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## CONTENTS

### REVIEW ARTICLE

*Jiří Patočka, Anna Strunecká*

**The most important microtubule natural inhibitors .....3**

### ORIGINAL ARTICLES

*Jiří Kassa, Jiří Bielavský*

**A comparison of the efficacy of new monopyrindinium oximes with the oxime HI-6 against mevinphos in mice.....9**

*Ioannis Ilias, Georgios Panoutsopoulos, Nikiforos Filippou, Anna Dima, Ioulia Christakopoulou, Paraskevi Roussou*

**Soluble interleukin 2 receptors' levels versus thyroid hormones levels in nonthyroidal disease .....13**

*Jiří Ceral, Jiří Kvasnička, Josef Jandík*

**Changes of signal-averaged ECG in normal subjects after one year.....15**

*Pavel Žáček, Pavel Kuneš, Eva Kobzová, Jan Dominik*

**Thoracic electrical bioimpedance versus thermodilution in patients post open-heart surgery .....19**

### BRIEF COMMUNICATION

*Zbyněk Vobořil*

**Inguinal hernioplasty according to Lotheissen and McVay .....25**

### ANNOUNCEMENTS AND NOTICES

*Alexander Schirger*

**University students during historical developments of the University,  
650 years of the journey to knowledge and understanding.....29**

### HISTORICAL ARTICLE

*Zdeněk Nožička*

**History of Pathological anatomy in Hradec Králové .....33**

## THE MOST IMPORTANT MICROTUBULE NATURAL INHIBITORS

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*Summary:* Natural microtubule inhibitors represent chemically very variegated family of structures with strong effect on cytoskeletal functions and the use of them is one of the most frequent therapeutic strategies for carcinoma treatment. The survey of the most important natural microtubule inhibitors is summarized in this paper.

*Key words:* Cytoskeleton; Microtubule; Inhibitor; Carcinoma; Minireview

### Introduction

The cytoskeleton of eukaryotic cells is a filamentous network formed by microtubules, microfilaments and intermediate filaments. The cytoskeletal network is responsible for the mechanical properties of the cell that modulate functions such as cell shape, locomotion, cytokinesis, and translocation of organelles. Experimental evidence suggests that there are many important functions of dynamic cytoskeletal network besides the regulation of cellular mechanics. The cytoskeleton also provides connections between cellular structures and presents a large surface area for interactions of various proteins and signaling molecules. Modulation of cytoskeletal network may influence cell signaling, ion channels and intracellular calcium levels. The reorganization or degradation of all cytoskeletal filaments is associated with apoptosis. Cytoskeleton is thus essential for regulation of cellular functions, cell integrity, and viability. The relationships between direct mechanical effects of modulations of cytoskeletal structures and cellular functions remains to be elucidated.

The aim of this minireview is to characterize the most important compounds of natural origin which interact with microtubules. Microtubules are tubulin polymers involved in many cellular functions (10), one of which being the formation of the mitotic spindle required for chromosome moving to the poles of the new forming cells during cell division (2). The importance of microtubules to cellular functions makes them a sensitive target for biological microtubule poisons. All compounds which interact with microtubules in the sense of their stabilization or disorganization are called microtubule inhibitors. They have cytotoxic effect and may kill the cell. Since microtubules are required to carry out mitosis in cell proliferation, microtubule inhibitors would primarily attack cancer cell which divides more frequently than healthy cell. Therefore many of them are very

important anti-cancer compounds. The use of this poisons is one of the most frequent therapeutic strategies for carcinoma treatment. In addition to well known microtubule poisons such as vinblastin, colchicin, and taxol, already now many new natural toxic compounds are used as outstanding scientific tools in biological experiments and serve the purpose of model structures for synthesis new compounds with expected effect.

### Microtubule system

Tubulin is a protein whose quaternary structure is composed of two polypeptide subunits,  $\alpha$ - and  $\beta$ -tubulin. Several isotopes have been described for each subunit in higher eucaryots. Microtubule functions are based on their capacity to polymerize and to depolymerize. This process is a very dynamic and is attend with rapid shortening or elongation of this cell structures. Tubulin is a GTP-binding protein and the binding of this nucleotide to the protein is required for microtubule polymerization, whereas the hydrolysis of the GTP bound to polymerized tubulin is required for microtubule depolymerization. Microtubule stability in healthy cell is regulated by the presence of some proteins called microtubule-associated proteins (MAP) which facilitate microtubule stabilization. The cellular mechanisms regulating microtubule assembly is highly sensitive to the concentration of  $\text{Ca}^{2+}$ . The low cytosolic  $\text{Ca}^{2+}$  level characteristic of the resting state of most eucaryotic cells promotes microtubule assembly, while the localized increase in  $\text{Ca}^{2+}$  cause microtubule disassembly (13). Microtubules forms through polymerization of protein dimers, consisting of one molecule each of  $\alpha$ - and  $\beta$ -tubulin. Dimer and polymer are in a state of dynamic equilibrium, so that the network can respond flexibly and quickly to functional requirements. The polymer forms a fine, unbranched cylinder, usually with internal and external diameters of 14 and 28 nm, respective-

ly, the so called microtubule (Fig. 1) (22). Assembly is initiated by the binding together of  $\alpha$ ,  $\beta$ -dimers to form short protofilaments, 13 of which subsequently arrange themselves side by side to form the microtubule. Subsequent growth of the microtubule is polar, occurring mainly at the so-called plus end of the protofilaments through the addition of further dimers. Addition involves GTP, which is bound to the dimer, being cleaved to GDP, which remains attached to the tubulin. The binding site for GTP is on the  $\beta$ -subunit. When the cell becomes enriched with GTP-tubulin dimers, hydrolysis to GDP-tubulin falls behind the rate of assembly and an  $\alpha$ ,  $\beta$ -tubulin-GTP cap forms at the plus end of the protofilaments blocking further growth of the microtubule.

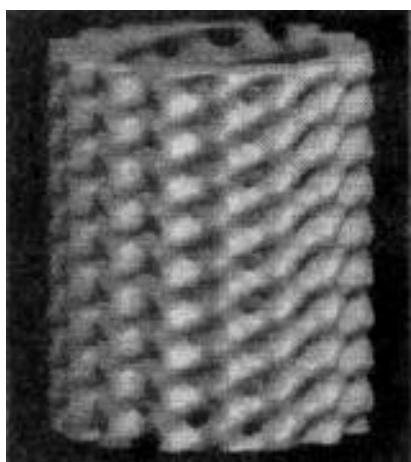


Fig. 1: The structure of microtubule polymer cylinder, usually with internal and external diameters of 14 and 28 nm, respectively.

### The classification of microtubule natural inhibitors

Microtubule inhibitors represents chemically very varied group of compounds from different biological sources with strong effect on cytoskeletal functions and strong toxicity. Microtubule functions in cell depend on the capacity of tubulin to polymerize or the capacity of microtubules to depolymerize.

Compounds which are able to influence these processes, i.e. microtubule inhibitors (also anti-tubulin agents, antimitotic agents, etc.), can be divided into four group according to their mechanism of action. 1. Compounds which bind to GTP site, 2. compounds which bind to colchicine site, 3. compounds which influence as microtubule-stabilizing agents, and 4. compounds which do microtubule network disorganization.

#### 1. Compounds bind to GTP site

Typical representatives of this group of microtubule poisons are vinca alkaloids, compounds derived from

*Catharanthus roseus*, a plant from warm climate. The most important compounds of this group are **vinblastine** and **vincristine**, compounds composed from a tetracyclic structure of catharantine and a pentacyclic structure of vindoline (41). Both structures appear to be important for both **vinblastine** and **vincristine** activity. The analysis of the localization of vinblastine-binding site on tubulin has indicated that it occurs at the central region of the beta-tubulin subunit (37). In this region is also GTP binding site and it has been shown that vinca alkaloids and other related molecules can prevent the binding of GTP to tubulin. **Vinblastine** is mainly useful for treating lymphocytic and histiocytic lymphoma, Hodkin's disease, Kaposi's sarcoma, and advanced breast or testicular cancer. **Vincristine** is used mainly to treat acute leukemia, neuroblastoma, rhabdomyosarcoma, Hodkin's disease and other lymphomas. Semisynthetic derivatives of vinca-alkaloids with lower toxicity are now at different phases of clinical trial, for example **vindesine** or **vinorelbine**, which are tested in breast cancer. Other microtubule inhibitors are **dolastin** isolated from the sea hare (*Dolabella auricularia*), compound with both pyrrolidine and thiazoline moiety in the molecule, **griseofulvin**, an antibiotic produced by *Penicillium griseofulvum*, **maytansine**, a macrolide compound from rainforest plant *Maytenus serrata* and others family Celastraceae (34), famous ethnomedicine known in western Amazonia as chuchuhuasi.

**Halichondrin B** is the most potent compound of a class of polyether macrolides isolated in low yield from four different sponge genera - *Axinella*, *Halichondra*, *Lissodendoryx*, and *Phakellia* (35). **Halichondrin B** acts on tubulin by similar mechanism as **vinblastine** (4,11). From the fungus *Rhizopus chinensis* was isolated other cytotoxic macrolide compound, **rhizoxin**, with significant antineoplastic activity in several murine and human tumor models (6). **Cryptophycin A** is a new antimicrotubule agent, active against some drug-resistant cells (44) and with potent antiproliferative effect and with excellent antitumor activity against mammary, colon, and pancreatic adenocarcinomas (33). A highly cytotoxic macrocyclic lactone polyether has been isolated from a Spongia species and named **spongistatin**. This compound inhibited the glutamate-induced polymerization of tubulin and it is a potent inhibitor of the binding of vinblastin and GTP to tubulin (3).

#### 2. Compounds bind to colchicine site

**Colchicine** is alkaloid found in the autumn crocus (*Colchicum autumnale*) and also in other plants. Autumn crocus was used in the antiquity for the treatment of gout, but the main interest for the study of colchicine came when it was observed that this drug could stop cell proliferation in mitosis, by preventing the formation of the mitotic spindle. Structurally, colchicine is a tropolone derivative with three rings A, B and C. Ring A is a six-carbon ring with three methoxy groups, whereas B and C are seven-carbon rings (19). Several unfavorable characteristics have been observed for

the binding of colchicine to tubulin. The reaction is very slow, temperature-dependent and essentially irreversible. The binding of colchicine to tubulin becomes faster and reversible when a methyl group replaces the acetyl group present on the amine of the B ring, yielding the compound known as colcemide.

On the same site as colchicin bind also **podophyllotoxin**, plant compounds obtained from *Podophyllum peltatum*. **Podophyllotoxin** is a tetracyclic compound with four rings A, B, C, and D, linked to an aromatic ring with three methoxy groups. This alkaloid is, like **colchicine**, a drug that prevents microtubule polymerization. It has been used for topic treatment of some benign skin tumors. Some synthetic derivatives of podophyllotoxin appear to be more active than podophyllotoxin alone in the treatment of leukemias and solid tumors.

#### 3. Microtubule-stabilizing compounds

Among these compounds, the best known one is **taxol** (paclitaxel), tetracyclic compound obtained from the bark of the Pacific yew (*Taxus brevifolia*). In the structure of taxol there are two aromatic rings and a tetracyclic-structure containing an oxetane ring which is required for the activity of the drug (18). The primary action of this compound is to stabilize microtubules, preventing their depolymerization. In this way taxol should block proliferating cells between  $G_2$  and mitosis, during the cell cycle. The binding of **taxol** appears to occur at different localizations at the amino terminal of  $\beta$ -tubulin, but binding to the middle region of an  $\alpha$ -tubulin has also been reported (28). **Taxol** has been used mainly for the treatment of breast and ovarian cancer but also it has been tested for other types of tumors such as lung cancer, head and neck cancer and melanoma. Several disadvantages have been indicated for application of this very actual anti-cancer compound. One of them, the relative low amount that can be obtained from the bark of the Pacific yew and its relatively rare incidence restrict to the forests of the Pacific Northwest of the USA and Canada. This problem has been partially solved by chemical synthesis of this compound (32). Another disadvantage is the low solubility of **taxol** in water, thus, this drug must be delivered dissolved in oil and this solvent could effect to cardiac functions or promote allergic reactions. Also, this problem has been partially solved by synthesizing some **taxol** analogs with a higher solubility in water (32). Interesting semisynthetic analogue of **taxol** with clinical use is **docetaxel** (Taxotere), compound which contains a taxane ring linked to an oxetan ring at positions C-4 and C-5 and to an ester side chain at C-13.

