijra

Roy’s most recent novel, *The Ministry of Utmost Happiness* (2017) is, for sure, not a direct reaction to colonialism as theory and practice, but it can come under the banner of postcolonialism for at least two reasons: it is created in a postcolonial country in “the period after independence” as well as it “represents the period from the inception of colonialism to the present [...] intent on affirming difference, cultural plurality and the consideration of the process of history” [14]. Furthermore, by choosing a hijra for the main character, and narrating his(her) story, Roy depicts the place of the exponents of the third gender in the contemporary Indian society, which is, obviously, the result of colonial influences (i.e. the postcolonial legacy) [18,19]. At the same time by doing that she “(...) insert(s) the often ‘absent’ colonized subject into the dominant discourse in a way that it resists/subverts the authority of the colonizer” [20]. Moreover, the choice of the protagonist who represents the third nature is a postulate to take into consideration the *Other* in his singularity, to reflect on his living conditions, difficulties, battles, needs and desires.

A literary, expressive representation of a hijra named Anjum in *The Ministry of Utmost Happiness*, is very probably inspired by a real person, Mona Ahmed, “India’s Most Iconic Trans Person” [21], described by Dayanita Singh, world-renowned photographer with these words: “She wanted to tell the story of being neither here nor there, neither male nor female, and finally, neither

a eunuch nor someone like me. She would always ask me, tell me: what am I?" [22, 23]. Question to which the protagonist of *The Ministry* seems to give an answer: "It doesn't matter. I am all of them (...). Who says my name is Anjum? I'm not Anjum, I'm Anjuman. I'm a mehfil [24], I am a gathering. Of everybody and nobody, of everything and nothing (...)" [1].

Born as a fourth of five children in an orthodox middle-class Muslim family, the baby is assigned male at birth. The other three are girls, so the birth of a boy is the ultimate reward for the patriarchal family. They call him Aftab, which means 'Sun' [25]. The happiness of Aftab's mother, however, is not destined to last for long. The next morning, she discovers "nestling underneath his boy-parts, a small, unformed, but undoubtedly girl-part" [1]. The element that turns out to be determinant for the whole Aftab's life, causes disbelief and concern of his terrified mother, who understands that her child is beyond doubt, "trapped in between" [1]: "In Urdu, the only language she knew, all things, not just living things but all things – carpets, clothes, books, pens, musical instruments – had a gender. Everything was either, man or woman. Everything except her baby. Yes of course she knew there was a word for those like him –Hijra. Two words actually, "Hijra" and "Kinnar" [27]. But two words do not make a language. Was it possible to live outside language? Naturally this question did not address itself to her in words, or as a single lucid sentence. It addressed itself to her as a soundless, embryonic howl" [1].

Growing up, Aftab is more and more inclined towards girlish behaviour, what provokes a teasing explosion of other children: "He's a She. He's not a He or a She. He's a He and a She. She-He, He-She Hee! Hee! Hee!" [1]. For this reason when his sisters and younger brother go to school, Aftab is spending long hours on the balcony of his home until one morning when his attention is drawn by "(...) a tall, slim-hipped woman wearing bright lipstick, gold high heels and a shiny, green satin salwar kameez (...)" and he desperately wants to be her. Her, and not just an ordinary woman with burqa, but this special woman dressed and walking "the way she did only because she wasn't a woman." [1]. Aftab, who is never comfortable with his boy's body, but who does not know what or who he wants to be, in this very moment understands that it is not his girl-element that is an appendage. Listening to his inner voice he follows the delightful creature and then he enters the realm of hijras, where the beautiful Bombay Silk lives with seven others like her in the Khwabgah, the House of Dreams. Aftab's happiness is so extreme that he is completely regardless of what Nimmo, one of them, says about hijras, who – apparently - are made by God as an experiment of a living creature incapable of happiness, expelled from society, seen as queer and a castaway [1].

Well determined Aftab abandons the family home and by undergoing a transition (gender reassignment) she is reborn as a hijra called Anjum ('Stars') [25]. Thanks to her captivating nature, she immediately integrates with the residents of the Khwabgah, which becomes her home. Becoming a part of a hijra family, she breaks ties with the former world, which she never felt belonging to. Over the years Anjum (or maybe Anjuman, (Urdu, 'Meeting, Gathering' [25]) turns into the most famous, desirable "a sought-after lover, a skilled giver of pleasure" [1] hijra in Delhi. She has her dream (patched together) body, men, friends, she even fulfils a dream of being a mother (adopting a girl-child).

But one day, in the age of forty-six, after thirty years of being a part of the House of Dreams community, a traumatic experience marks her irreversibly. During the journey to Gujarat one of her friends is murdered because of being Muslim [30]. Anjum comes back to Delhi totally alienated from the House of Dreams and from the people around her. She realizes that not only to be a hijra makes human life precarious and endangered. Feeling lonely, outlying, frustrated and exhausted she decides to live in seclusion. For the second time she leaves everything and she goes again in search for herself and her own place in the world. She finds it in a suburb graveyard, where she lives “like a tree” [1], trying to reconstruct her personal paradise: “Only a ten-minute tempo ride from the Khwabgah, once again Anjum entered another world. It was an unprepossessing graveyard, run-down, not very big and used only occasionally. Its northern boundary abutted a government hospital and mortuary where the bodies of the city's vagrants and unclaimed dead were warehoused until the police decided how to dispose of them” [1].

Overwhelmed with sense of desolation and distress, Anjum chooses apparently the least cozy and friendly place, what emphasizes her state of highly depressive sense of emptiness: “On her first night in the graveyard, Anjum placed her Godrej cupboard and her few belongings near Mulaqat Ali’s grave and unrolled her carpet and bedding between Ahlam Baji’s and Begum Renata Mumtaz Madam’s graves. Not surprisingly, she didn’t sleep. Not that anyone in the graveyard troubled her-no djinns arrived to make her acquaintance, no ghosts threatened a haunting.” [1].

And yet, despite dejection and all the adverse circumstances, Anjum does not give up. She recreates her life out of the hijra *Gharana* [31], without any support of the group. In a modest corner cut out on one grave, little by little she builds her own “Jannat” (Urdu, “Heaven”) [25] leaving out of it the “Duniya” [32], the rest of the world. “Over time Anjum began to enclose the graves of her relatives and build rooms around them. Each room had a grave (or two) and a bed. Or two. She built a separate bathhouse and a toilet with its own septic tank. For water she used the public handpump.” [1].

And then, the rest of the world comes back to her: “Gradually, Jannat Guest House became a hub for Hijras who, for one reason or another, had fallen out of, been expelled from, the tightly administered gird of Hijra Gharanas. As word spread about the new guest house in the graveyard, friends from the past reappeared (...)” [1]. Thus, some few abandoned, neglected graves turns into not just a guest house for the gang of homeless (people and animals) to whom Anjum gives generously a shelter, but a kind of sanctuary visited by hordes of 'pilgrims'. Here Anjum with her big heart created a second Khwabgah, or place „where special people, blessed people, came with their dreams that could not be realized in the Duniya.“ [1].

Postcolonialism and Multiplicity of Precariousness

The Ministry of Utmost Happiness is not a biography of a hijra, or a propaganda story of a fighter for sexual minority rights. It is not a strictly historical novel or a made-up fiction. It is nothing of them and everything of them, to paraphrase what Aftab-Anjum says about him/herself. It is a novel which depicts the multiple crisis of humanity, politics, ecology, nation in the multi-cultural, multi-ethnic, multi-religious country in the age of the postcolonial national-liberation movement. The hybrid narrative form reverberates with cries of the victims of an extreme social

discrimination, among which there are people of the third nature (one could say: hybrid nature), left to themselves in some no man's land to beg for their place in society. The homophobic society, being itself a legacy of the colonial Victorian mores [33], treats people of the third identity as the incomplete, not fully human beings. Hijras are frequently the object of various unjustified persecutions, mockery and offences, to which they eventually get used to: "She didn't turn to see which small boy had thrown a stone at her, didn't crane her neck to read the insults scratched into her bark. When people called her names -clown without a circus, queen without a palace -she let the hurt blow through her branches like a breeze and used the music of her rustling leaves as balm to ease her pain" [1].

The character such as Anjum can be seen as an epitome of struggling for the recognition of one's own rights, for social acceptance of the otherness. Arundhati Roy with her characteristic insight depicts a harsh reality of the contemporary Indian society in a moment of its political and social crisis with a deep empathy for the humiliated life of the people like Anjum: of hijras who are between two genders, a secret, outcast community craving for its sanctioned, stable, no more precarious position within the society. "Ordinary people in the Duniya – what did they know about what it takes to live the life of a Hijra? What did they know about the rules, the discipline and the sacrifices? Who today knew that there had been times when all of them (...) had been driven to begging for alms at traffic lights? That they had built themselves up, bit by bit, humiliation by humiliation (...)" [1].

The Duniya represents here the outside world, where people of the third gender identity either don't exist or are not accepted because of their non-belonging to the mainstream. This outside world is unfriendly, competitive and hostile for those who for some reason constitute a minority and do not sign up in the general scheme. In this Duniya the hijras community constantly has to fight for the civil rights of its members who are still treated with indifference, being considered a shame of the nation. That is why they are so nostalgic thinking about their glorious past confirmed in both epos and in numerous historical sources [34]. Like Ustad Kulsoom Bi, a hijra guru in the Khwabgah, who gets so moved by the notice transmitted in a TV program about the court of eunuchs in a late Mughal history at New Delhi's historic Red Fort: "The moment passed in a heartbeat. But it did not matter. What mattered was that it existed . To be present in history, as nothing more than a chuckle, was a universe away from being absent from it, from being written out of it altogether .A chuckle, after all, could become a foothold in the sheer wall of the future" [1].

After this good past of being entrenched part of the culture, even as a "chuckle" and after thousands of years of mythologically and historically sanctioned existence, the hijras occupy an ambivalent position being both worshiped and feared , even by those in a high position who "(...)like everyone else (...) fear (...) being cursed by a Hijra" [1]. Their role is reduced to offering blessing (or curses) to the newlyweds and newborn children, and to dealing with sex trade. Commonly considered social outcasts they can exist only in this odd no man's land, avoided by all the others. But like their controversial body which is both male and female or "neither[of] man nor [of] woman" [13], they are from one side reluctantly accepted and from the other one hallowed. Thus, they must fight two battles: with the outside world against marginalisation and stigma and

with their own body which “had suddenly begun to wage war on [them].” [1]. Because “the riot is inside [them]. The war is inside [them]. (...). It will never settle down. It can’t” [1].

Conclusion

Like the “patched-together” body of Anjum, also the novel of Arundhati Roy can seem to be a patchwork sewn from various apparently disconnected stories of not connected people who have only one element in common: they all are in an unprincipled way marginalised. When finally, the stories merge and the representants of different precariousities meets together “Somehow everything became a little easier to bear” [1]. Therefore, Anjum, who thanks to her strength and humanity constructs in the graveyard not only a funeral service for all the excluded and persecuted but also her own paradise, the Ministry of Utmost Happiness, to which all the underprivileged, humiliated, powerless and rejected are invited, unites those heterogenous people who have a common goal, such as to live peacefully and with dignity in the hostile, intolerant, inhuman world in the age of globalization.

The social exclusion of hijras and numerous ethnic, religious and other minorities is the direct, infamous legacy after British colonialism and the example of the existential dimension of precariousness. Arundhati Roy portraying a hijra together with other social sufferers deconstructed some commonly widespread myths and prejudices giving a voice to those who are not listened, who are not taken into consideration in a multicultural democratic society.