A new class of microtubule-stabilizing compounds have been isolated from the bacterium *Sorangium cellulosum*. These macrolide compounds were called **epothilones**, because their typical structural units are epoxide, thiazole, and ketone (27,42). **Epothilone** occurs in two structural variations, **epothilone A** and **epothilone B**, the latter containing

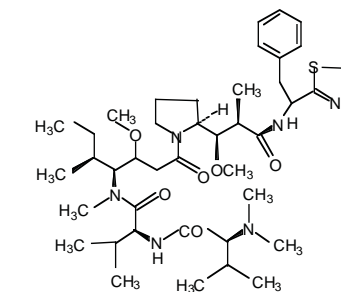
an additional methyl group (23). **Epothilone A** is the main product of bacteria metabolism, the yield of **epothilone B** amounting to 20-30 per cent of the yield of **epothilone A**. Despite the small different in chemical structure, in most test systems **epothilone B** has been approximately ten-time more effective. These compounds show a striking effect on stabilizing polymerization of microtubules and they are easily obtained on large scale by a fermentation process (14). Both **epothilones** show a very narrow spectrum of activity (19) and halts cells, as does **taxol**, in the  $G_2$ -M phase (23). The Total synthesis of **epothilones** was reported in many laboratories (5,43,45,48).

Of recent interest is the discovery of the marine-derived compound, **discodermolide**, whose anti-mitotic mechanism of action includes the polymerization and stabilization of microtubules in a method analogous to that observed with the structurally unrelated compound **taxol** (29,30).

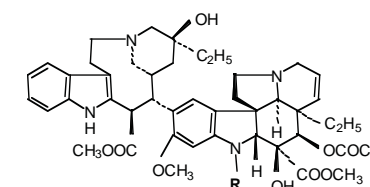
#### 4. Compounds with disorganization effect on microtubule network

Some natural marine compounds with anti-tumoral activity were found to disorganise the microtubule network (12). There are **ecteinascidin 743**, tetrahydroisoquinoline alkaloid isolated from the marine ascidian, *Ecteinascidia turbinata*, (15,24), several members of the family of **lamellarins**, for example **lamellarin Q**, polyaromatic alkaloids isolated from marine tunicates belonging to the genus *Didemnum* (36), as well as cyclic depsipeptides of the family **didemnins**; the most known compound of this family is **didemnin B** (31). **Didemnins** were isolated from the marine tunicates *Tridemnum solidum* and *Aplidium albicans* (47) and many very biologically active compounds of this family were prepared also synthetically or semisynthetically (40).

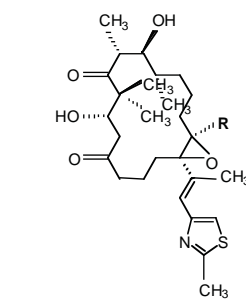
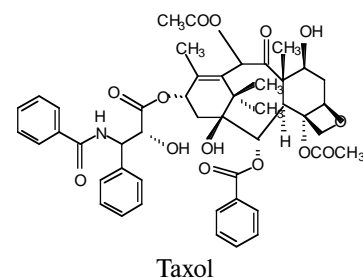
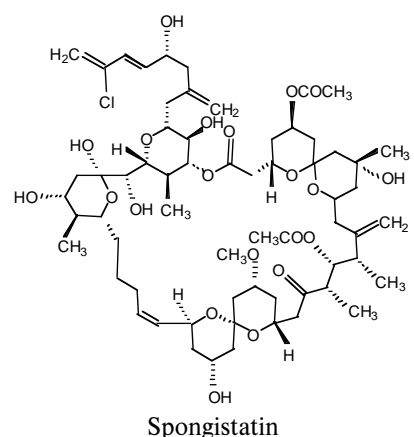
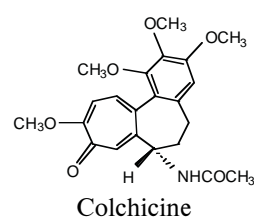
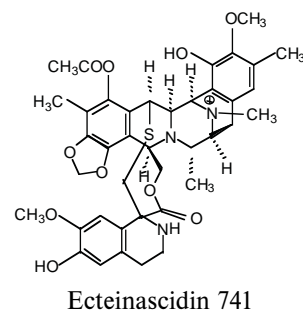
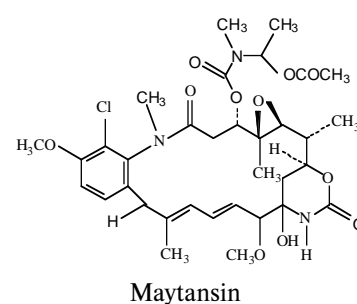
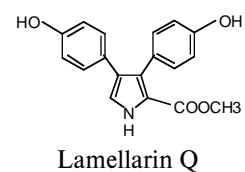
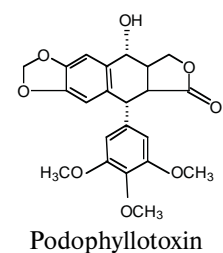
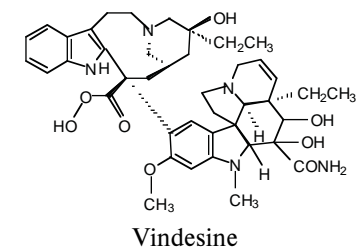
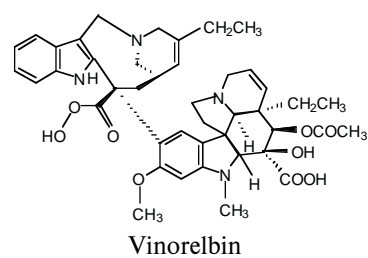
#### Microtubule-structures



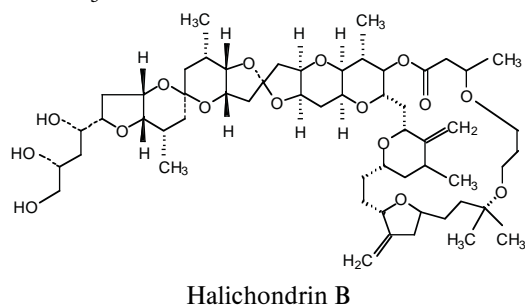
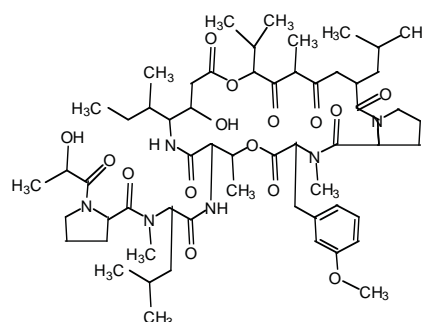
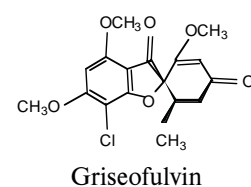
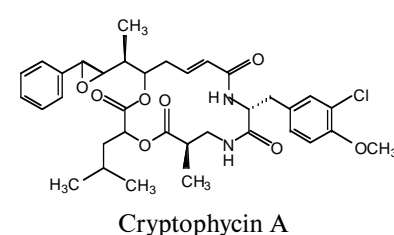
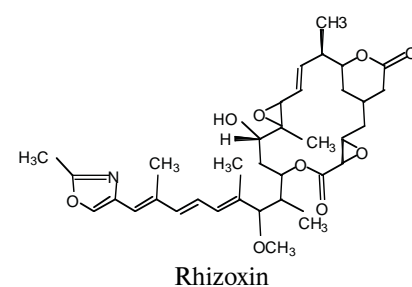
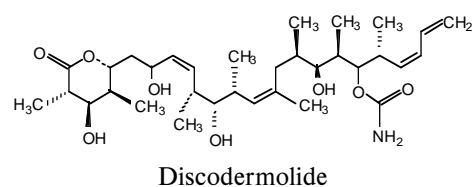
Dolastatin



Vinblastine R = CH<sub>3</sub>  
Vincristine R = CHO



R = H  
R = CH<sub>3</sub>



## Applications of microtubule inhibitors in future research

There are many demonstrations that mechanical forces mediated by cytoskeleton play a vital role in assembling cellular structures. Moreover, recent experimental evidence demonstrate the multiple interactions between cytoskeletal structures and ion channels, calcium fluxes, and events connected with signal transduction. The process of microtubule assembly proceeds in a cell-free system and the effects of various inhibitors can be thus studied even in the test tube. The use of various natural microtubule inhibitors provides the possibility to study the mechanisms of assembly and disassembly of cytoskeletal structures as well as the role of cytoskeleton in spatial and temporal integration of vital cell functions.

The regulation of microtubule assembly depends on the ability of tubulin heterodimers to bind GTP. The GTP bound to the  $\beta$ -polypeptide is hydrolyzed to GDP plus phosphate (26). The course of assembly and disassembly of microtubules is therefore affected by the action of GTPases. A number of heterotrimeric GTPases or small GTPases of the rho family move on the cytoskeleton after cell activation (9,46]. Moreover, a direct transfer of GTP from tubulin to the  $\alpha$  subunit of the Gs and Gi protein has been reported (39). On the other hand it has been reported that microtubules can also assemble in the presence of nonhydrolyzable GTP analogues. These observations may demonstrate that such interactions do not entirely serve to microtubule reorganization, but may be also related with cell signaling pathways. The use of various microtubule inhibitors which bind to GTP site could contribute to the study of this suggested role of microtubules in eucaryotic cells.

Polymerization of tubulin heterodimers is regulated by Ca<sup>2+</sup> concentration. Under low Ca<sup>2+</sup> concentration characteristic for the cytoplasm of most eucaryotic cells, much of the tubulin is assembled into microtubules. Localized increases in Ca<sup>2+</sup> concentration cause microtubule disassembly (13). The alteration of microtubule structure by colchicin has been reported to enhance the activity of Ca<sup>2+</sup> channels in Lymnaea neurons and mammalian hippocampal pyramidal neurons (25). An intact microtubule system is required for the IP<sub>3</sub>-dependent Ca<sup>2+</sup> release from intracellular stores (17). The inhibitory effect of colchicin in saponin-permeabilized platelets has been reported (8).

Disruption of microtubules by colchicin has been reported to increase conductance of Cl<sup>-</sup> channels in skeletal muscle (21), and decreased conductance of snake twitch fibre end plates (20). The mechanism of this functional change and the role of cytoskeletal structures in the transmembrane ion transport is not known. On the other hand, taxol had no effect on the ion channels in hippocampal neurons (38).

Disruption of microtubular structures by taxol leads to increased phosphorylation and to the cell death (7,16). Degradation of tubulin can occur very early in the course of apoptosis. It has been reported in neuronal cells treated with

glutamate (1). Although the relation between the microtubule system and the apoptotic program remains unclear, the disruption of microtubule turnover undoubtedly leads to cell death.

## Conclusions

Microtubule inhibitors from different natural sources represents chemically very variegated group of compounds with strong effect on cytoskeletal functions and strong toxicity. The use of this poisons is one of the most frequent therapeutic strategies for carcinoma treatment. Drugs like **vinblastine** and **taxol**, have wide clinical use, although they have some drawbacks. The discovery of new compounds such **epothilones**, **halichondrins**, **didemmins**, etc., could overcome some of the problems found with the use of the earlier drugs. In addition, already now these natural toxic compounds are used as outstanding scientific tools in biological experiments and serve the purpose of model structures for synthesis new compounds with expected effect.

*We would like to thank Miss Kateřina Ševčíková from the Institute of Chemical Technology, Prague, for technical assistance.*

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## A COMPARISON OF THE EFFICACY OF NEW MONOPYRIDINIUM OXIMES WITH THE OXIME HI-6 AGAINST MEVINPHOS IN MICE

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**Summary:** 1. The therapeutic efficacy of three new monopyridinium oximes (2,4-PAEtM, 2,5-PAEtM, 2,5-PAAM) and the bispyridinium oxime HI-6 was evaluated in combination with benactyzine against acute poisoning with the organophosphorus insecticide mevinphos in mice. 2. When mice were treated two min after mevinphos poisoning, no significant differences in the therapeutic effectiveness of tested oximes were observed. They increased the 24h LD<sub>50</sub> values of mevinphos about three times in comparison with non-treated intoxicated animals. 3. On the other hand, there were significant differences in their therapeutic efficacy when they were administered 30 sec following mevinphos challenge. The monopyridinium oxime 2,5-PAEtM seems to be the most efficacious against mevinphos toxicity. 4. Use of new monopyridinium oxime 2,5-PAEtM appears to be the improvement in the antidotal treatment of poisoning with organophosphorus insecticide mevinphos in comparison with HI-6.

**Key words:** *Mevinphos; Monopyridinium oximes; HI-6; Benactyzine; LD<sub>50</sub>; Mouse;*

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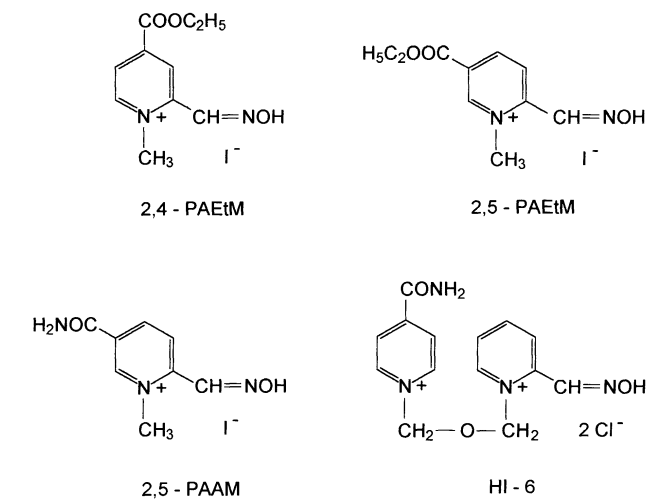
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### Introduction

Organophosphorus insecticides (OPI) have become the most widely used class of insecticides in the world. The use of OPI in agricultural fields has replaced more resistant chlorinated hydrocarbon compounds. The choice of OPI is based on their properties of low bioaccumulation and high rate of biodegradation. They are also used in large quantities because of their high potential for insect knockdown capacity (1,5). In spite of relatively low toxicity in comparison with highly toxic nerve agents, they have passed occupational hazards to workers employed in the application of these insecticides. Careless handling of OPI and their voluntary exposure with suicidal intent are the main reasons for intoxication (12,17).

One of the most toxic OPI, mevinphos (2-methoxycarbonyl-1-methylvinyl dimethylphosphate), is used for its high efficacy against various insect species (4). The 24h intramuscular (i.m.) LD<sub>50</sub> of mevinphos for mice is 0.79 mg/kg body weight (18).

OPI induce clinical signs including salivation, diarrhea, lacrimation, tremors, convulsions and respiratory distress. Death from exposure to OP compounds is generally due to respiratory failure from excessive airway secretions, constriction of the airways and a loss of central respiratory control (13). Antidotal treatment of poisoning with OPI usually consists of anticholinergic drugs to counteract the accumulation of acetylcholine (ACh) and oxime reactivators to reactivate OPI-inhibited acetylcholinesterase (EC 3.1.1.7) (3).



**Fig. 1:** Chemical structures of the oximes used

To improve the efficacy of antidotal treatment of acute poisoning with OPI, three new monopyridinium oximes (2,4-PAEtM, 4-ethoxycarbonyl-2-hydroxyiminomethyl-1-methylpyridinium iodide; 2,5-PAEtM, 5-ethoxycarbonyl-2-hydroxyiminomethyl-1-methylpyridinium iodide and 2,5-PAAM, 2-hydroxyiminomethyl-5-carbonyl-1-methylpyridinium iodide) (Figure 1) have been synthesized at the Department of Toxicology of Purkyně Military Medical Academy in Hradec Králové.

The purpose of this study was to compare the efficacy of three new monopyridinium oximes (2,4-PAEtM, 2,5-PAEtM, 2,5-PAAM) and the bispyridinium oxime HI-6 with anticholinergic drug benactyzine against multiple lethal doses of OPI mevinphos in mice.

## Methods

Male mice (20-24g) obtained from Konárovice were housed in an air-conditioned room (20-22°C) on 12-h light/12-h dark cycles and were allowed access to food and tap water ad libitum. The principles of laboratory animal care were followed and the handling of animals was made under the supervision of the Ethics Committee of the Medical Faculty of Charles University and the Military Medical Academy in Hradec Králové.

The monopyridinium oximes (2,4-PAEtM, 2,5-PAEtM, 2,5-PAAM) were prepared by quaternization of tertiary bases by methyl iodide in the medium of dimethylformamide and purified by crystallization from ethanol. Chemical structures of products obtained after synthesis were identified by an elemental analysis and NMR. The chemical purity of products of synthesis assessed by TLC was more than 98%.

Mice were treated i.m. with oxime in equieffective doses (5% or 10% LD<sub>50</sub>) in combination with benactyzine (BNZ) at a dose 8.4 mg/kg 30 sec or two min following mevinphos (Spolana Neratovice) poisoning. LD<sub>50</sub> values and 95% confidence limits were calculated by probit analysis of death occurring within 24h after i.m. administration of mevinphos at five different doses with six mice per dose (15). The efficacy of antidotal mixtures tested was expressed as protective ratio (LD<sub>50</sub> of mevinphos in protected mice/LD<sub>50</sub> of mevinphos in unprotected mice).

## Results

The LD<sub>50</sub> values of all oximes tested are shown in Table 1. Generally, the monopyridinium oximes are significantly less toxic for mice than the oxime HI-6.

**Table 1:** Toxicity parameters of oximes tested.

OXIMES	LD <sub>50</sub> (mg/kg)
HI-6	671.3 (627.4 - 718.3)
2,4-PAEtM	1560.3 (1187.2 - 2050.6)
2,5-PAEtM	1381.6 (1267.6 - 1505.9)
2,5-PAAM	1264.4 (1160.3 - 1377.8)

The therapeutic efficacy of the monopyridinium oximes as well as the oxime HI-6 is presented in Table 2 and 3. When the oximes in combination with BNZ were administered two min after mevinphos poisoning, the 24h LD<sub>50</sub> values of mevinphos in treated mice were increased approximately three times in comparison with the 24h LD<sub>50</sub> values in non-treated mice. No significant differences between effectiveness of the oximes tested were observed (Table 2).

**Table 2:** Therapeutic effect of oximes administered at 2 min after poisoning on the LD<sub>50</sub> value of mevinphos.

TREATMENT	DOSE OF OXIME	LD <sub>50</sub> (95% confidence limits) of mevinphos (mg/kg)	Protective ratio
—	—	0.79 (0.70 - 0.89)	—
HI-6 + BNZ	5% LD <sub>50</sub>	2.44 (2.03 - 3.13)	3.1
	10% LD <sub>50</sub>	2.71 (2.53 - 2.91)	3.4
2,4-PAEtM + BNZ	5% LD <sub>50</sub>	2.40 (2.17 - 2.65)	3.0
	10% LD <sub>50</sub>	2.43 (2.19 - 2.67)	3.1
2,5-PAEtM + BNZ	5% LD <sub>50</sub>	2.41 (2.20 - 2.65)	3.0
	10% LD <sub>50</sub>	2.91 (2.59 - 3.26)	3.7
2,5-PAAM + BNZ	5% LD <sub>50</sub>	2.38 (2.04 - 2.78)	3.0
	10% LD <sub>50</sub>	2.30 (1.99 - 2.61)	2.9

On the other hand, when mice were treated 30 sec following mevinphos intoxication, the efficacy of all tested oximes was significantly increased and there were some differences in their therapeutic effect. The 24h LD<sub>50</sub> values of mevinphos in mice protected with monopyridinium oxime 2,4-PAEtM or 2,5-PAEtM in combination with BNZ were increased 12 - 20 times in comparison with the 24h LD<sub>50</sub> values in unprotected mice while the 24h LD<sub>50</sub> values of mevinphos in mice protected with 2,5-PAAM plus BNZ were increased 6 - 8 times in comparison with the LD<sub>50</sub> values in unprotected mice only. The effectiveness of the bispyridinium oxime HI-6 in combination with BNZ varied between them. The monopyridinium oxime 2,5-PAEtM seems to be the most efficacious oxime according to the 24h LD<sub>50</sub> values (Table 3).

**Table 3:** Therapeutic effect of oximes administered at 30 sec after poisoning on the LD<sub>50</sub> value of mevinphos.

TREATMENT	DOSE OF OXIME	LD <sub>50</sub> (95% confidence limits) of mevinphos (mg/kg)	Protective ratio
—	—	0.79 (0.70 - 0.89)	—
HI-6 + BNZ	5% LD <sub>50</sub>	9.73 (9.10 - 10.40)	12.2
	10% LD <sub>50</sub>	12.09 (10.83 - 13.48)	15.1
2,4-PAEtM + BNZ	5% LD <sub>50</sub>	10.09 (8.06 - 12.74)	12.8
	10% LD <sub>50</sub>	13.85 (12.90 - 14.90)	17.5
2,5-PAEtM + BNZ	5% LD <sub>50</sub>	13.89 (13.30 - 14.53)	17.6
	10% LD <sub>50</sub>	16.05 (15.09 - 17.40)	20.4
2,5-PAAM + BNZ	5% LD <sub>50</sub>	5.16 (4.84 - 5.49)	6.5
	10% LD <sub>50</sub>	6.20 (5.89 - 6.52)	7.8

Following antidotal treatment of mevinphos-poisoned mice at two min after intoxication, the similar intensity of

clinical signs and symptoms attributable to ACh accumulation at cholinergic sites (salivation, lachrymation, convulsion of skeletal muscles and respiratory depression) were found. When antidotal treatment was administered 30 sec following mevinphos challenge, a slight clinical improvement of mevinphos-poisoned mice treated with monopyridinium oxime 2,5-PAEtM or 2,4-PAEtM in comparison with the other oximes tested was observed.

## Discussion

The oxime HI-6 has been shown to be very effective against some highly toxic OP compounds not only because of its high reactivating potency but also because of its other antidotal mechanisms based on antimuscarinic, antinicotinic and ganglion blocking actions as well as on restoration of neuromuscular blockade and beneficial effects on cardiovascular and respiratory systems (2,11,16,19). On the other hand, HI-6 efficacy against OPI is not so high. It is not more effective than other currently available oximes in diminishing acute toxicity of OPI (6,8,18).

Our results confirm that the new monopyridinium oximes studied are relatively efficacious against mevinphos toxicity. Their effectiveness differs from each other when they are administered shortly (30 sec) following mevinphos poisoning. Above all, they can be used in relatively high doses in the case of OPI poisonings because of their very low toxicity for mammals. Our data demonstrate that the monopyridinium oxime 2,5-PAEtM appears to be significantly more efficacious than other oximes tested including HI-6.

Our data also suggest that it is necessary to treat mevinphos-poisoned animals as soon as possible because of the rapid onset of life-threatening OPI-induced cholinergic crisis (13). The efficacy of antidotal treatment of mevinphos-induced poisoning significantly decreases if the time interval between poisoning and treatment increases. Thus, not only poisoning with highly toxic OP compounds (3) but also intoxication with some OPI must be treated as soon as possible (9,18).

In conclusion, our data indicate that only monopyridinium oxime 2,5-PAEtM has definite advantages over HI-6 in the treatment of mevinphos poisoning in mice because of its high therapeutic efficacy and low toxicity for mammals.

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## SOLUBLE INTERLEUKIN 2 RECEPTORS' LEVELS VERSUS THYROID HORMONES LEVELS IN NONTHYROIDAL DISEASE

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**Summary:** Serum soluble interleukin-2 receptor levels, basal thyrotropin, total thyroxine, total triiodothyronine and free triiodothyronine were assayed in 29 - otherwise healthy - patients with pulmonary tuberculosis before initiation of anti-tuberculosis treatment and after two weeks of therapy. Twenty seven out of 29 patients presented low-normal total triiodothyronine levels, showing a statistical elevation after anti-tuberculosis therapy. Total triiodothyronine levels before anti-tuberculosis therapy were inversely correlated with levels of serum soluble interleukin-2 receptors. Further investigation on the relationship between soluble interleukin-2 receptor's levels and thyroid hormones in non-thyroidal disease can be envisaged.

**Key words:** Receptors; Interleukin-2; Thyroid hormones; Tuberculosis

### Introduction

Antigen stimulation of resting T-cells triggers synthesis and secretion of interleukin-2, as well as the membrane expression of interleukin-2 receptors (1). Normal peripheral blood mononuclear cells and certain lines of T- and B-cell origin release, after its membrane expression, a soluble form of interleukin-2 receptor (sIL-2R); this appears to be a consequence of cellular activation of various cell types that may play a role in the regulation of the immune response (1,2). Patients with autoimmune diseases, haematologic malignant diseases or overt thyroid disease often show high sIL-2R serum levels (3,4,5), however the course of sIL-2R levels in non-thyroidal disease affecting thyroid function has not been adequately explored. Recent studies have shown that patients with tuberculosis (TB) present with low to normal free and total triiodothyronine levels, which rapidly elevate after anti-TB treatment (6,7,8). The aim of the present study was to evaluate thyroid function parameters and versus sIL-2R- $\alpha$  (one of the receptor's subunits) in patients with pulmonary TB before and after initiation of anti-TB therapy.

### Materials and methods

Sera from 29 ambulatory patients (19 men, 10 women, mean age $\pm$ SE: 36 $\pm$ 3 years, BMI $>$ 26) with sputum-smear positive focal pulmonary TB were assayed for sIL-2R- $\alpha$  (Quantikine hIL-2sR EIA assay, R&D Systems, Oxon, UK,

normal limits 676-2132 pg/mL), serum basal thyrotropin (Gammacoat hTSH IRMA, INCSTAR, Stillwater, Minnesota, USA, normal limits 0.40-3.10  $\mu$ IU/mL), total serum thyroxine and triiodothyronine (Amerlex T4 and T3 RIA, Kodak diagnostics, Amersham, UK, normal values at 5.0-14.0 ng/dL and 0.50-1.90 ng/mL respectively) and free triiodothyronine (Free T3 Clinical Assay, INCSTAR, Stillwater, Minnesota, USA, normal limits 1.50-3.20 pg/mL). The intra- and interassay coefficients of variation of these commercially available assays were less than 6%. Measurements were executed twice for each patient; the first sample was obtained before anti-TB therapy and the second after two weeks of isoniazid (300 mg/day), rifampicin (600 mg/day) and pyrazinamide (30 mg/kg/day) treatment. No other medications were administered during the study period.