Appendix

The third gender persons were also seen as ‘a breach of public decency’, ‘the vilest and most polluted beings’ suspected to deal with ‘revolting’ practices.[22]. Considered the ‘*habitual sodomites*’ and gender deviants, they were placed under the Criminal Tribes Act, and as such, were subjected to obligatory registration. And so over the years of meticulous registering and stigmatization under British rule, hijras were pushed out of the social sphere.

1864: Section 377. Unnatural offences: ”Whoever voluntarily has carnal intercourse against the order of nature with any man, woman or animal shall be punished with imprisonment for life, or with imprisonment of either description for a term which may extend to ten years, and shall also be liable to fine” [22].

1871: Criminal Tribes Act, Part II. Eunuchs:

- 24. Registers of eunuchs and their property.- The Local Government shall cause the following registers to be made and kept up by such officer as, from time to time, it appoints in this behalf.

(a) a register of the names and residences of all eunuchs residing in any town or place to which the Local Government specially extends this Part of this Act, who are reasonably suspected of kidnapping or castrating children, or of committing offences under section three hundred and seventy-seven of the Indian Penal Code, or of abetting the commission of any of the said offences; and

(b) a register of the property of such of the said eunuchs as, under the provisions hereinafter contained, are required to furnish information as to their property.

'Eunuch' defined.- The term 'eunuch' shall, for the purposes of this Act, be deemed to include all persons of the male sex who admit themselves, or on medical inspection clearly appear, to be impotent.

- 25. Complaints of entries in register. - Any person deeming himself aggrieved by any entry made or proposed to be made in such register, either when the register is first made or subsequently, may complain to the said officer, who shall enter such person's name, or erase it, or re kin it, as he sees fit.

Every order for erasure of such person's name shall state the grounds on which such person's name is erased.

The Commissioner shall have power to review any order passed by such officer on such complaint, either on appeal by the complainant or otherwise.

- 26. Penalty on registered eunuch appearing in female clothes.- Any eunuch so registered who appears, dressed or ornamented like a woman, in a public street or place, or in any other place, with the intention of being seen from a public street or place,

or dancing in public, or for hire.- or who dances or plays music, or takes part in any public exhibition, in a public street or place or for hire in a private house, may be arrested without warrant, and shall be punished with imprisonment of either description for a term which may extend to two years, or with fine, or with both.

- 27. Penalty on registered eunuch keeping boy under sixteen.- Any eunuch so registered who has in his charge, or keeps in the house in which he resides, or under his control, any boy who has not completed the age of sixteen years, shall be punished with imprisonment for a term which may extend to two years, or with fine, or with both.
- 28. Maintenance and education of boys whose parents cannot be found.- The Magistrate may direct that any such boy shall be returned to his parents or guardians, if they can be discovered. If they cannot be discovered, the Magistrate may make such arrangements as lie thinks necessary for the maintenance and education of such boy, and may direct that the whole or any part of a fine inflicted under section twenty-seven may be employed in defraying the cost of such arrangements.

The Local Government may direct out of what local or municipal fund so much of the cost of such arrangements as is not met by the fine imposed, shall be defrayed.

- 29. Disabilities of registered eunuchs.- No eunuch so registered shall be capable-

- (a) of being or acting as guardian to any minor,
- (b) of making a gift,
- (c) of making a will, or
- (d) of adopting a son.

- 30. Power to require information as to registered eunuch's property.- Any officer authorized by the Local Government in this behalf may, from time to time, require any eunuch so

registered to furnish information as to all property, whether movable or immovable, of or to which he is possessed or entitled, or which is held in trust for him.

Penalty for refusing such information.- Any such eunuch intentionally omitting to furnish such information, or furnishing, as true, information on the subject which he knows, or has reason to believe, to be false, shall be deemed to have committed an offence under section one hundred and seventy-six or one hundred and seventy-seven of the Indian Penal Code, as the case may be.

- 31. Rules for making and keeping up registers of eunuchs.- The Local Government may, with the previous sanction of the President in Council, make rules for the making and keeping up and charge of registers made under this Part of the Act.”

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b) In India all three sexes are considered biological and naturally existing as a result of conception. "A male child is produced by a greater quantity of male seed, a female child by the prevalence of the female; if both are equal, a third-sex child or boy and girl twins are produced; if either are weak or deficient in quantity, a failure of conception results." [7] (3.49).
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b) *Keśava* (Sans.), having long or much or beautiful hair [6].
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- [27] *Kinnara* = *kim* + *nara*, (Sanskrit) 'what sort of man?', originally perhaps a kind of monkey.[4]; किन्नर *kinnar* [4], m. 1. *mythol.* a being with a human body and horse's head, reckoned among the *gandharvas* (as divine musicians or singers). 2. *mythol.* a class of demigods attached to Kuvera, the god of wealth. 3. name of a community of singers and musicians [28]; *kinnar* (Hindi), 1. centaur. 2. sheer, transsexual, trans [29]. As well as the word hijra, the name *kinnar* is intended for determining all the variabilities of individuals who are not typical from the heteronormative point of view. They can be naturally intersexed (or hermaphrodites by birth), transgenders, transvestites, impotents, homosexuals and so on.
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b) In the Mughal courts (1526-1761), the hijras were known for enjoying unestimable prestige and power: "They can get whatever they desire- fine horses to ride, servants to attend them outside, and female slaves inside the house, clothes as fine and smart as those of their master himself" (Francisco Pelsaert (1595-1630), Dutch merchant, commander of the ship *Batavia* (Dutch East Indies Company) [36].
c) "Mughal Court in Delhi, one of the most magnificent in Asia, concentrated around the person of the emperor, the highest dignitaries and the harem. A real crowd of eunuchs performed all the most honorable duties, such as treasure management, wardrobe or jewels. (...) In a culture based on pleasures and music. poetry and ceremonies, also the care of the harem belonged to the eunuchs. Those ladies' closest companions served them, managing their finances, getting them lipsticks, perfumes, and sometimes men [37].
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TO BE OR NOT TO BE...? – A BRIEF REVIEW OF *SALMONELLA TYPHIMURIUM* INFECTION

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Abstract:

Salmonella infections cause wide range of clinical manifestations. *S. enterica* subspecies *typhimurium* is one of the most common etiological factor of an inflammatory diarrhea and is considered to be a heavy burden for medical service worldwide. Although the research on its pathogenesis mechanism is in-depth and comprehensive, many issues are still unknown. Better understanding of *Salmonella* infection steps, regulation of virulence genes and interaction with host immune system are necessary to create a fast diagnosis as well as a successful treatment for salmonellosis.

Keywords:

Salmonella typhimurium, pathogenesis, virulence factors

Introduction

Salmonella is a genus of Gram-negative bacteria, facultative anaerobic bacilli unable to form endospores [1]. It is belonging to the *Enterobacteriaceae* family similarly as its close relative – *Escherichia coli*. *Salmonella* spp. cells are rod-shaped and motile (polytrichial arrangement of flagella) [2, 3]. Taxonomic classification is elaborate (Fig.1) and has been changing over last 20 years. During clinical identification, serotyping is commonly used to detect *Salmonella* strains. There are two species: *Salmonella enterica* and *Salmonella bongori* [1]. Over 2500 serovars of *S. enterica* have been identified and classified into 6 subspecies [4] (Fig. 1).

Salmonellosis (infection caused by *Salmonella* spp.) is transmitted *via* the fecal–oral route. Bacteria reach the host organism through contaminated food or water. Uncooked eggs and chicken are regarded as the main source of infection [5]. The manifestation of symptoms in *Salmonella* infection is different depending on the serovar and the host. For example, in humans the majority of *Salmonella* serovars cause inflammatory diarrhoea and nausea, although the main manifestation of *Salmonella typhi*, *Salmonella paratyphi* and *Salmonella sendai* infection is fever [4].

S. enterica hosts are warm-blooded vertebrates. Serovar Typhimrium (*Salmonella typhimurium*) is the most common etiological factor of inflammatory diarrhea in human in developed and developing countries [1]. In United States of America over 40.000 cases of

salmonellosis are diagnosed annually. However, this number might be seriously underestimated [1, 6]. Diarrhoea diseases because of *Enterobacteriaceae* infections are considered to be one of the most important cause of death among children under 5 [3].

Calves and mice are used in research on *Salmonella typhimurium* as models of gastroenteritis in human [1].

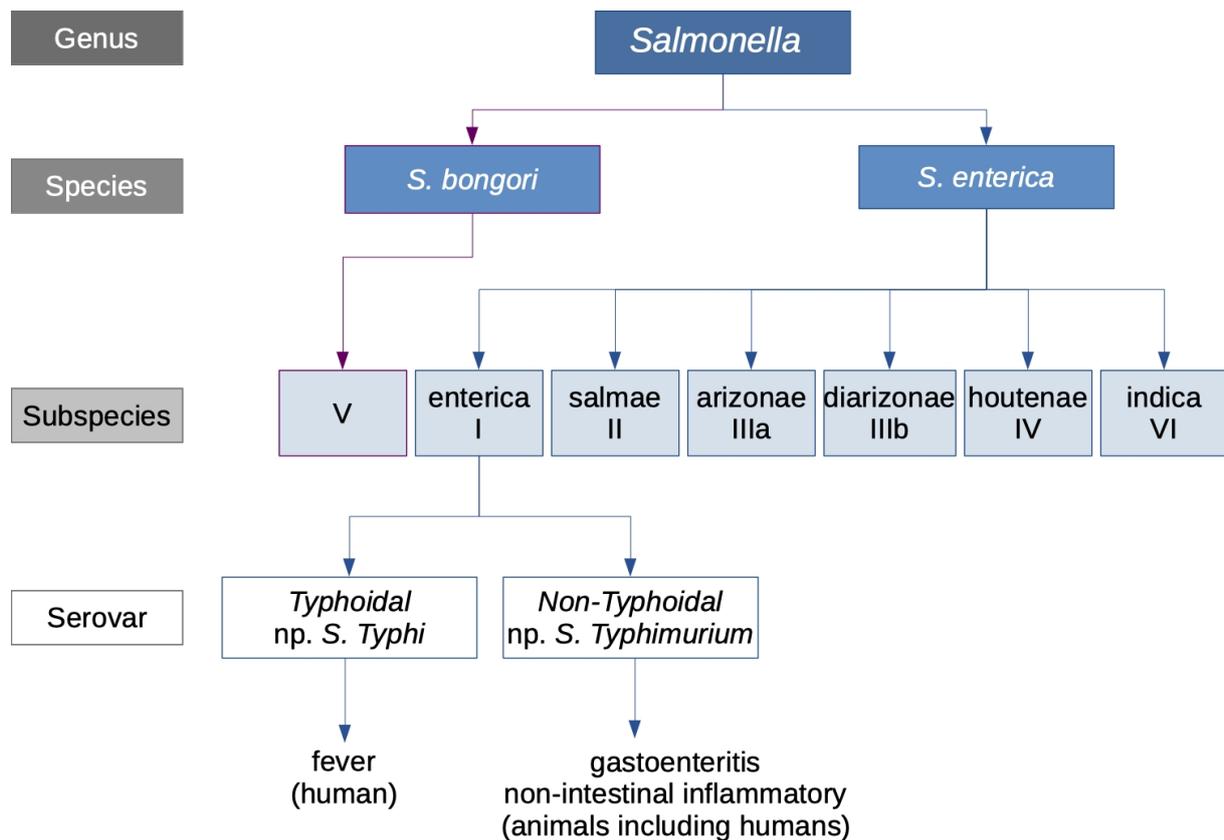


Fig. 1. Taxonomic classification of the genus *Salmonella*
Source: [7]

Phatogenesis

Bacteria as well as host organisms have developed a wide range of adaptation mechanisms against each other. *Salmonella* strains contain numerous virulence factors to persist and replicate in extremely difficult environments like a phagosome or an acidic stomach. Many mechanisms of *Salmonella* virulence result in innate and adaptive immune response activation. My main aim is to briefly describe how many obstacles *S. typhimurium* has to overcome to effectively infect the host.