The patients' HIV status was assessed, with their consent, using a commercial assay (HIV-1/HIV-2 3<sup>rd</sup> Generation Plus EIA, Abbott GmbH, Deikenheim, Germany) and none was HIV (+). Subsequent *Mycobacterium tuberculosis* cultures did not reveal in vitro resistance to first-line anti-TB drugs; all the patients completed a standard nine-month anti-TB regimen and made an uneventful recovery. The patients did not have a history of thyroid and/or autoimmune disease.

Between groups comparison of measured values was made with the Kruskal-Wallis non-parametric ANOVA test while correlations between sIL-2R- $\alpha$  and the other thyroid function parameters were done with Spearman's rank correlation test.

## CHANGES OF SIGNAL-AVERAGED ECG IN NORMAL SUBJECTS AFTER ONE YEAR

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**Summary:** Repeated signal-averaged electrocardiograms (SA ECG) were recorded twice with a mean interval of 13 months in 11 healthy volunteers in order to acquire basic information on long-term changes of SA ECG. After one year the duration of filtered QRS remains the most stable parameter of SA ECG on the contrary to parameters describing end of fQRS - i.e. both HFLA and RMS. Moreover fQRS seems to have better specificity in comparison to HFLA and RMS. An estimation of significant long-term changes in individual parameters of SA ECG was obtained. According to our results, only changes in QRS  $\pm$  13 ms, fQRS  $\pm$  8 ms, HFLA  $\pm$  22 ms and RMS  $\pm$  17  $\mu$ V should be considered significant when found in a long-term follow-up of patients with a heart disease.

**Key words:** Signal-averaged electrocardiography (SA ECG); Late potentials; Long-term changes; Healthy volunteers

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### Introduction

Late potentials appear to be a hallmark for sustained ventricular arrhythmias (1). Signal-averaged electrocardiography (SA ECG) helps in stratifying the risk of developing a sustained ventricular arrhythmia in patients who are recovering from myocardial infarction (2). With the present knowledge, it appears that late potentials seem to be more closely related to the underlying morphological substrate for arrhythmias than the clinically occurring arrhythmia per se. Abnormal signal-averaged ECG reflects abnormalities in ventricular activation caused by separation of myocardial bundles and the distortion of their parallel orientation by fibrosis (3).

There are several studies on the long-term changes in SA ECG in patients after myocardial infarction (4,5) and one study of patients with right ventricular dysplasia (6). But assessment of changes of SA ECG was not based on a comparison with a control group. Moreover there has been no study of the long-term changes in SA ECG in normal subjects. In order to acquire such basic information we performed a prospective study of signal-averaged ECG in 11 normal subjects. Such a study should be, in our opinion, the first step in evaluating long-term changes of SA ECG in different group of patients.

### Materials and methods

11 men of relatively young age  $32 \pm 6$  years were studied. For inclusion into the study, each subject had to feel healthy and be active. All patients had to have a history and

a physical examination neither of which was suggestive of cardiac disease, and a normal surface standard electrocardiogram. Repeated signal-averaged surface electrocardiograms were recorded with a mean interval of  $13 \pm 1$  months.

The recording and signal averaging and processing was performed with a system from Arrhythmia Research Technology, model 1200 EPX, based on the method previously described by Simson (1). Standard orthogonal bipolar X, Y, and Z leads were used to analyse 250 cycles with a noise  $\leq 0,4 \mu$ V. The recorded signals were amplified, averaged and filtered with a Butterworth bidirectional filter (range 40 to 250 Hz). The signal obtained from the 3 leads were then combined to form a vector magnitude ( $V = \sqrt{X^2 + Y^2 + Z^2}$ ), a measure that sums the high-frequency content from all three leads, termed „the filtered QRS complex“. Three indices were measured: 1. the duration of the filtered QRS (fQRS), 2. the root mean square of the terminal 40 ms of the filtered QRS (RMS) and 3. the period for which the filtered QRS remains  $< 40 \mu$ V (HFLA). Abnormal values for these three parameters were defined according to current recommendation as fQRS  $> 114$  ms, RMS  $< 20 \mu$ V, and HFLA  $> 38$  ms (2). Abnormal late potentials were defined by presence of two criteria out of the three.

All data were expressed as mean  $\pm$  one standard deviation (SD). In order to gain criteria for significant changes for all measured parameters we doubled and rounded up standard deviation of mean change of each of the parameters. Any change in case of QRS, mfQRS, HFLA higher by 1 ms and in case of RMS higher by 1  $\mu$ V was considered to be significant (table 1.).

### Results

One patient was hyperthyroid and presented initially with total serum thyroxine and triiodothyronine above the assays' normal limits (16.1 ng/dL and 2.45 ng/mL respectively, with thyrotropin at 1.52  $\mu$ IU/mL), remaining so after anti-TB treatment began (with serum thyroxine at 16.0 ng/dL, triiodothyronine at 2.74 ng/mL and serum thyrotropin at 2.20  $\mu$ IU/mL). His free triiodothyronine and s-IL-2R- $\alpha$  remained normal in both samplings.

Overall hormone and s-IL-2R- $\alpha$  measurements results are presented in table 1. Mean thyrotropin  $\pm$  SE remained well within normal limits at the first and at the second sampling. Mean total thyroxine  $\pm$  SE did not show any statistically significant differences between measurements. The observed elevation in total triiodothyronine levels (noted in 27/29 patients), after two weeks of anti-TB treatment, was statistically significant (Kruskal-Wallis  $p=0,05$ ). Mean  $\pm$  SE free triiodothyronine showed an increase after the initiation of anti-TB treatment, however these mean values were within normal limits and differences were not statistically significant. Mean  $\pm$  SE s-IL-2R- $\alpha$  levels were higher before treatment compared to mean values after treatment began, but not up to statistical significance and within normal limits. With the exception of the hyperthyroid patient, s-IL-2R- $\alpha$  levels were inversely correlated with total triiodothyronine levels before treatment (Spearman's rank correlation R: -0.62,  $p: 0.017$ ), while no correlation was found at the second sampling, after two weeks of anti-TB therapy (Spearman's R: -0.11,  $p: 0.70$ ). Serum thyrotropin, total thyroxine and free triiodothyronine were not correlated with s-IL-2R- $\alpha$  neither before nor after the initiation of anti-TB therapy.

**Table 1:** Overall hormone and soluble interleukin-2 receptor measurements results of the patients (n=29) included in the study

Measured parameter	1 <sup>st</sup> sampling (mean $\pm$ SE)	2 <sup>nd</sup> sampling (mean $\pm$ SE)
Thyrotropin (in $\mu$ IU/mL)	1.07 $\pm$ 0.15	1.09 $\pm$ 0.15
total thyroxine (in ng/dL)	9.91 $\pm$ 0.41	9.89 $\pm$ 0.41
total triiodothyronine (in ng/mL)	0.98 $\pm$ 0.06	1.29 $\pm$ 0.08*
free triiodothyronine (in pg/mL)	1.73 $\pm$ 0.05	1.91 $\pm$ 0.07
soluble interleukin-2 receptor (in pg/mL)	1832 $\pm$ 174	1805 $\pm$ 129

\* comparison of parameters' results between samplings significant at the  $p=0.05$  level (Kruskal-Wallis non-parametric ANOVA).

### Discussion

This study's patients initially presented with low to normal serum total triiodothyronin levels, which, following anti-TB treatment, showed a small but statistically significant elevation. This is a finding compatible with the low T-3 syndrome (euthyroid sick syndrome) encountered in non-thyroidal disease (8). A significant negative correlation was observed between serum total triiodothyronine and s-IL-2R- $\alpha$  in patients with TB, a non-thyroidal disease, before administration of anti-TB treatment. Consistently high

levels of s-IL-2R have been found in patients with untreated Graves' disease and toxic adenoma (4), while low levels of s-IL-2R have been consistently measured in hypothyroid post-thyroidectomy patients (4) and reported in cases of autoimmune thyroiditis (10). Levels of s-IL-2R have been shown to be affected essentially in severe cases of TB and in immunocompromised patients (9). The patients of this study were not immunocompromised and made an uneventful recovery, so in this setting, we can also speculate (given the small overall variations), a relation between thyroid hormones and s-IL-2R in the low-T3 syndrome. Since the measurement of s-IL-2R has already been proposed as an indicator of disease activity in Graves' disease (11) and an early response marker in thyrotoxicosis' treatment (5), further relevant studies can be envisaged, in order to assess the behavior and clinical utility of s-IL-2R levels versus thyroid function parameters in non-thyroidal disease.

### Conclusion

Soluble serum interleukin-2 receptor alpha levels were found to be inversely correlated with total triiodothyronine levels in 29 -otherwise healthy- patients with pulmonary tuberculosis and the low-T3 syndrome before the administration of antimicrobial therapy. Further studies can be envisaged, in order to assess the behavior and clinical utility of this receptor's levels versus thyroid function parameters in non-thyroidal disease.

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## Results

On the basis of the previously defined criteria, late potentials were found in 2 out of 11 volunteers (18%) in the first measurement. After 1 year the signal averaged ECG of both previously positive volunteers were found to be within normal limits, but one subject (9%) whose SA ECG was originally normal was classified as late potentials positive.

Interestingly, in all cases of positive SA ECG, late potentials were present due to coincident abnormal values of HFLA and RMS. fQRS was well within normal limits in all measurements. Both RMS and HFLA were in all 22 measurements 4 times abnormal.

Mean changes of measured parameters, and calculation of final values of changes considered abnormal are shown in table 1.

**Table 1:**

	mean change $\pm$ SD	2 SD	borderline values	abnormal changes
QRS (ms)	1,9 $\pm$ 5,6	11,2	$\pm$ 12	$\pm$ 13
fQRS (ms)	2,3 $\pm$ 3,1	6,2	$\pm$ 7	$\pm$ 8
HFLA (ms)	0,5 $\pm$ 10,3	20,6	$\pm$ 21	$\pm$ 22
RMS ( $\mu$ V)	0,78 $\pm$ 7,87	15,74	$\pm$ 16	$\pm$ 17

**Changes of measured parameters of SA ECG after one year in healthy volunteers (n=11) and calculation of changes considered to be abnormal.**

According to our results we consider as significant a change of the standard QRS duration  $\pm$ 13 ms, a change of fQRS  $\pm$ 8 ms, a change of HFLA  $\pm$ 22 ms and a change of RMS  $\pm$ 17V.

## Discussion

Using the currently recommended method in 11 healthy volunteers, we found the abnormal late potentials in 2/11 (18%), which is slightly higher than reported in previous studies (7,8). This difference may be caused by the different method of detection of late potentials, but also by the small size of our group of volunteers. Interestingly, the abnormal late potentials in our group did not remain stable over the longer period, they either appeared or disappeared without any apparent changes in the health status of the study participants. An important finding is that the abnormal late potentials were always diagnosed by simultaneous abnormalities of RMS and HFLA. In addition, we observed a low long-term stability of these parameters suggesting their poor long-term reproducibility. On contrast to RMS and HFLA no abnormal value of fQRS was observed in our study. FQRS was found to be the most stable parameter over time. In this way our work gives rise to some doubts about currently recommended criteria for evaluation of SA ECG. In order to eliminate the false positive results the duration of the filtered QRS should be preferred to the other two recommended parameters of SA ECG. Our results on long-

term stability of fQRS closely correspond with previous studies which found fQRS to be the most reproducible parameter of SA ECG in a short-time (6,9).

To our knowledge the estimation of the significance of long-term changes of the SA ECG parameters is the first attempt to obtain such criteria. In previous research just the occurrence of abnormal late potentials was used to describe changes in SA ECG. Such studies were done in patients after myocardial infarction (4,5). But by this simple way of evaluation changes in late potentials may be under- or overestimated. For example prolongation of fQRS from 95 ms to 113 ms is definitely a significant change without meeting defined criteria for late potentials. But a change as small as 1 ms may be sufficient to meet recommended criteria e.g. prolongation fQRS from 114 to 115 ms. Blomström-Lundqvist et al. (6) have arbitrarily defined the changes in late potentials as 10  $\mu$ V or more for RMS and 10 ms or more for HFLA under 25  $\mu$ V to be significant. Our results clearly show their suggested criteria to be unacceptable. The most important limitation of our study is the limited size of the group of volunteers. At the beginning of the study we considered the size to be sufficient as we expected only a small variability of the studied parameters in time. Although limited by the size of the group, the findings demonstrate that in the long-term follow-up, only rather large differences in individual parameters of SA ECG ( $\Delta$ QRS  $\pm$ 13 ms,  $\Delta$ fQRS  $\pm$ 8 ms,  $\Delta$ HFLA  $\pm$ 22 ms and  $\Delta$ RMS  $\pm$ 17  $\mu$ V) are likely to be caused by myocardial damage.

## Conclusion

The duration of fQRS appears to be the most stable parameter of SA ECG on analysis of the long-term changes of SA ECG parameters. The fQRS is clearly superior to both RMS and HFLA. We obtained an estimate of significant long-term changes of parameters of signal-averaged ECG which might be useful in evaluating changes of SA ECG in different groups of patients. We consider fQRS to be the most useful parameter of SA ECG for assessment of long-term changes of SA ECG.

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## THORACIC ELECTRICAL BIOIMPEDANCE VERSUS THERMODILUTION IN PATIENTS POST OPEN-HEART SURGERY

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**Summary:** Thoracic electrical bioimpedance cardiography is a non-invasive, continuous and low-cost method of estimation of cardiac output and other haemodynamic parameters. Though subject to continuous technological refinement controversial opinions exist on its validity in subsets of critically ill patients, patients with heart disease or after cardiac surgery. A comparison study between thermodilution (TD) and bioimpedance (TEB) was performed in 28 patients undergoing elective cardiac surgery (CABG, aortic or mitral valve replacement or combined procedures). 128 pairs of cardiac index estimates at specific time points during 20 hours at the postoperative ICU were evaluated. A poor correlation ( $r = 0,26$ ,  $p < 0,05$ , bias  $-0,07 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^2$ , precision  $+ 1,1 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^2$ , 95% limits of agreement  $-2,27 - 2,13 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^2$ ) between TD and TEB cannot support the routine use of TEB monitoring in early postoperative period after open-heart surgery. Possible reasons of lack of agreement in this population are discussed. Further studies with technically improved bioimpedance cardiographs will be needed.

**Key words:** Cardiac output; Thermodilution; Electrical bioimpedance; Cardiac surgery; Intensive care unit

### Introduction

Knowledge of changes of central haemodynamics in patients after cardiac surgery is of crucial importance for optimal therapy. Cardiac output, cardiac index (CI) and other indices characterizing left ventricular performance together with data of systemic and pulmonary vascular resistance give us information about the adequacy of oxygen transport - the most important function of cardio-pulmonary system.

Cardiac output and other derived parameters can be measured invasively by Fick method, dye dilution or thermodilution. Fick method and dye dilution are employed mostly in catheterization laboratories and are not suitable for clinical haemodynamic monitoring because of their technical difficulties. Thermodilution (TD) by means of right heart catheterization by Swan-Ganz pulmonary artery catheter is method most frequently used for routine and repeated bedside measurement of cardiac output despite its possible risks and costs. Cardiac output can be estimated non-invasively by Doppler echocardiography but the method is unsuitable for routine monitoring because it is time-consuming and operator-dependent.

Transthoracic electrical bioimpedance (TEB) is an attractive alternative providing non-invasive, continuous, real-time, time-unlimited and cheap monitoring of central haemodynamics. Though the technology has been refined in recent years controversies still do exist about its validity in clinical

settings. The aim of this study was to determine the correlation and agreement between measurements of CI by means of TEB and TD and thus, in case of good correlation and agreement, indicate possible areas where thermodilution could be interchanged or replaced by bioimpedance.

### Material and methods

The population studied were adult patients undergoing elective cardiac surgery at university cardiac surgery centre who had Swan-Ganz pulmonary artery catheter inserted either before induction of anaesthesia or in the course of operation. The decision about the right heart catheterization was upon anaesthesiologist's consideration based on patient's history, preoperative status and haemodynamic situation in the course of operation. The study was approved by the institutional Ethics Committee.

A total of 37 patients representing the usual incidence of cardiac procedures were monitored non-invasively by thoracic bioimpedance cardiograph. Ten patients were excluded of the cohort because of cardiac pacing (4), motoric disturbance (3) and low-quality impedance signal (3). Finally 28 patients were enrolled in the study having undergone following procedures: coronary revascularization (CABG - 19, including 1 miniinvasive coronary bypass grafting - MIDCAB), aortic valve replacement (4), mitral valve replacement (1) and combined procedures - aortic

valve replacement with coronary revascularization (3). All patients were in sinus rhythm. All but one were intubated and artificially ventilated at the beginning of the monitoring period and were intentionally extubated no sooner than 4 hours after the arrival at ICU.

Thermodilution measurements were performed upon the arrival of the patient from operation room to ICU and then after 4, 8, 12 and 20 hours respectively. The value of CI estimate was the average of four consecutive injections of saline solution of room temperature. If any of the trials differed more than 10 percent it was deleted and additional injection was performed. The measurements were processed, stored and printed by the Marquette Electronics Inc. software.

Thoracic electrical bioimpedance measurements were performed in a way of continuous monitoring by non-invasive bioimpedance cardiograph Hotman AH/HHC (Hemo Sapiens, Irvine, Ca, USA). Eight solid gel electrodes were applied on the skin at the area of neck and thorax according to the scheme (Fig. 1). The bioimpedance cardiograph displayed the real-time continuous CI estimate as well as other haemodynamic parameters (respiratory rate, heart rate, stroke index, non-invasive blood pressure, end-diastolic index, ejection fraction, left stroke work index, inotropic state index, ejection phase contractility index, thoracic fluid conductivity). All measured data were stored by the cardiograph in a form of patient's record.

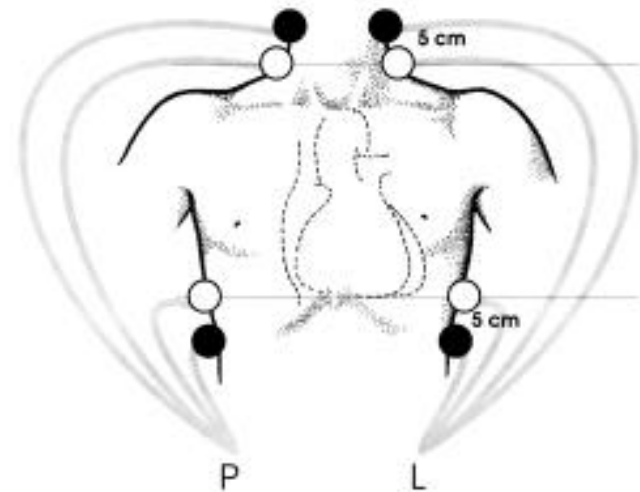


Fig. 1: Placement of TEB electrodes (black - current electrodes, white - sensing electrodes).

From all measured parameters only CI estimates were studied. As the bioimpedance CI estimate results from slightly oscillating reading that is updated every 1 minute the average value of 15 minutes record at corresponding time points was taken as a counterpart to be paired with the thermodilution measurement.

The paired data were processed by Excel 8.0 software (Microsoft). The correlation coefficient  $r$  was calculated between the methods and data analysis introduced by Bland-Altman was performed.

## Results

Together 128 pairs of CI estimates (thermodilution/bioimpedance) were obtained (25, 26, 27, 26 and 24 at each time point respectively). The range of readings was 1,3 - 6,7  $\text{l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$  (TEB) and 1,8 - 5,6  $\text{l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$  (TD).

A correlation was sought between TD and TEB readings. The correlation coefficient  $r$  calculated for all pairs of data obtained was 0,26,  $p < 0,05$  (Fig. 2). Then a correlation was sought between specific subsets of data. The correlation coefficient for CABG patients only was  $r = 0,30$ ,  $p < 0,05$ . On the same basis correlation was determined between paired measurements at specific time points 1 - 5. In these subsets  $r$  was determined 0,25; 0,33; 0,23; 0,34; 0,21;  $p < 0,05$  respectively, indicating thus the best between-method correlation in measurements 12 hours after the end of open-heart surgery.

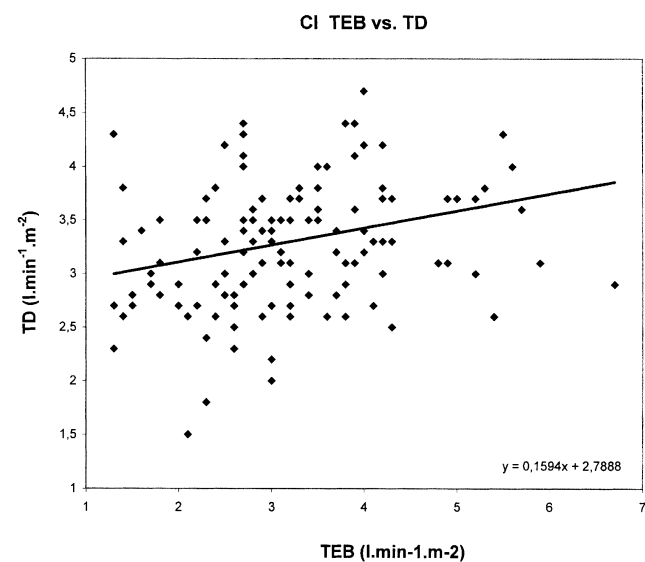


Fig. 2: Correlation coefficient between TEB and TD CI estimates during 20 hours after open-heart surgery ( $r = 0,26$ ,  $p < 0,05$ ;  $n = 128$ ).

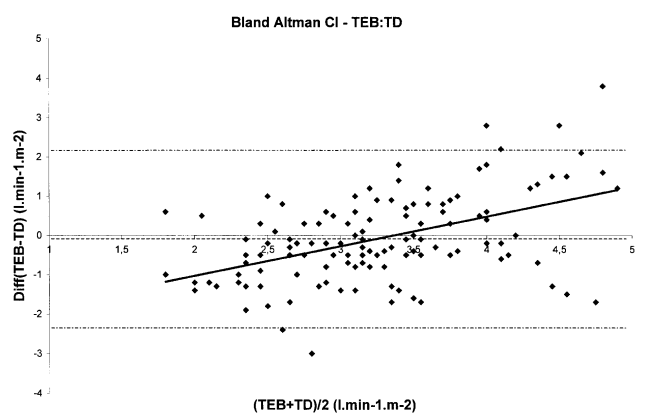


Fig. 3: Bland-Altman analysis of 128 pairs of CI measurements (TEB vs. TD); bias  $-0,07 + 1,1 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ ; 95% limits of agreement  $-2,27 - 2,13 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ .

A data analysis that was introduced in 1986 by Bland and Altman and since then has been widely used as the only correct procedure in determining the agreement of two methods, neither one of which is absolutely precise, was performed. The distribution of all data in Bland-Altman's plot display bias  $-0,07 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ , with precision (SD)  $\pm 1,1 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$ . The 95% limits of agreement defined as  $\pm 2 \text{ SD}$  were  $-2,27 - 2,13 \text{ l}\cdot\text{min}^{-1}\cdot\text{m}^{-2}$  (Fig. 3).

## Discussion

Though the changes of impedance synchronous with cardiac cycle were noticed already by Vigoroux (1888) and Cremer (1907), the theoretical grounds were layed and first modern devices for measurement of thoracic electrical bioimpedance were constructed in U.S.A. in late 60-ties for the purpose of N.A.S.A. Nyboer in the beginnings of 50-ties described first equation for computation of stroke volume which was later modified by Kubicek in 60-ties:

$$SV = \rho \times (L/Z_0)^2 \times LVET \times (dZ/dt)$$

where LVET = left ventricular ejection time,  $dZ/dt$  = maximal velocity of change of impedance during systole ( $\text{Ohm/s}$ ).

In 1981 Sramek presented a new equation for computation of SV which eliminated blood resistivity and thoracic length:

$$SV = VEPT \times VET \times IC$$

where VEPT = physical volume of electrically participating tissue calculated from sex, height and weight correlated to age, VET = ventricular ejection time, IC = index of contractility ( $\text{s}^{-1}$ ).

Sramek's formula was later modified by Bernstein (1986) who introduced correction factor delta to eliminate some disproportions in patients with borderline weight.

Thorax represents an electrically inhomogeneous volume conductor. High-frequency electrical current of low intensity (50 - 100 kHz, 0,2 - 5 mA) is distributed via two pairs of electrodes on the surface of the neck and at the level of diaphragm. Both the basic level of impedance and its dynamic changes are measured by two pairs of sensing electrodes situated inside the electrical field. The dynamic impedance changes are synchronous with heart rate and are caused predominantly by changes of descending thoracic aorta throughout the heart cycle. Descending thoracic aorta due to its longitudinal orientation is the main electrical current pathway in thorax. Changes of its impedance are caused by changes of its volume thus reflecting the cyclic intravascular changes of pressure originated in heart performance. Second contribution to the dynamic impedance signal  $dZ$  is the change of flowing blood conductivity with regard to its velocity. At the moment of highest velocity (systole) the blo-

od displays the highest conductivity that is caused by most of the erythrocytes being aligned parallel to the stream.

The devices for measurement of transthoracic electrical bioimpedance have been substantially improved in the course of development. The dramatic onset of hardware and software sophistication enabled the modern bioimpedance cardiographs to be constructed as compact, easy-to-handle multifunctional devices suitable for longitudinal real-time bedside monitoring of central haemodynamics.

Due to its favourable characteristics TEB is frequently employed by investigators in anaesthesiology, gynaecology or nephrology. On the other hand, serious controversies still do exist about the validity of bioimpedance measurements in clinical conditions. The on-going efforts of investigators are motivated by the need of a device performing reasonably precise, non-invasive and cheap measurements. Works with different results were published comparing TEB with other modalities of cardiac output estimation in experimental model as well as in various patients populations.