Stomach

Bacteria reach stomach through contaminated food or water [2]. Ingesting at least 50.000 *Salmonella* cells is necessary to cause a disease. First symptoms emerge between 6 and 72 hours after the bad luck meal [6]. After entering the stomach the acid tolerance response (ATR) is

activated. Its function is maintaining acid-base homeostasis inside bacterial cell – the cytoplasm has drastically higher pH level than the surrounding stomach contents [8].

Small intestine

The most optimal scenario for *S. typhimurium* after reaching small intestine is traversing the mucus layer [1]. This protects bacteria from being removed from the small bowel with the rest of digestive track content. Adhesion to enterocytes is the next step. However, in murine model *S. typhimurium* prefer infecting M cells and Peyer’s patches (PPs) [1]

One of the most crucial protein – type 3 secretion system (T3SS) is encoded within *Salmonella* pathogenicity island 1 (SPI-1). It enables bacteria to enter the mammalian cell. T3SS translocates some virulent proteins (product of *sopEΦ* and few encoded within SPI-1 and SPI-5) through the plasma membrane [9, 2]. The structure of the host cell’s cytoskeleton is being rearranged as a result of signaling pathways’ disruption [9]. It starts the formation of membrane ruffles and *S. typhimurium* is surrounded and then engulfed by the cell (Fig. 2). The vesicles with bacteria inside are called *Salmonella-containing vacuoles* (SCVs) [10]. It is the only intracellular place where bacteria can replicate [2].

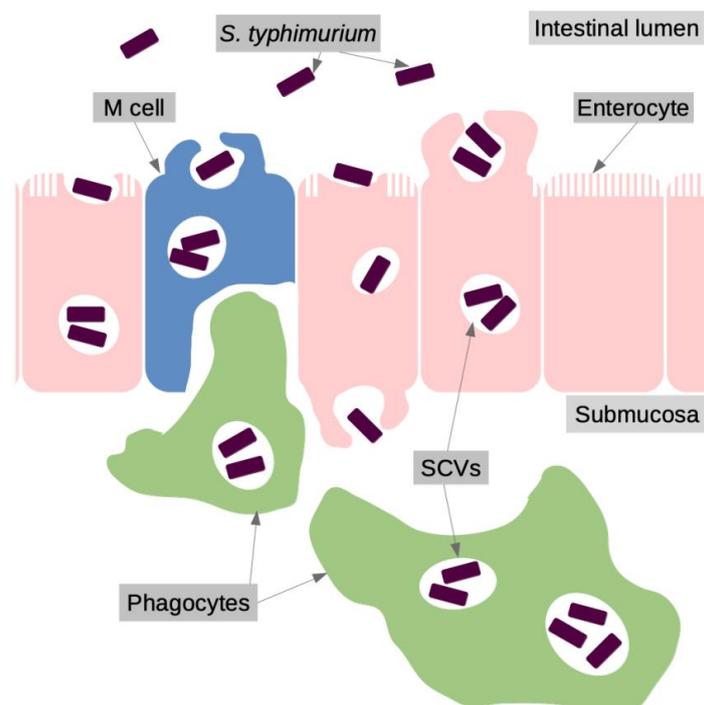


Fig. 2. Infection of *S. typhimurium*. Bacteria attach to the enterocytes (pinkish cells) and M cells (blue), induce rearrangements in their cytoskeletons’ structure and are enclosed within vesicles (SCVs). Next step of invasion is engulfment by the phagocytes (green cells)
Source: [1, 2]

During SCVs maturation *Salmonella* induce aggregation of F-actin molecules around vesicles. This phenomenon is named *vacuole-associated actin polymerization* (VAP). The main functions of actin meshwork are: (i) maintenance of vacuole’s integrity and (ii) protection from fusion with lysosome [11]. SCVs are localized in perinuclear space near Golgi apparatus what enables them to

fuse with cell's vehicles which contain nutrients. As a result, *Salmonella* cells obtain energy source and fragments of membrane. Environmental conditions are optimal for bacteria replication inside SCVs [12].

Complex metabolism with plurality of alternative biosynthesis and katabolic pathways is characteristic for enteropathogens including *Salmonella*. They are able to obtain energy from various organic compounds and immediately adapt to different environments. The second key to *Salmonella* success is choosing the best energy source among the available ones [13].

In infected cells, long filamentous and membranous structures have been identified and they are referred to as *Salmonella*-induced filaments (SIFs). They are beginning in SCVs and spread across the entire cytoplasm. SIFs' role in pathogenesis is unknown [1].

To hit the road...

After traversing the intestinal mucus layer *Salmonella* may be engulfed by 3 types of phagocytes: (i) macrophages, (ii) neutrophils, (iii) dendritic cells (DCs) [2]. Genes encoded within Gifsy-2 prophage, SPI-2 and few more (whose products are responsible for biosynthesis and nutritional substances uptake) enable *S. typhimurium* to persist and replicate inside macrophages *in vitro* [12].

In research on murine model the majority of bacteria transfer the mucus layer using M cells. Then they reach Peyer's patches and mesenteric lymph nodes, finally – a liver and a spleen. In general bacteria are translocated *via* lymphatic vessels inside dendritic cells [14].

Liver and spleen

The main function of virulence genes identified on the pSLT plasmid is promoting maintenance and replication in the liver and spleen [15]. In the latter one *Salmonella* cells have to coexist with huge amounts of macrophages. Experimental data shows that genes inducing chemical modification in LPS structure and promoting polymyxin (non-ribosomal polypeptide, antibiotic) resistance may be engaged in saving *S. typhimurium* from phagocytes' killing mechanisms [16].

In the next step of infection, bacteria start to produce lipid A which induces the production of cytokines and amplifies the oxidative burst in macrophages and neutrophils. Acute immune response may lead even to a host's death [1].

Host immune response

Cytokines are small proteins which play important role in cell signaling including cells of the immune system. In *Salmonella*-related infections, a wide spectrum of proinflammatory pathways is engaged through the activation of the innate and adaptive immune response [6]. Experimental results show that *Salmonella* may cause dramatic growth in a cytokine secretion from epithelial cells, macrophages and dendritic cells [17- 19].

IFN- γ and TNF- α are crucially important in the immune response against *S. typhimurium*. The first one control bacteria replication in the early stage of infection [20]. Production of IFN- γ is mainly localized in GALT (gut-associated lymphoid tissue) and the spleen. TNF- α amplifies the nitrogen oxide production by neutrophils and macrophages [21]. In naive *Salmonella*-infected mice TNF- α is secreted from phagocytes [22].

The majority of cytokines affect a faster defeat of the pathogen by the host organism. However, these small proteins may be called a double-edge sword. Some of them have negative or even destructive effect on host cells. *Per exemplum* MCP-1 and CCL-3 may protect bacteria from being killed as well as cause destruction of tissues through a tremendously boosted immune response [6].

Experimental data shows that products of genes *sopB*, *sopE* and some encoded within SPI-1 promote neutrophil activation, resulting in diarrhea and inflammation [23]. During salmonellosis, enterocytes are considered to amplify secretion of the IL-8 what also boost their ability to phagocyte [24]. Polymorphonuclear neutrophils (PMNs) recruitment seen in the histopathological examination of an intestinal tissue is a clear-cut hallmark of intestinal disease [6].

Virulence factors

S. typhimurium life strategy requires a majority of virulence factors (Tab. 1). They are necessary to overcome physical and immunological obstacles that the pathogen faces in the host as well as tackle the difficulties that come along with competition for a niche with commensal bacteria, high osmolarity, drastic changes of the pH level and energy source availability. *Salmonella* has to elastically adapt to changeable environmental conditions in extremely variable localizations at different stages of infection [2, 3].

The majority of virulence genes are located within conserved *Salmonella* pathogenicity islands (SPIs), some within pSLT, while another – in the bacterial chromosome. Products encoded within all five SPIs are involved in *Salmonella* pathogenesis. Adhesins, fimbria proteins, products of *spv* operon located on pSLT and factors connected with biofilm formation play an additional role [1, 6, 25].

Salmonella pathogenicity islands

SPI-1. *Salmonella* pathogenicity island 1 encodes proteins (called effectors) involved in mammalian cell invasion. They are engaged in cytoskeleton rearrangement and formation of membrane ruffles which enable bacteria to be engulfed. T3SS (encoded within SPI-1) is responsible for effectors' translocation. Genes of few chaperons were also identified [26]. Products of SPI-1 genes have 3 main effects:

- polymorphonuclear neutrophils (PMNs) recruitment (SipA effector),
- induction of NF- κ B signaling as a result of MAPKs' (mitogen-associated protein kinases) activation [27],
- caspase-1-mediated IL-1 β /IL-18 activation, host cells' death (SipB effector) [28].

After indirect contact between *S. typhimurium* and a host cell SipB effector is translocated into the second one's cytoplasm. The protein binds caspase-1 which causes a proinflammatory secretion of cytokines: IL-1 β oraz IL-18. Cell's death resembles apoptosis as well as necrosis and is named “pyroptosis” [6].

SPI-2. *Salmonella* pathogenicity island 2 is divided into two parts: the smaller one is the *ttrRSBCA* operon (encoding proteins involved in tetrathionate reduction), the bigger one consists of seven open reading frames (ORFs) of unknown function. SPI-2 is crucial to infection's success – it

enables *S. typhimurium* to persist and replicate inside epithelial cells and phagocytes [29]. SPI-2 genes products are also engaged in a cyclooxygenase induction and impact cytokines production [6].

SPI-3, SPI-4, SPI-5. Researches on the rest of *Salmonella* pathogenicity islands were not as in-depth as SPI-1 and SPI-2. There are a lot of identified open reading frames without a clearly defined function. However, they are important for the process of adhesion and long-term persistence of *Salmonella* [2].

Tab. 1. Virulence genes of *S. typhimurium*; based on [1, 30, 31]

Virulence gen (localization)	Function of encoded protein	Site of action	Specific environmental conditions
<i>atr</i>	<i>acid tolerance response</i> , ATR	stomach	low pH
fimbrial genes (chromosome) <i>sipA</i> (SPI-1) <i>svr</i> (SPI-4) <i>sopB, pipA, pipB</i> (SPI-5) <i>sopE</i> (chromosome)	invasion; apoptosis; chemokine production	small intestine	osmolarity pH oxygen
<i>entF</i> (chromosome)	iron uptake		iron
<i>mgtCB</i> (SPI-3)	magnesium uptake		
<i>sseF, sseG</i> (SPI-2)	replication inside macrophages	macrophages	cation levels P _i levels
<i>sodC</i> (Gifsy-2 prophage)	defence against macrophages' killing mechanisms		
PmrA-dependent Pags (chromosome)	Resistance to cationic levels; defence against macrophages' killing mechanisms		
<i>spvABCD</i> (pSLT plasmid)	replication	liver and spleen	cation levels pH
<i>waaN</i> (chromosome)	stimulation of proinflammatory cytokines production; cells' death induction		

pSLT plasmid

Among thousands of *Salmonella* serovars only a few have plasmids encoding virulence factors. However, almost every clinical strain of *S. typhimurium* owns at least one – named pSLT (95 kb) [2]. 8 kb-long region of the plasmid is highly conserved and contains five genes –*spvRABCD*. Their precise role in pathogenesis is imprecise. SpvB is cytotoxic protein, the SpvC is important in proinflammatory response, SpvA was detected in bacteria outer membrane and SpvD – outside the cell [32].

Conclusions

Salmonella is one of the most in-depth researched bacteria among human pathogens. Despite this, many mechanisms that govern its pathogenesis remain not entirely understood. Better understanding of *Salmonella* infection steps, virulence genes regulation, and interaction with host immune system are necessary to create a fast diagnosis as well as successful treatment for salmonellosis. Scientists should investigate how host’s adaptive immune system responds to *Salmonella* infection. Understanding this may lead to designing a treatment based on an immunity boost without danger of antibiotic resistance [6].

The bacterial cancer therapy is the idea of using particular bacteria species in oncology. It may be surprising and unbelievable because a lot of pathogens are tumor promoters rather than suppressors. They may disrupt cell signaling and accelerate cell division rate [33]. However, many facultative anaerobic bacteria have ability to colonize solid tumors *in vivo*. *S. typhimurium* is one of the most promising species in this regard. Some *Salmonella* strains have enormous potential for a tumor targeting therapy [34].

Salmonella spp. are bacteria of many faces. Research regarding the genus *Salmonella* is very promising and may lead to not only significant improvements in the understanding of microbial pathogenesis, but also to biotechnological advancements such as in the case of the aforementioned cancer treatment. Hence, even more in-depth study of these organisms should be at the forefront of microbiological research for the years to come.

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PIERRE BOURDIEU'S STRUCTURAL CONSTRUCTIVIST THEORY OF POLITICS AND RALF DAHRENDORF'S CONFLICT THEORY IN THE RESEARCH ON LOCAL SELF-GOVERNMENT

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Abstract:

The aim of the article is to show Pierre Bourdieu's structural constructivist theory of politics and Ralf Dahrendorf's conflict theory in the analysis of selected aspects of the functioning of the local self-government. This article focuses on the results of the author's own research that has been carried out in several rural communes from the Lesser Poland voivodeship. These theoretical concepts have been used to explain a range of relationships between officials and residents of local communities. Bourdieu's structural constructivist theory of politics has found application in the analysis of self-assessments of commune heads, opinions of residents regarding local government authorities as well as characteristics of commune heads and voters. The method of explaining social reality adopted in Dahrendorf's conflict theory was used to study election campaigns, reasons for the victories of current commune heads and to assess the chances of other candidates for their position.

Keywords:

commune head, electorate, commune, structural constructivism, conflict theory

Introduction

This article is an attempt to capture certain aspects of the relationship between residents and commune heads. The article concerns the issues discussed in two theoretical perspectives.

The first one is Pierre Bourdieu's structural constructivist theory of politics. For the purpose of this analysis it was used to discuss the following: the image of an active voter; failures and successes of commune heads; opinions of residents regarding local government authorities; characteristics of commune heads and voters; the perception of politics through participation in formal aspects of the local community.

The second perspective that has been used to describe social reality is Ralf Dahrendorf's conflict theory. It explains the issues relating to: reasons for the victories of current commune heads; the course of election campaigns and their meanings; the chances of winning of not elected candidates and the impact their efforts have had on the local community.

Theoretical basis

The first theory used in this article is Pierre Bourdieu's structural constructivist theory of politics. In his research, he assumes that the objects of social cognition that constantly surround us are constructed by us. He rejects the view that people could only passively perceive the world around them. Social constructs always exist within modifiable structures closely related to people. The structures are used for practical purposes, and when they are used, more of them is created. They affect the behavior of actors, but they exist completely outside of them [1].

The set of the above-mentioned structures is called habitus by Bourdieu. Habitus should be understood as the rules that generate and organize everyday life practices that can be objectively tailored to the goals of the individual. They are characterized by the fact that they are implemented without deliberate planning and making no specific effort to achieve them. They are regulated and they occur regularly, but are not the result of acting according to the rules. Although they are collectively constructed, they are not the product of the plans of any particular person [1]. They can be understood as a set of all the factors that make up a given individual. This also applies to political preferences and a certain worldview.

Thanks to the habitus being a product of structure, social practices can be created. These practices are patterns of activities and social behavior, taking any form, which can only be limited by the imagination and capabilities of individuals. All these phenomena occur in the so-called field. According to Bourdieu, the field can be defined as a network or configuration of objective relationships between positions of social actors. Positions are determined objectively due to their existence and conditions which they impose on persons or institutions that deal with them. They determine their current and potential situation (situs) in the structure of various types of power (or capital) [2]. Bourdieu equates the concepts of authority and capital. According to Bourdieu, they are not the goal of individuals, but they allow access to the benefits offered by the field. In order to achieve benefits, the actors are forced to participate in a constant game the rules of which are strictly ordered. These rules define the logic of the field. What allows participants to win is the best possible use of capital / power and knowledge of the rules [2].

Social class is another important concept in Bourdieu theory. The social class consists of habitus, capital and position in a given field. According to Bourdieu they are the natural consequences of the human need to classify and organize reality [3].

The second theory used in this article is Ralf Dahrendorf's conflict theory. It assumes a coercive theory of society, according to which every society is subject to change. These changes are ubiquitous and incessant. Changes lead to disagreement and ongoing conflicts. Some members of society contribute to general disintegration and change by applying coercion to others [4].

Relationships of power and sovereignty are crucial for the existence of the social structure discussed in this theory. Coercion organizes everyday reality. However, power relations are not

immutable but are subject to constant change. Therefore, those who are currently in power want to maintain the current order. To this end, they use many techniques and control systems for other citizens [4]. Those who are subordinate have at least partly different interests from those in power. Dahrendorf divides these interests into open and hidden ones. All behaviors imposed on actors are hidden interests. Open interests do not appear until the individual fully realizes the purpose of his own actions [4]. In order to seize power, it is necessary to unite many individuals who will form a group of interest. The group of interest is organized and its members have a sense of belonging to this group [4].

According to Dahrendorf, after reaching the appropriate stage of organization, groups of interest engage in conflicts that result in structural changes [4]. Conflicts can take many forms. Political conflicts are usually resolved by means of elections. In this situation, the conflict is regulated by certain rules [4].

Ralf Dahrendorf's conflict theory refers to how the structure changes with the resolution of the dispute. Dahrendorf distinguishes three types of transformation: Complete or almost complete exchange in positions of power; partial change; minor changes. Minor changes are, according to Dahrendorf, the most important. They can quickly lead to another conflict because they only give the appearance of victory [4].

Method and selection of respondents

The research described in this article has been conducted with the inhabitants of four rural communes of the Lesser Poland voivodeship. The method in question was the in-depth interviews. They focus on talking to respondents about the issues contained in the previously developed guide [5]. This method had been chosen because it allows you to create an atmosphere of free exchange of thoughts.

The analyzed material consists of thirty-two recorded conversations. Twenty-eight of them were interviews with the inhabitants, and four interviews with the heads of the selected communes of the Lesser Poland voivodship. Individual interviews were conducted from January to April 2017. They were conducted by using two guides - for commune heads and village residents.

The selection of the sample took place in two stages. Firstly, communes were elected (as well as commune heads). Secondly, village residents were elected. Details of the municipalities themselves are not subject to analysis. However, they were taken care of to represent four different districts: krakowski, wielicki, myślenicki, nowosądecki. The respondents asked for anonymity, therefore their communes were assigned numbers from 1 to 4:

- Commune number 1 – district krakowski – current commune head since 2014;
- Commune number 2 – district wielicki – current commune head since 2010;
- Commune number 3 – district myślenicki – current commune head since 1990;
- Commune number 4 – district nowosądecki – current commune head since 2006.

The selection of the sample of voters took place in a much less rigorous manner. Seven people were asked to talk in each of the communes. The only requirements they had to meet were:

- Registration as an inhabitant in a given commune since the 2010 local elections;

- Voting in local elections;
- Possession of full electoral rights since 2010.

This allowed avoiding interviews with minors, visitors and those who were not interested in the local government matters.

Analysis of research results

The last part of the article shows selected aspects of the relations of commune heads with residents of four rural communes from the Lesser Poland voivodeship. They were shown in division into two theoretical approaches. Pierre Bourdieu's structural constructivist theory of politics and Ralf Dahrendorf's conflict theory were used here.

Pierre Bourdieu's structural constructivist theory describes self-assessments, opinions and characteristics of commune heads and voters.

An important issue for this article is the one which is concerning the profile of a typical active voter. Residents and commune heads were asked to characterize both persons voting for the current commune head and other candidates. In most cases, their responses were based on personal preferences. If the respondent voted for the current commune head, he ascribed exclusively positive qualities to himself and other voters alike. In such cases, commune heads were also praised. The voters pointed out that their representatives were very similar to the inhabitants and came from the same local environments. The respondents expressed negative opinions about voters and other candidates. The commune heads themselves answered much more calmly. In their descriptions, active voters are mainly young and educated people.

Another issue concerns descriptions of failures and successes of commune heads. Officials gladly answered this question. They did not mention failures, but focused on what was achieved. The commune heads pointed to the supervision of strategic investments, such as road and school repairs or the construction of waterworks as their successes. Mayors with longer experience described them as the achievements of a whole group of people. Two officials with just a few years of experience attributed merits only to themselves. When it comes to voters, division is much simpler, which means that it depends on the declared support for officials. Supporters of commune heads focused on how much has been done in recent years. People against the commune head indicated failures and shortcomings. There were also accusations of all kinds of abuse.

It is also important to summarize the respondents' opinions on the performance of functions by commune heads. Commune heads and voters were asked to assess the work of officials. The commune heads responded very modestly and assessed their work moderately well. In their opinion, most active voters cast their votes in their favor. Opinions of residents were more diverse. Voters declaring support of officials in other matters also in this case expressed only positive feelings. It was similar among opponents.

The last issue described in this part concerns the extent to which the participation of residents in the public life of the commune affects opinions on local politics. Commune heads were asked to assess the involvement of residents in the affairs of the local community. All the answers were similar. They described the participation of residents in all kinds of associations, clubs and

organizations. According to the commune heads, many residents had access and willingly used the opportunity to participate in the social life of the commune. The voters said quite differently because only a few people declared participation in any meetings or events organized in communes. If someone declared to participate in such events, they pointed out that they did not affect his views.

Pierre Bourdieu's structural constructivist theory of politics presupposes the existence of habitus as products of structure. This can easily be described by the example of local communities analyzed. This was manifested during conversations with respondents. The voters paid a lot of attention to whether the commune head was similar to them. It was about both lifestyle, tastes and way of speaking. The class aspect was also important. Voters sympathizing with officials pointed out that the commune heads and they were from the same social class. Another concept is the field which will constitute the commune in this case. In this approach, the commune head occupies a central position in the field. As each unit tries to defend its status, commune heads with only a few years of experience emphasize their merits. More experienced know that power itself is only a means to achieve the benefits offered by a given field. In this case it is a financial aspect and job satisfaction. They are also familiar with the logic of the field. That is why they stay in power for so long.

Another way of explaining social reality described in this work is Ralf Dahrendorf's conflict theory. It refers to election campaigns, winners and chances of other candidates.

One of the issues discussed in this passage are the reasons for the victory of commune heads in their and resident's opinions. Respondents were asked to indicate what determined the results of the last elections for the commune head. The commune heads drew attention to the satisfaction of voters with their achievements to date and great confidence. Everyone claimed very positive qualities. The voters who re-elected the same commune heads appreciated their work for the commune. The most interesting were the answers of respondents from the Krakowski district. Their commune head held the position only since the last elections. In the eyes of the inhabitants, he began his career as an ambitious employee and gradually gathered more and more followers around him. He was credited with being a real leader.

The next issue concerns the course of election campaigns and the meanings attributed to them. This topic was often raised during interviews. The commune heads were describing their campaigns as planned and substantive measures. They mentioned organizing meetings, hanging posters and visiting the homes of their potential voters. In this case, the inhabitants had a completely different opinion. In each commune, residents mentioned a fierce campaign. They described incidents related to the breaking of posters, mutual defamation and a hostile attitude. These issues were described as a conflict of enemies. The interviews presented disputes for which the candidates devoted a lot of energy and resources.