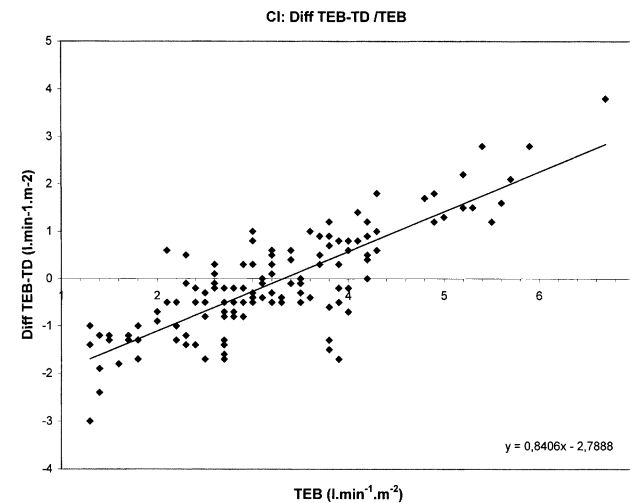
Whereas the first trials were performed mostly on healthy volunteers confirming thus the basic concepts of the method a number of authors tried to validate bioimpedance cardiography in various clinical conditions against proven methods of CO estimation. The population for these studies was therefore recruited among those patients whose diagnostics and/or treatment necessitated the measurement of CO (thermodilution, indirect Fick, dye dilution, Doppler). Typically, patients undergoing diagnostic catheterization, open-heart surgery or staying at the ICU from various reasons were studied. The majority of these studies found an overall good correlation between methods ( $r = 0,65 - 0,9$ ) and low bias values with varying values of precision. Shoemaker documented in multicenter trial the use of TEB in series of high-risk surgical patients with possibility of tracking the typical patterns of survivors vs. non-survivors via longitudinal bioimpedance cardiography monitoring (1, 2). Similarly, Bishop published good results in patients with gunshot wounds (3), Belardinelli in patients with ischaemic cardiomyopathy (4), Spiess during orthotopic liver transplantation (5), Northridge and Talarico in acute myocardial infarction, etc.(6 - 9). The possibility of early detection of rejection episodes after heart transplantation was documented in the decrease of specific TEB acceleration index (10). On the contrary, others found only fair correlation between thermodilution and TEB and limits of agreement too wide for reasonable clinical application (12, 13).

The use of TEB in patients undergoing cardiac surgery is another appealing issue. The candidates for open-heart surgery - CABG predominantly - form relatively uniform group of patients who are well diagnosed, treated according to routine schemes and frequently with the use of pulmonary artery catheter. Hypothetical replacement of TD by TEB, if allowed under certain conditions, would mean a considerable diminishment of invasive burden as well as decrease of costs. However, the results of several studies are deeply controversial. Doering reports poor agreement in her

series of 34 elective cardiac surgery patients (CABG, mitral valve replacement, combined valve and coronary surgery) being monitored non-invasively for 22 hours after operation (14). Thomas discourages the use of TEB in the first 12 hours after cardiac surgery (15). Similar negative conclusions were published by Spahn and Sageman (16, 17) whereas good correlation was experienced by Ferraro, Hraška, Schwann and recently in a multicenter COST study (18 - 20).

Despite controversial opinions on validity of TEB in clinical settings there is an agreement in defining the areas where TEB due to its inherent drawbacks is unsuitable for use. These are sepsis, tachycardia over >180/min, extreme obesity or height, excessive patient movement, dilatation of aorta, LBBB (21). Patients with aortic or mitral valve regurgitation are falsely underestimated because only forward flow is taken into consideration. The effect of improper position of lower pair of sensing electrodes on the CO estimate was demonstrated by Jewkes (22).

On the other hand, it has to be reminded that thermodilution is not a reference method and bears a lot of inherent imprecision as well (23). It is known for underestimating in low-flow states. In general, the flow-dependent relation between the two methods remains unclear. While overestimation by TEB in low-flow states is claimed by some (12) and also in high-flow states by others (22), a systematic underestimation was also reported (16). We found almost linear underestimation at low flow and overestimation at high flow (Fig. 4).



**Fig. 4:** Low-flow vs. high flow TEB readings - relation to simultaneous TD: low-flow TEB readings underestimate TD whereas high-flow TEB overestimates TD ( $r = 0,87$ ,  $p < 0,05$ ;  $n = 128$ ).

In our experience, transthoracic electrical bioimpedance monitoring can be easily accomplished providing comfortable real-time information and unlimited longitudinal record. Care has to be taken of correct placement of lower pair of sensing electrodes because the level of diaphragm lays more cranial in supine patients than it might be expected.

Patients who were 100 % paced (via epimyocardial electrode placed at the time of operation) were not suitable candidates for TEB monitoring because aberrant QRS formation disabled correct detection of systole and led to erroneous CI estimate. Three MIDCAB patients were excluded from the study because of restlessness and motoric disturbances commonly seen after this type of procedure.

Nevertheless, correlation between two methods of CI estimation was poor what is expressed in  $r = 0,2$ ;  $p < 0,05$ . The result did not change significantly when CABG patients were studied solely ( $r = 0,30$ ,  $p < 0,05$ ) or only measurements from specific time points were examined separately ( $r = 0,21 - 0,34$ ;  $p < 0,05$ ).

Bland and Altman introduced their statistical method in 1986 as the only correct tool in determining the agreement of two methods of which neither one is absolutely precise. The differences in readings by TD and TEB were plotted against the average of both methods. The between methods bias was very low  $-0,07 \text{ l.min}^{-1}.\text{m}^{-2}$ . The precision however was  $\pm 1,1 \text{ l.min}^{-1}.\text{m}^{-2}$ . The 95% limits of agreement were  $-2,27 - 2,13 \text{ l.min}^{-1}.\text{m}^{-2}$ , what represents interval unacceptable for clinical purposes.

It is evident that TEB technology encounters distinct problems in open-heart surgery patients. An acute dysbalance in thoracic fluid content after cardio-pulmonary bypass may be the leading cause. Different patterns of thoracic resistance in patients operated with vs. without cardio-pulmonary bypass, which were expressed shortly after cardiac surgery, were documented in the work of Máttar (24). The structural and functional changes of thorax, amount of fluid in pericardial and pleural cavities together with presence of chest tubes make the correct bioimpedance calculation difficult. Other misleading situations as arrhythmias, low-flow or high-flow states and artificial ventilation - the influence of which is poorly understood - are frequently present after open-heart surgery.

### Conclusion

Transthoracic electrical bioimpedance though controversially accepted is an established method for non-invasive monitoring of central haemodynamics. The bioimpedance cardiographs have been subject to continuous refinement of calculation algorithm. Newly released monitors are awaited for clinical evaluation. As numerous studies were performed in various clinical subsets using different types of bioimpedance cardiographs and employing different formulas (Kubicek, Sramek, Sramek-Bernstein) further technological improvement as well as clearly defined areas of clinical application still have to be sought. If justified by comparison study it would be very attractive modality due to its non-invasiveness, continuous real-time estimate and low costs in situations where information on central haemodynamics was needed but invasive approach was discouraged from multiple reasons. At this moment, however, our data cannot support this hypothesis.

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## INGUINAL HERNIOPLASTY ACCORDING TO LOTHEISSEN AND McVAY

Zbyněk Vobořil

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*Summary:* Inguinal hernioplasty represents one of the most frequently performed operation in surgery. In the article the author describes the technique of inguinal hernioplasty according to Lotheissen and McVay. He has an excellent experience when using this procedure. This communication tried to focus the attention of surgical community on this method.

*Key words:* Inguinal hernioplasty; Inguinal hernia

Inguinal hernioplasty represents one of the most frequently performed surgical interventions, being only second in frequency after appendectomy. In spite of the fact that hernioplasty is frequently performed by surgical residents, this does not mean that treating patients with hernias is an easy task, as we often realize when encountered with recurrence. Current surgical practice stresses the importance of meticulous reconstruction of deep inguinal layer. This is by no means a new concept. Back in 1804 Cooper suggested that transversal fascia discovered by him protects against hernias. It was not until 1941 that McVay and coworkers proved on preparations that transversal fascia is not attached to inguinal, but to pectineal (Cooper's) ligament (Fig. 1). He noticed that when transversal fascia is attached to Cooper's ligament there may be no reason for disturbing this spatial relationship during inguinal reconstruction, and there is no reason joining deep structures and fascia to inguinal ligament. By proposing the procedure McVay essentially rediscovered and made popular an older technique described by Lotheissen. Georg Lotheissen, an Austrian surgeon, was the first to use Cooper's ligament for repair of inguinal hernia in 1898. He discovered an effective method of reconstructing posterior wall of inguinal canal which plays an important role in the origin and treatment of inguinal hernias. The technique, most often referred to as McVay's method, which should rather be called technique after Lotheissen and McVay, slipped into oblivion and is currently not widely used.

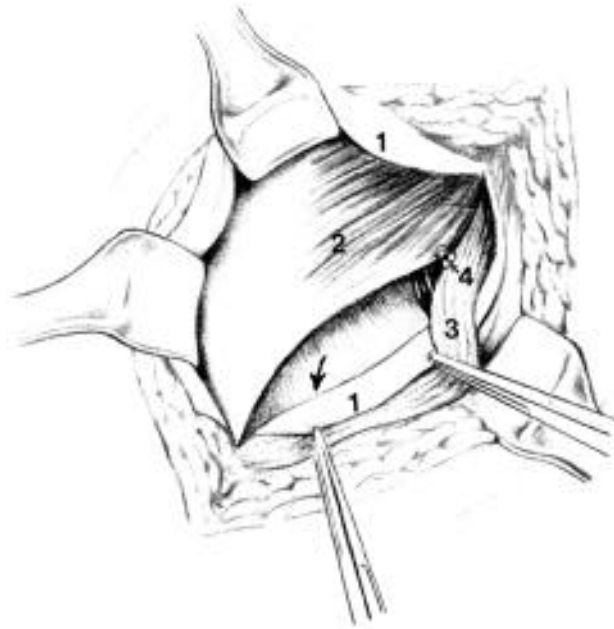
At the Department of Surgery of Faculty of Medicine and Teaching Hospital we are using the technique presented in cases of important defects of the posterior wall of inguinal canal, and in surgical management of recurrent hernias, including femoral hernias.



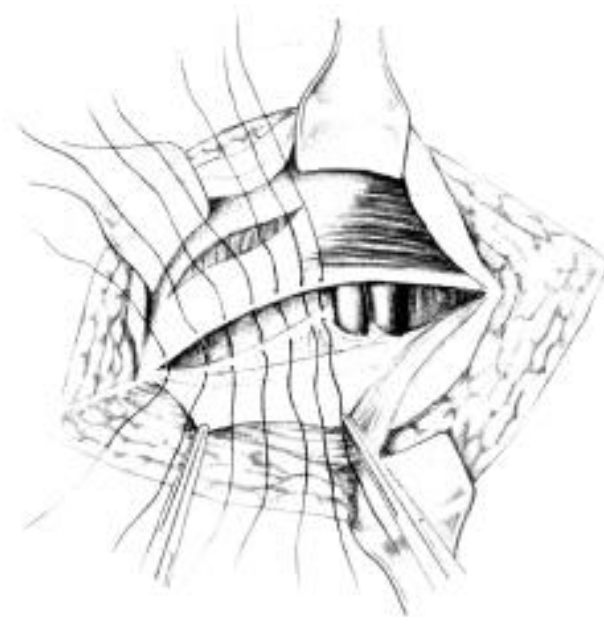
**Fig. 1:** View of lower abdominal wall quadrant from inside. Transversal fascia attaches to Cooper's ligamentum. 1 - lig. inguinale, 2 - lig. pectinale Cooperi, 3 - tuberculum pubicum.

### The procedure

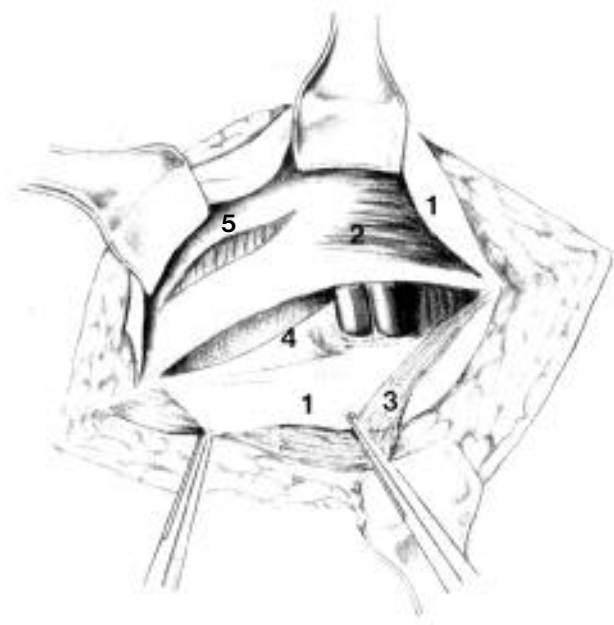
The procedure requires exact identification of anatomical structures and meticulous work. The procedure is identical to standard techniques up to the moment of closing the inguinal sac. Anulus inguinalis internus is contracted and then moved laterally.