The third and final issue concerns determining the chances of unsuccessful candidates and the impact of their efforts. Commune heads were reluctant to comment on their political rivals. They limited their opinions only to statements that they were well known to them. The vast majority of voters described the commune heads as favourites in elections. Despite the failures, campaigns of those who were not elected have made a big impact on whole commune. Residents considered them

as a source of motivation for commune heads. Thanks to the opposition, they constantly strove for the good of the inhabitants.

According to Ralf Dahrendorf's conflict theory, everyday reality is based on constant conflict. It perfectly fits the issue of commune heads elections. The relations of power and sovereignty and cyclical changes are also pivotal here. For the commune of the krakowski district, it meant a change in the position of the dominant power. The commune head of the village gathered his election committee and together they stood against the current local authorities, achieving success. However, this involved putting a lot of energy and resources into campaigns. During the campaign all available methods of electoral agitation were used. Since the fight was fought for the highest available position in the commune, one can speak of a complete change in the position of power.

Conclusion

This article presents selected aspects of the relationship of commune heads and voters of four communes of the Lesser Poland Voivodeship. They were described by means of two theories. Using Pierre Bourdieu's structural constructivist theory issues regarding peer reviews of local government representatives and residents of municipal communities were clarified. The similarities of habitus which are sets of factors constituting the identity of each individual were pivotal. The more the commune heads were recognized as members of the community, the better they were rated. Ralf Dahrendorf's conflict theory concerned issues directly related to elections. Thanks to its application, it was possible to grasp aspects accompanying changes in the highest positions of communes authorities.

Although the above issues are closely related to the specificity of the region, they serve to show universal mechanisms for Polish rural communes. The considerations described in the above article should be treated as a starting point for larger projects. The author's analysis presented here is a step towards a new, bottom-up study of the specificity of local governments.

Currently, it is implemented by the author in order to capture pre-election relations at the level of the voivodeship assembly.

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EFFECTS OF CAFFEINE ON HUMAN HEALTH

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Abstract:

Coffee is one of the most commonly consumed beverages in the world. The available evidences reported that caffeine intake has a protective effect against heart failure hypertension, cardiac arrhythmia, stroke, and all-cause mortality. Another studies suggest that high caffeine intake can increased urinary calcium excretion and inhibition of osteoblast proliferation which has negative impact on bone metabolism. For many years caffeine has been used as an adjuvant in the treatment of pain by combining it with OTC analgesic medications. Caffeine includes the perception of alertness and wakefulness, both rested and tired people. It is also effective in reducing the risk of Alzheimer's Disease. Its health benefits include liver disease as well, especially related to progression of liver fibrosis to cirrhosis, hepatocellular carcinoma and virological responses to antiviral therapy in chronic hepatitis C. Therefore, in patients with following disease, daily coffee consumption should be encouraged.

Keywords:

caffeine, heart failure, impact, benefits

Introduction

Caffeine use is increasing worldwide. Caffeine is an organic chemical compound that can be found in several plant raw materials. Coffee, tea, caffeinated soda, and energy drinks are important sources of caffeine in the diet. It is estimated that caffeine in various forms is consumed by nearly 90% of the adult population every day. There is increasing public and scientific interest in the potential health consequences of habitual intake of these caffeine-containing beverages (CCB). Epidemiological studies support a beneficial role of moderate coffee intake in reducing risk of several chronic diseases. For people who value their *balanced diet* and healthy lifestyle scientific studies shows that moderate coffee drinking is completely safe and does not pose any health risk [1].

Materials and methods

The aim of the study is to statistical data the literature and present the current state of knowledge on the impact of the caffeine on human health based on statistical data and literature review. Additionally, during the literature review on PubMed and Google Scholar platforms, keywords such as caffeine, heart failure, impact, benefits were used.

The pharmacokinetics of caffeine

Caffeine is a well-known stimulant that affects numerous neurotransmitters and hormonal signaling pathways. This substance contains three methyl substituents, which facilitate her penetration to central nervous system [1]. Caffeine is an antagonist of several adenosine receptors (A1, A2A, A2B, A3). Those receptors are located in the central and peripheral nervous systems, as well as in various organs, such as the heart and blood vessels. Adenosine is a compound that accumulates in a neuronal synapse during constant wakefulness. This molecule by binding with receptors stimulates many biochemical pathways, mostly those for energy transport and signaling. Cellular response that is produced is responsible for increasing drowsiness or worsening memory. Caffeine by blocking adenosine receptors prevents this effect [2]. Moreover, the antagonistic effect on adenosine also promotes the release of the neurotransmitter such as monoamine or acetylcholine, which gives a stimulant effect [3].

Absorption and Metabolism

Caffeine is absorbed swiftly and completely by the small intestine within 45-60 minutes after ingestion and rapidly distributed throughout other tissues [4, 5]. Absorption by the small intestine does not seem to vary by sex, genetic background, environmental factors, or other variables [6]. Maximum caffeine concentration in saliva is after 45 minutes and in blood after about 2 hours [5, 7]. Caffeine has a relatively long biological half-life. In healthy adults the time required for the body to eliminate one-half of a dose is between 3 and 7 hours [2]. These values may vary widely among individuals according to different factors such as age, pregnancy or condition of internal organs. During pregnancy this time can be 8-16 hours longer [8, 9]. In newborns, because of their immature kidneys and liver, the half-life time lasts between 65 and 130 hours [10]. Smoking decreases the half-life by 30–50%, while oral contraceptives can double it [11].

Caffeine is metabolized to 1,7-dimethylxanthine (paraxanthine) mainly in the liver by the cytochrome P450 system in particular by the CYP1A2 enzyme. CYP1A2 is characterized by high genetic variability. At least 150 single polymorphism can accelerate or decelerate caffeine metabolism [12] The vast majority of caffeine is eliminated from the plasma by the kidneys (85-88%), although fecal excretion also takes place to a limited extent (i.e. about 2-5%) [6, 13].

Caffeine impact on cardiovascular system

Cardiovascular diseases (CVD) are one of the most common causes of death in the world. Caffeine works in various mechanisms, but inhibition of the adenosine receptor seems to be the most important in perceived cardioprotective properties [14]. Researches have confirmed that A1

receptor activation has a number of effects in the cardiovascular system. It affects the reduction in heart rate and atrial contractility, and the attenuation of the stimulatory actions of catecholamines on the heart. The A_{2A} receptors are involved in vasodilation in the aorta and coronary artery. The blockade of these receptors by caffeine can contribute to the protective effect of coffee in CVD [15]. Several studies and reviews of prospective and randomized studies involving many subjects have demonstrated either a beneficial or a neutral effect of coffee and caffeine consumption contained in it with CVD, hypertension, cardiac arrhythmia, stroke, and all-cause mortality [16].

The previous perception that coffee drinking *could cause arrhythmia* has not been supported by recent studies. One of research studies indicate that the acute ingestion of high doses of caffeine did not induce arrhythmias in patients with chronic systolic HF at rest and during a symptom-limited physical exercise [17]. Other review and meta-analysis of 434 patients with preexisting arrhythmias showed no worsening with coffee consumption [18]. *With regard to* coffee consumption on the incidence of atrial fibrillation (AF), two large prospective cohort studies, with two reviews and meta-analyses, showed no significant effect of coffee consumption on the incidence of AF [16].

Infarction means necrosis of an organ or tissue caused by ischemia. An acute myocardial infarction (AMI) is usually caused by a rupture of the atherosclerotic plaque in a coronary vessel, a vessel that brings blood to the heart. A total of 50 observational (40 cohort and 10 case-control) studies evaluated the potential relationship between caffeine intake at various and acute myocardial infarction relative risk. Most of the available studies (37 of 50) demonstrated no statistically significant increased risk or a significantly decreased risk (protective effect) of AMI with caffeine consumption compared to non- or low- consumption. The majority of the evidence indicates that caffeine intake does not increase the risk of AMI. Some evidence suggests that some sensitive subgroups, including those with a specific genotype (e.g., CYP1A2 or COMT variants) may be more susceptible to the potential CVD effects of caffeine [19].

Heart failure is a condition in which the heart is not able to pump the amount of blood into our body's organs. The most common conditions that can lead to heart failure are CVD, high blood pressure, and previous AMI. Five cohort studies evaluated the potential relationship between caffeine and heart failure. The available evidence suggests that caffeine intake is not associated with an increased risk of heart failure. Moreover, the largest cohort study reported a protective effect against heart failure among some study participants [19]. Literature suggests that moderate caffeine intake is not associated with increased risks of total cardiovascular disease, arrhythmia, heart failure. Contrary, consumption of moderate amounts of caffeine is associated with a reduced risk of cardiovascular diseases and may even be protective.

Caffeine impact on bone metabolism

Although moderate caffeine intake is believed to have a positive effect on human health, its impact on bone metabolism remains controversial. Studies suggest that high caffeine intake can cause increased urinary calcium excretion, inhibition of osteoblast proliferation and delayed tissue repair, increasing the risk of fractures, osteoporosis, periodontal disease, and affecting the success of bone reconstruction procedures [20]. Several studies conducted in recent years have shown the

correlation between caffeine intake and calcium absorption. The majority of them showed that moderate consumption of this substance does not harm bone health. However, excessive doses of caffeine may cause higher calcium excretion, thus increasing the risk of osteoporosis [21]. In experimental conditions, caffeine at high doses has been shown to inhibit osteogenesis, increase osteoblastic apoptosis, and differentiation of osteoclasts. In animal models, high caffeine intake inhibits bone tissue growth and causes a decrease in bone mineral density [22]. Caffeine may have a beneficial effect on the skeletal system of rats after the removal of ovaries, slightly inhibiting the development of bone changes caused by estrogen deficiency. It increases bone mineralisation and improves the strength and structure of the spongy bone [23]. Chronic caffeine intake also decreases growth hormone secretion. Thus, it is conceivable that caffeine may disrupt bone growth during the peripubertal period. Studies on animal models have shown that in the group of rats which were given caffeine, it caused a significant decrease in body mass gain. This was accompanied by proportional decreases in lean body mass and body fat [24]. Another study conducted on rats showed that the negative effect of caffeine on bones depends on the growth rate. When the growth rate is faster, the side effects are more severe. However, high caffeine intake in young adults may also lead to severe bone mineral loss, which may increase the risk of osteoporosis and fractures at a young age [25]. The results of the study conducted in the Asian population suggest that drinking coffee ≥ 4 cups a day is associated with an increased risk of femoral neck fracture. On the other hand, drinking 2-3 cups of coffee a day may reduce the risk of femoral neck fracture in postmenopausal women [22]. Hallstrom et al. reported that the consumption of ≥ 560 mg of caffeine (≥ 8 cups) was not associated with higher percentage of hip fractures in the comprehensive assessment of long-term coffee consumption compared to the risk of fractures and BMD in women [26]. The same researcher also demonstrated that consumption of caffeine ≥ 237.5 mg/day was associated with a 4% lower BMD of the proximal femur compared to low or nonconsumers of coffee in a large population of Swedish men aged 72 years. Barbour et al. reported that higher caffeine intake of 520.7 mg/day was associated with lower cortical and trabecular volumetric BMD in men ≥ 69 years of age. Another study reported that the rate of bone loss at the spine was higher in a group of high-caffeine consumers compared with low-caffeine consumers [27].

Effects on the Central Nervous System

Except for its influence on cognitive performance, it increases the perception of alertness and wakefulness, both in rested or fatigued individuals. These effects are dose-related. While lower concentrations of caffeine stimulate the locomotor activity, the use of high doses can also induce an anxiogenic-like effect [28, 29]. It has been found that panic disorder patients (PD) show an increased sensitivity to the anxiety-generating effects of caffeine. Since caffeine acts as an adenosine receptor antagonist, the correlation between this disease and the gene polymorphism for the A2 adenosine receptor was found [30]. Other studies also showed that high caffeine intake is positively associated with various psychiatric disorders including generalized anxiety disorder, panic disorder, antisocial personality disorder, major depression, alcohol, cannabis or cocaine abuse [31]. In addition, data presented by the O'Neill's team emphasized that caffeine consumption during adolescence has an impact on the expression of behaviours associated with anxiety and

neuroendocrine functioning. This disorder persists in adulthood, even in the absence of continuous consumption of caffeine [32]. However, other studies showed that caffeine exerts little impact on complex judgment and risky decision-making [29].

As it comes to the effects of caffeine on attention performance, it affected both reaction times and accuracy on a variety of simple tasks. It also had an impact on enhancing higher-order processes which were involved in the active monitoring and coordination of behaviour [33]. Study conducted by Konishi's team have investigated the effects of a single instance of caffeine intake on executive functions. They proved that the single intake significantly shortened the response time in the Harsh-braking test. It confirmed the finding that caffeine increases mental arousal [34]. For many years caffeine has been used as an adjuvant in the treatment of pain by combining it with OTC analgesic medications, such as APAP, ASA, and IBU. By the antagonization of adenosine A1 and A2 receptors it can cause seizures and cerebral vasoconstriction. This action is implicated in headache relief. In many studies, acute caffeine intake has been shown to cause spontaneous analgesia in headaches [28]. The appropriate use of the caffeine may significantly enhance the effectiveness of analgesics and nonsteroidal anti-inflammatory drugs (NSAIDs) in the treatment of patients with migraine and tension-type headache (TTH). Additionally, it may reduce the amount of doses of acute medication needed to successful treatment of migraine attack [35]. Moreover, caffeine and caffeine-containing analgesics are current first-line treatments in the treatment of hypnic headaches. This ailment is characterized by waking patients from a night's sleep at a fixed time, usually in the early morning [36].

Effects on the Alzheimer's Disease

Alzheimer's disease (AD) is the most common neurodegenerative disease causing dementia in the elderly population. Caffeine exerts its effects through different pathways, including the antagonism of adenosine receptors, inhibition of phosphodiesterase and activation of ryanodine receptors. Sufficient caffeine consumption both in middle-aged people and in the elderly has been correlated with decreases in risk of cognitive impairment and AD. Additionally, treatment with dietary antioxidants, such as caffeine, may be effective in reducing the risk of AD in individuals carrying the ApoE ϵ 4 allele [37, 38, 39]. The analysis of Wu et al. stated that the lowest risk was observed at the daily intake level of 1–2 cups of coffee [40]. Additionally, Eskelinen's follow-up study found that moderate consumption (3- 5 cups) of coffee substantially reduced the risk of AD (62%- 64%) and dementia (65%- 70%) later in life, compared to low coffee consumers (0- 2 cups) [38]. Lindsay et al estimated that regular coffee consumption among 4615 healthy older adults (>65 years of age) was associated with a 30% decreased risk of developing AD over 5 years [41].

Caffeine impact on liver diseases

Coffee consumption was associated with improved serum gamma glutamyltransferase, aspartate aminotransferase and alanine aminotransferase values in a dose dependent manner in individuals at risk for liver disease [42]. Recently, coffee consumption has been inversely related to progression of liver fibrosis to cirrhosis and even hepatocellular carcinoma. We recently reviewed the retrospective studies investigating associations between coffee consumption and

changes in liver enzymes, liver fibrosis, and HCC in patients with a variety of chronic liver diseases [43]. These data have been confirmed in a recent prospective trial using noninvasive measurement of liver fibrosis in a healthy population, in which advanced liver fibrosis was inversely associated with coffee consumption, even when controlled for a variety of potentially relevant confounding variables [44]. Liver myofibroblasts, which are the effector cells in liver fibrosis, express the A2a adenosine receptor, which is coupled to a variety of phenotypic changes that are profibrogenic. Most importantly, A2a activation links directly with matrix production. Caffeine as an active ingredient of drinks and drugs is a pan-antagonist of adenosine receptors (including A2a) [45]. The recent article by Arauz et al. is one of the first to compare decaffeinated and caffeinated coffee. Using common bile duct ligation, a model of biliary cirrhosis, the investigators showed that direct and indirect markers of liver fibrosis were blocked by caffeinated coffee or caffeine, but not by decaffeinated coffee. However, it is critical to note that the rats were "overdosed" if the scaling between rats and humans is linear [46]. Moreover, several studies have examined the impact of coffee consumption in chronic hepatitis C patients [47]. Coffee was associated with improved virologic responses to antiviral therapy. Freedman et al. demonstrated that greater than 3 cups of coffee daily was an independent predictor of improved virologic response to retreatment with peginterferon plus ribavirin in patients with hepatitis C who failed initial treatment [48].

Summary

Caffeine intake has a positive effect on reducing heart failure hypertension, cardiac arrhythmia, stroke, and all-cause mortality, many neurological disorders such as Alzheimer's Disease and liver diseases. However, it can increased urinary calcium excretion and has negative impact on bone metabolism

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ARCHITECTURAL CHALLENGES IN DESIGNING APPLICATIONS FOR AUGMENTED REALITY GLASSES

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Abstract:

The article discusses the classification of augmented reality glasses and the approach to creating application architecture for these devices. The actual process of making architectural decisions leading to the construction of a working production application such as mobile outdoor navigation is presented. The optimal method of making this kind of decision was formulated, giving its real effectiveness in the context of project errors.

Keywords:

Augmented Reality, AR glasses, software architecture, software design

Introduction

Development of software for augmented reality glasses computer devices requires a new decisions in the field of user experience and extensive knowledge of the technical capabilities of this type of device. In this extremely dynamically changing field, it is necessary to select basic classes of devices and formulate an effective design method for these devices of the appropriate application architecture. In the article we present both the classification of glasses and the method of making design decisions leading to the successful creation of solutions of this type.

Smartglasses classification

Below there is a classification of devices belonging to the group of smart glasses, computer equipment worn on the user's body. The classification created by the author is based on differences in the scope of: possibilities of data processing power as independent computers, having the function of analyzing the position of the eyepiece in space (SLAM), the ability to display and position 3D objects in space, owning or not own computer.

This allowed to create 4 main groups of devices:

1. Eyewear data stream Displays: AR Displays.
2. Augmented Reality Eyewear Computers: AR Glasses.
3. Mixed Reality Eyewear Computers: Mixed Reality Glasses.
4. Eyewear Mixed Reality Displays: Mixed Reality Displays.

In practice, each of these groups of devices requires different software design decisions. Below is a description of the device classes given.

Eyewear data stream displays: AR Displays

Devices belonging to this group are the most basic solution for presenting information to the user's eyes. They do not have their own computer with an operating system but only receive data packets from external devices.

The model eyepiece of this type is Epson BT-35E. This device connects via a HDMI port to video data streams. The displays present a binocular clean image in front of the user's eyes in a relatively high resolution. It is not possible to control this image - instead, you can send simple signals to the image transmitter type: Play / Stop. The eyepiece may have an IMU in order to, for example, rotate in a spherical film but may not perform operations other than just passive display.

Data stream displays can always be used where there is nothing more than just passing video stream from cameras and monitoring systems to the user's eyes.

Augmented Reality Eyewear Computers: AR Glasses

The typical of Augmented Reality eyepiece should meet the following requirements:

1. Own computer and own operating system
2. Display with FOV (field of view) covering at least 14 degrees of field of view
3. 9-axis IMU (accelerometer, compass, gyroscope)
4. Additional sensors: intensity of the surrounding light, pressure sensor, others
5. Vision camera
6. Ability to communicate via Bluetooth, wifi, USB
7. Microphone
8. Audio speakers

The technical parameters of these components differ from one model to the next. It is assumed that the eyepiece belonging to this group will correctly display pictograms, inscriptions, pictures, video but it is not intended to generate complex 3D objects that could be rotated in space.

The fields of view of Augmented Reality glasses are 9 to 23 degrees and the dominant resolution is 480px. Almost all AR glasses are monocular. This is probably due to the need to ensure energy efficiency with the generally acceptable human use of one eye. The light stream in these glasses is emitted from the side of the eyepiece. This does not allow to achieve really large AR vision because in the currently created optical systems only streaming from the top allows it (it is visible in MR glasses). These glasses have basic control options usually based on a touchpad in the eyepiece itself or external devices: a touchpad pinned on a cable, a Bluetooth joystick. Some of them have recognition of simple voice commands. An important element of their operation is a Companion application installed on a smartphone from which you can manage your eyepiece or use it as a virtual touchpad (Vuzix Blade).

Designers try to make these glasses not different from classic glasses (this type of effect was achieved in North Focals and Vuzix Blade glasses). If the equipment does not pretend to be ordinary glasses because it is, for example, an external display mounted on an ordinary eyepiece, then the designers' efforts are aimed at creating an elegant and aesthetic device. Google Glass 2.0 is a great example.

In terms of the number and type of sensors, these glasses do not have the function of SLAM (Simultaneous Localization and Mapping) and thus environmental analytics. These devices orient themselves in space using the IMU: accelerometer, gyroscope, compass + GPS. It is possible to enrich this orientation using real-time image analysis. Working time on these devices rarely exceeds 3 hours (on own battery). In the case of North Focals, the working time actually includes 18 hours, but it is paid for by a much smaller display and frequent switching to standby mode.

The group of basic devices of this type includes: Vuzix Blade, North Focals, Epson Moverio BT300 and BT350, Google Glass 2.0, Madgaze X5.

Importantly, the preparation of more complex solutions for these glasses usually requires processing in a mobile device of the smartphone type or direct communication with efficient API servers serving their services. The eyepiece should receive processed data so that the logic performed on it is maximally reduced.

There is a subgroup of AR glasses dedicated to the industries. It includes the Vuzix M300XL, Vuzix M400, VuzixM4000 and RealWare HMT1 models. In the case of industrial AR glasses, application architecture is mainly based on processing on the eyepiece itself, without the need for additional processing on the smartphone. The AR glasses are becoming a full-size mobile device that is expected to be processed like on an efficient tablet.

As a result of all these changes, AR Glasses are able to display e.g. 3D solids located in a marked space. This solid can be rotated but it is not possible to move around it in space.

The AR eyepiece has enough computing power to handle complex voice commands and analytics of user gestures performed in front of the eyepiece camera. This type of equipment will be a good device for industrial applications because it is able to perform complex data processing without requiring anything more than connecting to backend systems via wifi. In this type of device the field size outside the AR area is very important. Due to the risk of an accident, this space must be as unobstructed as possible.

Mixed Reality Eyewear Computers: Mixed Reality Glasses

A group of devices that are somewhat aware of their surroundings, i.e. powerful computers worn on their heads, analyzing in real time the place where the user was (SLAM).

The MR eyepiece has several cameras and sensors enabling it to accomplish this task.

The MR eyepiece has all the features of an AR eyepiece but also adds:

1. SLAM.
2. Additional cameras and sensors.
3. Additional control methods (gesture, head movements, eye movements).

Similarly to the previous group, processing this type of information raises requirements for computing power and energy consumption. The group of these glasses includes two models: Microsoft HoloLens 2 weighing 566 grams and relatively light because weighing 180 grams Thirdeye MR X2.

Microsoft HoloLens 2 has the richest on the market the ability to analyze the environment, can track the movements of the user's eyeballs, recognize gestures, sounds. It is a device designed for very demanding users. Thirdeye slightly subsides in some areas, adding a rather unique way of controlling the eyepiece by moving the head.

MR glasses aim to cover reality a lot, which is why they have wide fields of view, clear and colorful 3D objects. On the one hand, it is a sensational complement to the real world, and on the other, it is a serious disturbance of reception of the real world and its significant obscuring.