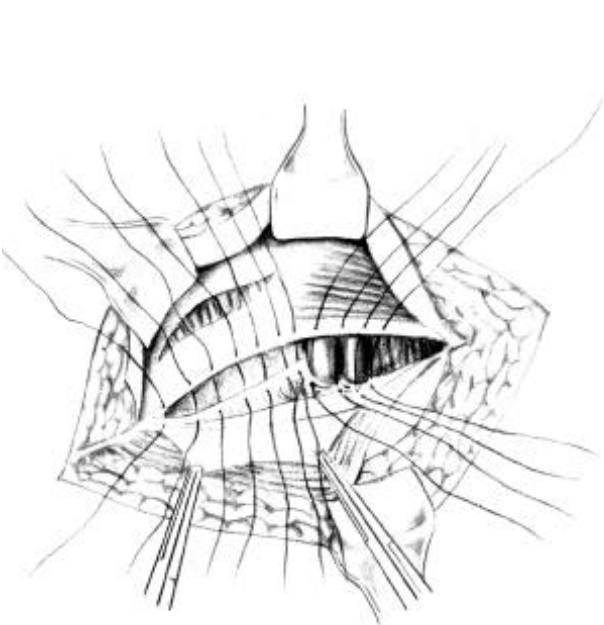
**Fig. 2:** Inguinal hernioplasty according to Lotheissen and McVay: preparation behind inguinal ligament caudally (arrow). 1 - aponeurosis m. obliqui abdominis ext., 2 - m. obliquus abdominis internus, 3 - funiculus spermaticus, 4 - rest of the hernial sac (ligated).



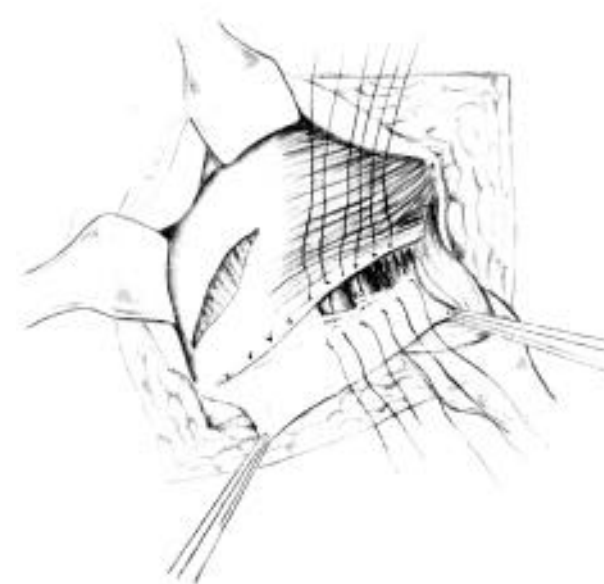
**Fig. 4:** Inguinal hernioplasty according to Lotheissen and McVay: hernioplasty is performed by suturing, superiorly, transversal fascia medially up to tendon conjoint, inferiorly to pectineal ligament and medially to tuberculum pubicum. The most lateral suture is placed in close proximity and medially to femoral vein.



**Fig. 3:** Inguinal hernioplasty according to Lotheissen and McVay: by preparation behind inguinal ligament caudally ramus superior and pecten ossis pubis with Cooper's ligament are made free. 1 - aponeurosis m. obliqui abdominis ext., 2 - m. obliquus abdominis internus, 3 - funiculus spermaticus, 4 - lig. pectineale Cooperi, 5 - Hockeystockschnitt.



**Fig. 5:** Inguinal hernioplasty according to Lotheissen and McVay: in the region above femoral vessels 2-3 sutures between fascia transversalis and connective tissue around femoral vessels, Thompson's band or deep inferior layer of inguinal ligament are placed (transient suture).



**Fig. 6:** The reconstruction of deep inguinal layer according to Lotheissen and McVay.

By preparation behind inguinal ligament caudally ramus superior and pecten ossis pubis with Cooper's ligament are made free (Fig. 2 and 3). Hernioplasty is performed by suturing, superiorly transversal fascia medially up to tendon conjoint, inferiorly to pectineal ligament and medially to tuberculum pubicum (Fig. 4). The most lateral suture is placed in close proximity and medially to femoral vein under careful visual control. In the region above femoral vessels we place 2-3 sutures between transversal fascia and connective tissue around femoral vessels, Thompson's band or deep inferior layer of inguinal ligament (Fig. 5). This part of the suture - transient suture - was considered by McVay to be the weakest point of the hernioplasty, and the line of the suture is moved here one layer more superficially to inguinal ligament.

In addition, a relaxing incision of both posterior sheaths of rectal muscle is performed (so called Hockeystockschnitt - Fig. 3-6), which makes possible performing the deep suture without tension.

Above the structure thus created the spermatic funicle is placed, and cremaster muscle is resected, followed by a suture of the exterior oblique muscle fascia to inguinal ligament.

### Patients

Between 1974 and December 1996 this procedure of hernioplasty after Lotheissen and McVay was performed at

the Department of Surgery in Hradec Králové in 225 cases. Long term results were evaluated in a cohort of 32 patients (29 males and 3 females, aged 21 to 74 years). The most numerous group of patients consisted of those with direct inguinal hernia (16 cases), indirect inguinal hernia was present in 10 cases, femoral hernia in 2 cases, a recurrent femoral hernia was present in 4 cases. The patients were followed for 2 to 19 years after surgery.

Among these 32 patients recurrence was observed in one case of a 65 year old man treated for recurrent direct inguinal hernia. Postoperative course was complicated by bronchitis with massive expectoration. The recurrence was diagnosed 9 months after surgery. A reoperation was performed and the patient is recurrence-free after 8 years.

### Conclusions

Inguinal hernia can be managed successfully using a variety of methods based on the type, extent and experience of the surgeon. A technique should be chosen, however, which pays attention to meticulous reconstruction of deep inguinal layers. The method according to Lotheissen and McVay is one of these procedures. At our institution we made an excellent experience using this procedure, and this communication tried to focus the attention of surgical community on this method.

Figures were drawn by Josef Bavor, Ph.D.

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**UNIVERSITY STUDENTS DURING HISTORICAL DEVELOPMENTS  
OF THE UNIVERSITY, 650 YEARS OF THE JOURNEY  
TO KNOWLEDGE AND UNDERSTANDING**

*Alexander Schirger*

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Mister Chairman, it was with a great deal of joy and a simultaneous sense of profound humility that I had accepted the invitation of Professor Daum and the organizing committee of the symposium on the University and Its Students, within the framework of the commemoration of the 650th anniversary of the founding of Charles University, to participate in today's program. Knowing there are probably many who would be able to offer these reflections with greater precision and perhaps a more sharpened focus particularly directed at the years when I was absent from the country, imparted a sense of hesitation. Nevertheless, I undertook the codification of my memories with a great deal of enthusiasm hoping to transmit to today's students not only reflections from the past but deductions for the future.

Having been born in Prague and having spent my early childhood in Prague, I had the privilege of becoming, very early in life, familiar with classical works of the Czech literature, like the book by Zikmund Winter on Mr. Kampanus or some of the works dealing with the period after the defeat of the Czech National Forces in 1620 on the White Mountain and its repercussions on the university. Little did I suspect, as a young boy reading what appeared to be essentially historical novels, that some of the same realities, in much harsher form, I will have the opportunity to witness reenacted in my own lifetime. Following the death of my parents, I returned to Prague as a citizen of the United States of America and continued my primary and secondary education there. Our otherwise peaceful life was interrupted by the events of September of 1938 when the nation was united and mobilized with maximum effort in a unified will to withstand, at whatever cost, the forces of aggression. The opportunity to exercise that will was denied to the nation by the leadership for reasons that I am sure will be debated for years to come. In those days in September of 1938, the students of Charles University were equal to others expressing readiness to defend freedom.

Czechoslovakia was abolished in 1939, by the voluntary session of Slovakia into an independent state and by the occupation of the remnant of the historic lands, that is Bohemia and Moravia, by the German army.

On October 28, 1939, during a peaceful demonstration, one of the Czech university students, Jan Opletal, was shot

and wounded. He died several days later, and on November 17, 1939, thousands of university students reverently and peacefully attended his funeral. This was deemed to be a provocation by the German authorities who then, that night, closed all schools of higher learning including Charles University for a six-year period. About a thousand students, who were engaged in the pursuit of acquisition of knowledge and betterment of themselves, were taken to concentration camps. Graphic testimony to this was when, on the morning of November 18, I awoke and found the building in which we lived occupied by SS troupers patrolling the entry and clearly occupying all the university premises. Thereafter, followed the five long years of war, until the hope for liberation by the western allies in 1945, which for the city of Prague and Charles University never took place since it had been decreed that such will need to be carried out by the Soviet Armed Forces. Immediately after liberation in 1945, there was a great deal of enthusiasm, that I had witnessed personally, by the students who had been denied an opportunity for five years to return to their Alma Mater. Provisional spaces had to be utilized and yet not all could be accommodated. It was then, as an American Citizen, I had enrolled into the School of Medicine of Charles University. At that time, without printed text books and, in fact, ironically largely in anatomy relying on German texts, we crowded into the large hall of Lucerna and anxiously listened to our professors' attempts to teach us the foundations of the basic sciences and medicine in a way that, at least for me personally, served me well throughout my professional career. It illustrated the fact that, while physical premises are important, it is the will to learn and the will to teach that is most important. The first lesson, perhaps, to learn and to carry away from those heady days of liberation in 1945. The International Congress of World Students, which convened in Prague in 1945, was unfortunately strongly hijacked by leftist forces and, thus, tainted in some respects the memories of those it was intended to honor and remember, the ones fallen either in the takeover of the university in 1939 or subsequently deceased in concentration camps.

Thereafter, ensued two and a half years of peaceful learning, and the course of the university returned to its usual business, which is education, acquisition of knowledge,

and the forming of academic bonds that were to last for life. We witnessed in the summer of 1947, as Czechoslovakia had the opportunity to join the Marshall plan that winds of change were again blowing and perhaps not auguring well for the university and its students. The communists tried to control some of the student organizations. In February of 1948, the then leadership of the Czechoslovak state failed for the second time by yielding to the forces of evil, this time coming from the left. It was the students of the university who were the sole voice of protest. I never forget the fact that when the unconstitutional Coup d'etat occurred, it was not the populus that was out in the streets, but it was the 40,000 students of Charles University who peacefully marched from all corners of Prague despite the fact that the communist police attempted to block their entry to the castle to present their petition to president Beneš. It always will be counted as one of the more disgracing acts of his presidency that he refused to see them in that critical moment of the nation.

Eventually, just like the troops in Jerusalem needed to workup their courage 2,000 years ago to arrest their victim, so here, it took the communist police several attempts to overcome their fright and the awe at observing students singing the national anthem, before finally, with rifle butts, they charged the crowd and dispersed them, arresting many. I had an eye witness account of this from my wife, who was running down the streets of Mala Strana in an attempt to escape both injury and arrest. I, myself, took refuge, as an American citizen, in the United States Embassy and witnessed what appeared to be the only and last protest of pro-democracy forces. While in 1939 the nation was facing five years of subjugation, in 1948 the prospects were much darker and longer. It, in fact, took 20 years before the first signs of spring arrived which, again, were crushed by the invasion of five eastern block nation's armies on August 22, 1968. While the resistance of the nation was more coordinated, it was the tragic fate of Jan Palach who expressed, in his act of self-immolation, the understandable, yet not commendable, feeling of frustration that the nation felt having its hopes of a new spring crushed again.

In the years between 1968 and 1989, it was, again, the university students who were vocal challengers to the establishment. On November 17, 1989, exactly 50 years after the Nazi onslaught against the students of Charles University and the closing of the university, the Czech student forces, again, assembled for a peaceful demonstration to commemorate the death of Jan Opletal and with the permission of the authorities, marched from the cemetery at Vyšehrad to Wenceslaus Square. At a critical point in route, this peaceful march was stopped by the security forces' onslaught against the students of Charles University. Fortunately, this time, in contrast to 1939 and 1968, the students' stand did not remain without support. The nation did not lose the symbolism of the forces of the current regime mimicking the forces of the Nazi aggressors 50 years ago in suppressing the university. On the next day, the play rights of the nation and the actors' union joined in a shut-

down of all public theaters, which turned into a forum of dialogue about the injustices of the regime. When the authorities tried to assemble the people's militia to subdue the students, the militia themselves realized that they would be shooting at their own children. It was at that critical point that the factory workers of the major iron works of Prague, CKD, joined the students' demonstrations, and thus, for practical purposes, sealed off the fate of the regime and gave rise to what has been come to be known throughout the world as the Velvet Revolution.