The surface observed outside the AR view in the Thirdeye eyepiece is small, it takes no more than 100 degrees from the conventional 180-degree viewing angle of a person without glasses. Therefore, business applications for MR glasses must consider a significant level of information reduction in user orientation in space and address the risk of an accident. HoloLens 2 glasses are open from the side, which allows the least to obscure the real world and reduce bumps and trips.

The lifetime of glasses with such capabilities is limited to 2-3 hours on one battery pack. The batteries can be replaced or the eyepiece must be connected to a power bank.

The MR eyepiece completes reality with complex 3D objects that can be rotated and viewed in any way. It is characteristic for these devices that the 3D object is hung in a certain place in space and you can walk around and transform it as expected.

Eyewear Mixed Reality Displays: Mixed Reality Displays

The intensive research and development works of two great investors, i.e. MagicLeap and Microsoft, inspired other companies to create surprisingly effective solutions in a different architecture. Similar to the companies mentioned above, the goal was to achieve a large Field of View ready to handle complex 3D objects - while having the SLAM function. However, the location of the computer was solved differently - by taking it out of the eyepiece block outside. A new branch of glasses was created that does not have its own computer consuming calculations performed by other units.

These devices use a mechanism of light streaming from above, using a set of small mirrors (in a similar form as it is in HoloLens). Thanks to this, a Field of View of 42-52 degrees was obtained at 720pix resolutions, which is a really sensational result. This was not without compromise. The optics of these glasses have characteristically obstructed upper parts of the field of view, which means that the user must unnaturally lift his head in order to see objects above him. The MR display is relatively light but stands out from the head. It is impossible to hide the large size of the optical system. The computer comes either from a smartphone or from a dedicated computer device, exactly as Epson solved in the models Moverio BT300, BT350.

Separation of processing into two devices is a step that may prove to be the key from the point of view of the low weight of such a eyepiece with relatively low nuisance of keeping a strong laptop

or smartphone with you. One should not forget about another important addition to such a set is a rich power bank.

Examples of devices of this type are Nreal, Madgaze Glow and OGlasses Real-X.

AR Glasses software architecture design approach

We will now carry out a decision-making process leading to key architectural decisions in the development of software applications. Below we describe how architectural decisions were made for the VEO Navigation application: land-sea navigation, online / offline supporting vehicles: yacht, kayak, cars, motorbikes, bicycles, walk, skier. The presentation of navigation data is carried out on several glasses belonging to our group of AR glasses but it is possible to run the application on any eyepiece with the Android 6.0+ platform having 9-axis IMU and Bluetooth.

Must-do requirements that had to be handled were:

1. Work online / offline - this forces the existence of large data sets on devices that support navigation.

2. Marine mode and land mode - this forces different styles of navigation on land and on water, different units of measurement, different dynamics of changes in the application during navigation, integration with on-board devices or lack thereof.

3. Data display on the AR eyepiece in the form of a 3D sphere with objects superimposed on the real world - the assumption of the project was to display data on the AR eyepiece, the decision was made in 2017, when there was no eyeglass on the market yet it was to be sure that it would do the job.

4. Navigation control should take place on the phone, due to the high complexity of operations that are performed when setting navigation, downloading offline files, searching for objects etc. It has been found that the simplified UI interfaces of the glasses will not be able to configure the navigation task efficiently and correctly read all route data. With the emergence of glasses capable of complex control of objects and data, this requirement has been moved to ordinary (not mandatory) requirements.

5. We cannot be dependent on global map and data providers in these Google maps due to licensing restrictions, price list volatility, architectural enforcement - it was necessary to base the architecture on publicly available data from OpenStreetMaps and own data.

Step 1: Technical and business feasibility. It consists in defining a list of key requirements, i.e. requirements that are not negotiable, and on this basis feasibility studies are conducted based on the technical capabilities of the devices on the market. On this basis, devices that are unable to perform specific tasks are rejected or the equipment is too expensive for our model user. In extreme cases, no eyepiece can meet the requirements, which will stop the project completely. This stage is carried out over several analytical cycles between Product Manager and Solution Architect. Iterations are carried out until the Product Manager is able to provide a valuable software product and the Architect finds feasibility on at least several devices at a price acceptable by the customer.

Step 2: Design system components. It consists in proposing the optimal structure of components with the allocation of the type and scope of processing on a particular one. Obtaining

knowledge to make such decisions is problematic and requires a number of studies. As a result, we get layers of the solution that are processed on individual devices as part of various components.

Step 3: Technology selection. It consists in the selection of technology and appropriate ready components on individual layers of the solution. Factors such as the functional scope of existing development environments and finished software components, the cost of software production, and the flexibility of future software in terms of changes are decisive.

Step 4: Integration methods. It consists in choosing integration methods between layers. We choose from RESTAPI methods, GraphQL API, class sharing between technologies, methods based on file transfer.

Step 5: Ensure flexibility of the solution. In this aspect, we assess how individual system components are able to switch to other technologies or devices - e.g. conversion of Android to IOS in a mobile device or replacement of one eyepiece for another. We try to correct the created architecture so as not to close the path of future development.

It is worth adding that the full decision-making process regarding the shape of the solution's architecture may take many months. It strongly depends on the devices available on the market and can last "in the background" throughout the entire 18-month project period. In fact, the creation of IT architecture never ends because on the dynamic IT market there are often smaller or larger technological breakthroughs opening new perspectives for created solutions. This approach is therefore iterative and we present it in Fig. 1.



Fig. 1: Approach to architecture design in an AR glasses project

Summary

Both the method developed and the decision-making process carried out in the project were assessed as optimal in terms of quality in the market conditions of this project. Measuring the quality of this process is possible and can be measured by the number of code refractors and the number of components listed. In the analyzed case, about 10% of the budget was consumed for the software refactor of a given component. This refactor twice resulted from an error as to the selected component (Graphhopper-Valhalla in the mobile application and once from the change of the authorization module in Backend). Several times with smaller errors in the approach taken by programmers. About 5% of the budget was consumed to remove the component and embed another. Some of these errors were due to gaps in knowledge during planning, and some were deliberate efforts to discover certain phenomena, and the effects of this research can be written off. However, losses were rare. The standard effect of the research was to use the initial worked out code for further stages of work.

Was it possible to avoid these excessive costs? If we had assumed that at least one full project of this type would have been carried out earlier, the answer is yes. The knowledge acquired during an exhaustive project in this field can be used to further minimize errors and reduce the delivery time of a similar project..

Literature

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THE STRUCTURE OF BOTANICAL NOMENCLATURE. A POLYCONFRONTATIVE STUDY

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Abstract:

In the article the author concentrates on the analysis of Latin botanical plant names in comparison to the common names in other languages, first of all taking into consideration the ways of realisation of so called specific epithet, which makes part of the species names in the system of binomial nomenclature. After explaining the basic rules of taxonomy and its history, the author presents the analysis of the way of realisation of the specific epithet in selected plant families native to the Netherlands and Poland. The polyconfrontative analysis of phytonyms in Dutch, Polish, English and Czech has been carried out to find the answers for the following questions: whether it is possible find any naming tendencies in the selected languages and to what extent the morphology of the specific epithet is compatible with the international Latin name. The analysis of the specific epithet takes into consideration the most common elements, i.e. shape, size, provenance, colour, animal, etc.

Keywords:

onomastics, specific epithet, binomial nomenclature, phytonyms, polyconfrontative studies

Introduction

The diversity of flora species worldwide is one of the factors of different approaches to plant naming in different languages. Before publishing of the Linnaeus' taxonomy there was practically no unanimous system of plant nomination. The proposed system of Latin nomination is to a considerable degree valid nowadays. It constituted a common base for plant identification, however, the common names of plant names have been preserved. Therefore, there concurrently function two systems of nomination: the Latin one (to some extent universal) and the native one.

This paper aims at presenting the possible realisation and comparison of the botanical nomenclature in Latin, Dutch, English, Polish and Czech, paying special attention to the specific epithet which constitutes a characteristic feature of the binomial nomenclature.

In 1753 Linnaeus published his *Species Plantarum*, which introduced so called binomial nomenclature, consisting of two elements, the first of them – the genus in the nominal form, and the second of them – the specific element, which basically is expressed via the means of an adjective.

That is also the period when Latin started to be used in naming newly discovered plant species. The attempts to create a unanimous system of nomination are still present nowadays. The most recent document is so called *Shenzhen Code (International Code of Nomenclature for algae, fungi, and plants)*, published in 2018 and containing the regulations concerning naming species native to natural environment. These rules were described in a straightforward way among others by Spencer (2007).

In the polyconfrontative studies on botanical lexicon the databases containing the registers of species native to a certain area seem very useful. Kaćki et al. (2012) present in their paper a database containing information about all vegetative types in Poland. *Heukels' Flora van Nederlanden* (Meijden, 2005) is the basic publication on flora in the Netherlands. It is the most complete database of information about more than 2000 species growing in the Netherlands. Undoubtedly, the most important advantage of this publication is the online database of plants found in the Netherlands (www.soorntenbank.nl). A similar source of data is the *Nederlandse Flora* (Eggelte, 2012), that describes more than 2000 wild plants, including practically the whole flora of the country. Due to its straightforward description of characteristic elements of particular species enabling their recognition it is a valuable source for studies on botanical lexicon. On the other hand, it is worth to mention the English written work *Plant names for the 21st century (...)* (Croft et al., 1999) as a source of botanical names. A comparable source of Czech plant names is the online database (botany.cz) created by botanists from many research institutes and containing over 10000 articles concerning among other the history of name changes.

A short story of taxonomy

In the vastness of plant species exceeding 300 thousand, there was a need to create a certain method to name and distinguish them. For this purpose the botanists use different taxonomic systems.

Taxonomy can be defined as a study and description of the organism changes, investigating the reasons and results thereof and creating system of classification on basis of gathered information, comprising also systematics. A taxon can refer to any taxonomic group, e.g. division, family or species. The description of a taxon presents its characteristic elements, that constitute the definition of a taxon (Stace, 1993:20).

The attempts to create a proper plant classification started in the ancient times. The Ancient created plant naming systems that were passed on to further generations. These, so called folk taxonomies, classifications that had spread both in primitive and civilized societies, developed due to the necessity of naming and without influence of science. The accuracy of folk taxonomies can be evaluated first of all on the basis of the amount of common names of plants and compliance of folk taxonomies with the taxons distinguished by contemporary researchers (Stace, 1993: 35-36).

The first written description of plant classification in a consistent form was created by Theophrastus (ca. 370-285 BC), who was recognised “the father of botany”. He classified only ca. 480 taxons, exploiting basically the most obvious features of plant morphology (trees, bushes, subshrubs, herbs), and subsequent the more specific features. Medicinal plants were in the scope of interest of living in the 1st century Dioscorides, who in his *De Materia Medica* described ca. 600

taxons, mainly on basis of his observations. Although the work was not very straightforward, it functioned for over 1000 years as a standard handbook of botany, which was still in use in 16th century. Therefore, it can be declared the first herbarium (Stace, 1993: 36).

In the Middle Ages there appeared practically no new publications in the area of botany, but only a few translations from Greek were published. The most characteristic features of herbaria were the illustrations (engravings) of plants drawn as they were seen and included in the texts. Some of the herbaria comprised the draft forms of classification, but their layout was artificial, in most cases alphabetical (Stace, 1993: 38).

By the end of the 16th century the attention of the researchers began to be concentrated on strictly cognitive study of plants and their systematics. A. Caesalpino (1519-1603), who published in 1583 *De Plantibus*, can be considered the first taxonom. In his publication he classified ca. 1500 species, taking into the consideration mostly the form of growth, fruit and seeds, but he also used numerous features of flowers and organs (Stace, 1993: 39).

A considerable contribution in the development of plant classification was made by Swiss botanists J. Bauhin (1541-1631) and G. Bauhin (1560-1624). Their most important work, that contributed to the development of taxonomy, was *Pinax Theatri Botanici* (1623). The most significant feature of the book was mentioned in the title (*pinax*=register). It is the first index of ca. 6000 plant species known to the author, including all synonyms that were given to the described species by previous researchers. It contributed in some degree to ordering the botanical nomenclature. In his work G. Bauhin distinguished such notions as genus, species and main taxonomic units. Moreover, he introduced for many species binary names consisting of a genus name and a single specific epithet (previously the specific epithet had a multi-word form). A copy of the second edition of *Pinax Theatri Botanici* from 1671 is nowadays the property of the Linnaean Society of London. Previously, it belonged to Linnaeus, and on the margins it contains the remarks to the names of plants. In most cases these are his own names of the plants. Therefore, it can be assumed that he picked up some ideas from this work (Stace: 39-40). The further publication that influenced the development of taxonomy is the *Institutiones Rei Herbariae* (1700) by J.P. de Tournefort. His work comprises the concept of *geni*, many of which were borrowed by Linnaeus to his system of nomination, and which function even nowadays (Stace, 1993: 40).

Carl Linnaeus (1707-1778) is considered the creator of modern taxonomy and of the system of nomenclature till present times. However, the most important achievement of Linnaeus is ordering the vastness of literature, systems of classification and new plants, that constituted a challenge for the 18th century botanist. Linnaeus wrote many works in the field of botany, but from the point of view of taxonomy the most important ones must be considered *Genera Plantarum* (1737) and *Species Plantarum* (1753). The works contain the plant classification according to so called “Gender System”, the biggest advantage of which was its simplicity, which, however, did not take into consideration the kinship of particular species (Stace, 1993:26). For the first time the system was presented in published in 1735 *Systema Naturae*, which contained the classification of all known animals, minerals and plants. *Genera Plantarum* contains a register and short descriptions of plant *geni* distinguished by Linnaeus and included in the works of Bauhin and Tournefort. Numerous described genus names were borrowed from their works, Linnaeus, however, introduced

many names inspired by classical literature or created in honour of famous botanists (e.g. *Dioscorea*, *Fuchsia*, *Bauhinia* etc.) (Stace, 1993: 41-43; Jarvis, 2019: 88-91). In contrast to *Genera Plantarum*, the *Species Plantarum* does not comprise the genus descriptions. Linnaeus barely enumerated different species giving their names and short descriptions, relating to previous publications containing information about a specific species together with its synonyms, habitat and provenance. Further, the description of each species in the form of a descriptive phrase started from the genus and consisted maximum 12 words. The most important element of Linnaeus’ work was adding on the margins, beside the descriptive phrase of each species, a one-word common name, that is the specific epithet. It is assumed that that they were initially used to index, due to economy of space, but quickly they proved themselves to be a very big convenience. They rapidly became standard names for each species. The specific epithet was as a rule a repetition of a keyword from the descriptive phrase, but it could also be created separately (Stace, 1993: 44).

Indubitably, *Species Plantarum* may be recognised as the source of contemporary botanical nomenclature, despite the fact that a considerable part of the work constituted a recapitulation of other botanists’ works. It accounted for creating the *International Code of Botanical Nomenclature*, in accordance with which the names for new plants are contemporary created.

The specific epithet

The specific epithet is a characteristic element of the binomial nomenclature that can be created on basis of different elements that may refer to different aspects, e.g. the plant morphology, environmental or other phenomena.

The first element that may be the basis for creating the specific element is the size. It can constitute both an element of a compound (as it can be seen in example (1) in the specific epithet in Polish, Czech and Latin) and an autonomous designation of the epithet (as in Dutch). The English equivalent does not adhere to this tendency describing rather resemblance to other species, particularly the flowering member of the *Solanaceae* family – the potato.

(1)

LAT *Galinsoga parviflora*,
NL kaal knopkruid, **klein** knopkruid,
EN gallant soldier, quickweed, **potato** weed,
PL żóółtlica **drobnokwiatowa**,
CS pět’our **malóúborný**, pět’our **malokvěťý**

In some cases the size described in the specific epithet may refer to the size indirectly (as in example (2) – to the size of a giant). It is worth noticing that the Dutch form of the specific epithet and also one of the English synonyms refer to the period of flowering of the plant, which falls in the end of September and October.

(2)

LAT *Solidago gigantea*,

NL **late** guldenroede,
EN tall goldenrod, **giant** goldenrod, smooth goldenrod, **late** goldenrod,
PL nawłóć **olbrzymia**,
CS zlatobýl **obrovský**

The next frequently occurring element in the specific epithet is the provenance of the plant. In most cases the forms in described languages are compliant with the Latin equivalent (see (3)).

(3)
LAT *Solidago canadensis*
NL **Canadese** guldenroede
EN **Canada** goldenrod, **Canadian** goldenrod
PL nawłóć **kanadyjska**
CS zlatobýl **kanadský**

However, there may appear some alternations in the (historical) way of naming the same geographical area, as seen in (4). The Dutch name refers to the first settlers in the region. What seems interesting, the Latin subspecies name also contains the phrase '*nova-belgiae*'. On the other hand there exists an alternative Latin name *Aster novi-belgii*, which refers to other country name, although the plant comes from North America. It is worth mentioning that also in Polish there exists a name for the subspecies containing the name of the other Dutch-speaking country. The problem with naming particularly in the case of this plant is a result of changes of the family it belongs to according to recent taxonomic findings.

(4)
LAT *Symphyotrichum novae-angliae* (*Aster novae-angliae/novi-belgii*)
NL **Nieuw-Nederlandse** aster
EN **New England** aster, hairy Michaelmas-daisy, Michaelmas daisy
PL aster **nowoangielski** (aster **nowobelgijski**)
CS astříčka **novoanglická**

There may also be found examples of partial equivalence of using the toponym in the specific epithet in the species names (see (5)). In this case, the other name is based on an alternative Latin name ('*vulgaris*'), what can be seen in the English and Czech examples.

(5)
LAT *Filago germanica* / *vulgaris*,
NL **Duits** viltkruid,
EN common cudweed / cottonrose,
PL nicennica **niemiecka**,
CS bělolist obecný

The other element appearing in the specific element are numerals. The numerals in the case of specific epithet are a part of a compound containing the element '-part' or '-leaved' (or other element of the plant morphology – see (6)). What seems interesting the Dutch form constitutes an interesting phenomenon. The compound form consists of the element '-part', but the numeral is replaced with a noun 'feather'. In Dutch there also functions an alternative form with the meaning 'winged'. Both of them refer to the morphology of the leaves, which may resemble small wings.

(6)

LAT *Bidens tripartita*

NL veerdelig tandzaad, gevleugeld tandzaad

EN **three-lobe** beggartick, three-part beggarticks, leafy-bracted beggarticks (...)

PL uczepek **trójlistkowy**

CS dvouzubec **trojdílný**

The other respectively frequent element of the specific epithet is the colour. The most popular colours seem to be 'white' and 'black'. As it can be seen in example (7), the equivalence can be full among the forms in the described languages. Or as in example (8), the equivalence can be only partial (here only between the Czech and Dutch). Colour can be a separate word or a part of an element, describing a part of plant morphology, what is illustrated in the Czech form of the specific epithet in example (8).

(7)

LAT *Petasites albus*

NL **wit** hoefblad

EN **white** butterbur

PL lepieźnik **biały**

CS devětsil **bílý**

(8)

LAT *Bidens frondosa*

NL **zwart** tandzaad

EN devil's beggarticks, devil's-pitchfork, devil's bootjack, sticktights, bur marigold, pitchfork weed, tickseed sunflower, leafy beggarticks, common beggar-ticks

PL uczepek amerykański

CS dvouzubec **černoplodý**

There can also be found examples of the usage of more than one colour in the form of the specific epithet (see the Latin, Polish and Czech examples in (9)). The Dutch form is, however, expressed through the hue of the colour (in this case - the 'light' one).

(9)

LAT *Laphangium luteoalbum*
NL **bleekgele** droogbloem
EN Jersey Cudweed, cat's paw
PL szarota **żółtobiała**
CS protěž **žlutobílá**

An interesting element of the specific epithet constitutes the shape. The shape may refer to different parts of plant morphology, e.g. the flower (10), the stem (11) or other part of the plant (12). The degree of equivalence may be different with different plants. As it can be seen in example (10) all the names can refer to the shape of the flower, that resembles a discus. However, the Polish name perceives the shape from a different perspective. In this case the name can be translated as ‘radiusless’.

(10)

LAT *Matricaria discoidea*, *Matricaria matricaroides*
NL **schijfkamille**
EN pineappleweed, wild chamomile, **disc** mayweed
PL rumianek **bezpromieniowy**
CS heřmánek **terčovitý**

As seen in example (11) the specific epithet can refer to the length of one of the plant parts, in this case – the stem. The Latin, English and Czech names contain the element “without”, which refers to the very short stem of the plant. The Dutch name refers to the length of the stem indirectly, through the phrase meaning ‘by the ground’. The only language that explicitly refers to the length of the stem is Polish (‘short-stemmed’).

(11)

LAT *Cirsium acaule*
NL aarddistel
EN dwarf thistle, **stemless** thistle
PL ostrożeń **krótkolodygowy**
CS pcháč **bezlodyžný**

The changes in botanical nomenclature resulted in creating alternative names functioning parallel. As seen in (12) the Latin botanical name the specific epithet may refer to the shape of the plant or may be the eponym. It is worth mentioning that the eponyms are not very frequent in forming the specific epithet, however, it is very frequent in the genus names.

(12)

LAT *Senecio fuchsi*, *Senecio ovatus*

NL schaduwkruiskruid
EN wood ragwort
PL starzec **Fuchsa**, starzec **jajowaty**
CS starček **vejčitý**, starček **Fuchsův**

The specific epithet may also refer to the general structure of the plant. Example (13) presents different approaches to the shape of the flower. The Polish name refers to the flower as a whole, the Dutch one refers to the length of the petals or the number thereof. The English name, on the other hand, describes the resemblance of the petals to long needles.

(13)

LAT *Carduus acanthoides*,
NL **langstekelige** distel, **veeldoornige** distel,
EN **spiny** plumeless thistle, **welted** thistle, **plumeless** thistle,
PL ostrożeń **nastrozony**,
CS bodlák obecny

Example (14) presents the use of an animal name in the specific epithet. All the described languages in this case use the name of the bird 'hawk' in the specific epithet. However, the English and Dutch at the same time refer to another animal – 'the ox', particularly a part of animal's body. The additional animal appears in Dutch in the specific epithet, but in English it constitutes the name of a genus.

(14)

LAT *Picris hieracioides*, *Helmintia echioides*,
NL echt bitterkruid, **ossetong-havikskruid**,
EN **hawkweed** **oxtongue**,
PL goryczel **jastrzębcowaty**,
CS hořčík **jestřábníkovitý**

The above discussed are the most frequent elements on basis of which the specific epithet may be derived. To the less frequent ones belong e.g. the period of appearance of a certain feature (mainly the flowering period), the habitat or the way of growing.

Conclusions

The Linnaean system of binomial nomenclature influenced to a certain degree the way of plant naming both in the botanical names and the common names. Some of the common names were used to create the botanical names, mainly to form the specific epithet. Therefore, the correlation of the Latin botanical names and the names of species in other languages can vary. The Latin botanical name may be preserved to a certain extent. The specific element may be formed on basis of different element. The most frequent are the size, colour, provenance, shape (or structure), an

animal. In the case of eponyms, popular in the genus names, the specific epithet does not make use of it on regular basis.

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