Czech students certainly cannot claim the heroism and the martyrdom of their co-sojourners in China who were crushed at Tiananmen Square. It is always going to be a testimony to Charles University and an example to the students of future generations that, when challenged with an unjust authoritarian regime, which was bent at suppressing basic human liberties, it was the students, in 1939, in 1948, and ultimately in 1989, who offered the voice that was at one time weak, at one time ineffective, and at one time a catalytic challenge to the nation that led to ushering in of the current era of freedom and prosperity. During this short span of history, there could be witnessed discrimination against students based on racial, ethnic, political, class origin, and religious belief. Whether this was carried out through the brut force or by means of the inquisitional-types of screening committees to whose ire our own chairman, Professor Daum, fell victim, mattered little. The end result was the same. Denial of access to higher education for reasons based on prejudice due to race, ethnicity, social origin, religious belief, or class origin. The price students of this land paid, as a result, is immeasurable. Yet not all was well on the other side of the curtain. When I attended the school of medicine here in Prague, about 50 percent of my classmates were women. When I returned to the United States, there were only two women in the class of 125 at Creighton University. Fortunately, this situation has been remedied with numbers in my home medical school approaching Parity. While we, in Prague, were in part, through circumstances largely color blind, I witnessed in the early 60's in Mobile, Alabama, that I United States Air Force captain, being African American, was asked to sit at the end of the bus. It took the civil rights movement and the ensuing legislation to remedy such and other injustices. Remedial measures were subsequently invoked to overcorrect consequences of past injustices by affirmative action, the value, wisdom, and justice of which have subsequently been both championed and questioned in animated debate and advanced and challenged in the courts. It is well to recall, here, that much of such challenge, in their most vocal form, would come from African American citizens such as the unsuccessful presidential candidate Allan Keith, who could identify with the Black African American political orator, Frederick Douglas, who stated, „Our oppressors have divested us of many valuable blessings and facilities for improvement in education, but thank heavens they have not yet been able to take from us the privilege of being honest, industrious, sober, and intelligent.“

A rich country-like America should seek to help its most disadvantaged members not because of what their ancestors endured but because they deserve a chance to reach their full potential here and now as human beings. As Leon Vilseter points out in *The Memory of Oppression*, oppression perpetuates itself. The real tragedy is that injustice retains the power to destroy long after it has ceased to be real. It is a posthumous victory of the oppressor when pain becomes a tradition. There is an unfair and difficult dilemma of the newly emancipated and enfranchised. An honorable life is not possible if they remember too little, and a normal life is not possible if they remember too much. As we contemplate the lawful, understandable, and sometimes misunderstood attempts to correct the barriers of racial inequality and later racial disharmony among university students, it is well for us to remember that solutions to problems of today might sometimes well be sought in the insights and ancient wisdom of those who preceded us. It is perhaps not inappropriate that as one reflects on the dys-synchronized influence of emphasis on race in our days, to recall, that in this hollow ground, some 650 years ago, a great European, Charles IV, proud of his Czech heritage but conscious of his continental responsibility, formulated ideas and put forth principles upon which it might be well for us to reflect today. As we scan the initial founding document of this illustrious university, it is well for us to admire the vision that he, in this brief document, put forth to the men of his time, his solicitude for citizens entrusted to his care. He felt it imperative that those of Czech native of these lands, not seek, as he put it, the fruits of sciences and wisdom by asking for alms in foreign lands but that they find in their own home a table fully prepared for generous hospitality. In addition, in his fond vision of this university, he also expressed the hope that others would join sons and daughters of this land to seek wisdom, advance in knowledge, and prepare themselves for the task of life not uncognizant of the environment which the beauty of the city, which he perceived even 650 years ago, offered as a milieu for good studies. He pledged his royal imperial favor to all and everyone who would wish to come to visit this university, to be lavishly endowed with privileges and freedoms similar to those enjoyed by the professors, teachers, and students of the universities of Paris and Bologna.

Translated in today's language, the farseeing founder of this illustrious university had pledged equal opportunity for all, native and foreign, based on the recognition of their dignity and on the obligation that this land and those entrusted with the grave responsibility to govern it even in the year of 1348 should provide equal opportunities to all based on their human dignity and the potential they harbor as human beings. Lessons well for us to contemplate and more importantly to put in practice throughout academia the world over. That, my dear friends, I believe would be the legacy of Charles IV, the farseeing founder of this illustrious university were he today address the topic I was asked to discuss. In recognizing the principles that he enshrined in the founding bull and applying them to the academic life to-

day, we shall pay the greatest and deserved homage to the man who, by several centuries, outdistanced through his vision the course of history.

It is true that knowledge is best advanced in peaceful times. It had been said by Horace that „inter arma silent musae.“ Thus, we look to the times of challenge and stress where our predecessors excelled often in heroism and while we view their acts with admiration, we also are cognizant of our own obligation to make the best use of the current day and make optimal use of the opportunities given to us. While doing that, we should never lose sight of the lessons of the past and be vigilant to the emergence of those forces that would challenge life, freedom, and human dignity. By doing so, we will remain students, professors, alumnae and alumni of Charles University, true to its mission given to us upon entry and reaffirmed upon graduation. May this university, carrying forth the ideas and vision entrusted to it by its founder, prosper in years to come and continue to serve as a beacon not only to this blessed land but also to lands and people beyond the shores of this continent. For this, I would like to conclude by offering my humble wishes that this comes true. Universitas Carolina Vivat, Crescat, Floreat.

Thank you very much.

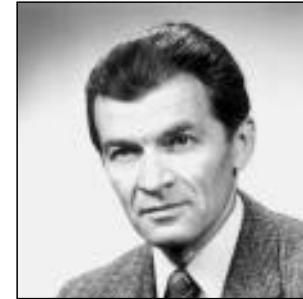
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Alexander Schirger, M.D., Professor of Medicine Mayo Clinic Medical School and Consultant in Medicine, Department of Medicine, Division of Cardiovascular Diseases was born in Prague, October 3, 1925. After the death of his parents he returned from the USA to Prague as a citizen of the United States of America and continued his primary, secondary and medical studies here. He graduated from the Faculty of Medicine, Charles University. In 1951 he returned to the USA. He is an internationally known internist, angiologist and researcher especially concerning the pathophysiology and therapy of hyper- and hypotension. He is the member of many scientific societies and was the president of the XIII<sup>th</sup> International Congress of Angiology in the USA. He has been cooperating closely with the Faculties of Medicine and Institutions in Czech Republic, such as the Foundation of Olga Havlová. He was a member of the Scientific Board of the Faculty of Medicine in Hradec Králové. He delivered several lectures at our faculty and was awarded the gold medal of the Faculty of Medicine. He was also invited to deliver the ceremonial speech on the occasion of the 50th anniversary of its foundation. As well, he was invited to participate in the Conference „The University and Its Students“ and the Rector of the Charles University awarded him the Jubilee Commemorative Medal coined on the occasion of the 650th Anniversary of Charles University in 1998. Thanks to his efforts, many medical students and doctors from the Czech and Slovak Republics have enjoyed the stay at Mayo Clinic, including some of our doctors and students.

L. Chrobák

## HISTORY OF PATHOLOGICAL ANATOMY IN HRADEC KRÁLOVÉ

Zdeněk Nožička



Prof. Z. Nožička \*1932

One of the three founders of pathological anatomy as a separate medical branch, Karl Rokitansky, was born in our city in the year 1804. Nevertheless, he had no relation to our department.

The pathological anatomical activity in Hradec Králové started as lately as 28 years old doctor of medicine Antonin Fingerland came to our city in the year 1928 when at the occasion of the tenth anniversary of our Republic Independence a new hospital was just being opened. Doctor Fingerland, who had been a disciple of professors Hlava and Šíkl, was appointed as both the senior dissector and the head of infectious diseases department. For many years he had been working completely alone but had never forgotten the publication activities. At the very beginning he used to compile regular annual reports of pathological department, enriched by analysis of autopsies. Later on, in the thirties, this allowed him on his own material to elucidate changes in some mortal diseases frequencies. At his own expenses he passed the holidays stay in histological laboratory of professor Masson in Paris, and the next year the bacteriological course in Pasteur's Institute. After coming back doctor Fingerland introduced BWR test, using **flint stone** liver obtained in dissection room as antigen. At the same time in the pathology lab he was performing basic bacteriological, biochemical and hematological tests for the whole hospital. Only one year after it the biopsy samples started to be examined in Hlava's institute in Prague, he arranged for the same service in Hradec Králové, too. It was in the year 1929. Doctor Fingerland in his far-sighted way also took care of the quality of laboratory tests performed. In the year 1934 he engaged 14 years old Růženka Rohoznická as a charwoman. Within the years he educated her as a legendary laboratory technician who for long decades became the guarantee of an outstanding histological technique. Still before the war the senior consultant Fingerland exploited his above mentioned reports for the piece of knowledge concerning the ever increasing number of deaths due to the pulmonary cancer and its relevance to cigarettes smoking that had widely spread among the population after the First World War.

It is impossible to pass without notice Fingerland's share in organizing the medical life within the East Bohemian Medical Region and, above all, his pioneer achievement - starting with the regular medical meetings where also some interesting casuistic communications - so called „little pearls“ - were given an account of.

During the war-time he arranged for two medical students of at then time closed Czech universities - Josef Vaněk and Vladimír Vortel - to work as lab technicians with famous pathologist professor Hamperl at the German Medical School in Prague, that was not affected by closing the Czech universities and went on in its functioning. After the war both persons mentioned became outstanding professors of pathology.

At the very end of the war at the Fingerland's laboratory there was determined the spotted typhus as a cause of deaths of prisoners from Terezin concentration camp who worked together with labour commando in the near-by village Libčany. There cannot be a trace of doubt how valuable was this diagnosis stating for taking the proper epidemiological measures. After the war in 1945 the senior consultant Fingerland habilitated by his work concerning Bodian's method for impregnating the granules in cells of glomus caroticum and in tumour cells derived from it. He was appointed to the head of Pathological Anatomical Institute in newly established Medical Faculty of Charles University in Hradec Králové. He also personally took part in creating this school. Since the year 1946 he became a professor. In the year 1948 he succeeded in constructing the new lecture hall in addition to the dissection lab building. Under the guidance of professor Fingerland our institute aimed its activities not only on the lecturing and health practice but also on research tasks. This was orientated, as opposed to other pathological anatomical institutions, on aetiological pathology of bacterial, mycotic and virus diseases, also thanks to the help of his co-workers, namely doctors Vortel and Herout. Professor Fingerland returned once again to the problems of smoking, that he intuitively looked into before the war, but this time in the exact, by numbers well founded research.

From Fingerland's interest in aetiological pathogenesis there arose some publications of absolute priority. Let us remind at least his (with cooperation with Vortel and Endrys) first description of herpetic oesophagitis, as well as his interpretation of stratified tuberculomas pathogenesis. Of great value there was also his observation (the fourth one world-wide) of herpetic encephalitis.

From Vortel' publications there are worth mentioning the priority of Proteus encephalitis in new-borns, as well as the very valuable work concerning the pathology of BCG vaccination. The later Herout's priority paper dealing with herpetic tracheitis and pneumonitis had also its origin from this complex. From other themes we should mention works devoted to nocardiosis, listeriosis, cryptococcosis, adenovirus infections and observations of atypical manifestations of tuberculosis in patients after transplantations. From the year 1963 there originate two, at that time very important, communications about Reye's syndrome from Dvořáčková, Vortel and Hroch.

One of the aetiologically directed activities paid an interest even a quarter of a century later. In sixties professor Vortel was engaged in problems of syphilitic lymphadenitis and by that time he introduced very demanding, nevertheless reliable method of silvering by Warthin-Starry. When in the middle of eighties Helicobacters-hunt broke loose, we had been already prepared and ready to join in the very earliest period of the research.

Bioptic business, initially performed by the chief of department only, was after the introduction of National Insurance system in the year 1950 transferred to junior consultants and divided into two rather separated spheres, the establishment biopsies (covering the needs of our teaching hospital), and regional ones (for the remaining hospitals of the East Bohemian region). That was why professor Fingerland was able to bestow all his powers on teaching and research activities.

Introduction of clinical-pathological conferences significantly contributed to the post-graduate education. The similar conferences on Mayo Clinic in Rochester, as known from publishing in New England Journal of Medicine, were always taken by professor Fingerland as an exemplar. These clinical-pathological conferences, the first of which took place in the year 1952, were being arranged each month in the filled lecture halls. Up to now there had been nearly 300 of them. It would be ungrateful not to mention a brilliant contribution of professor of Internal Medicine Jurkovič to their glare.

In the year 1951 the whole Faculty passed under the military control and was converted into the Military Medical Academy. By that time the normal run of our institute was unfavourably influenced by interdiction on professor Fingerland' lectures in the year 1953 as he was refusing by then power assertion of Olga Borisovna Lapešinská's opinion on the cells origin. This prohibition was revoked one year later only after a direct interference of the leader of Chief Political Executive of the Army - general Prchlík.

From middle fifties, from about years 1953 to 1954, there descends our institutional card-index system of necroptic and bioptic findings by the means of modified decimal classification. In spite of the fact that a certain initial role was played by Dr. Vorreith who was by then working in the institute, professor Vortel was the most decisive and for many years the only creator and supplier of these files. The most serious interest in them was always shown especially by cli-

nicians. It would be a great misunderstanding to consider this card-index system to be some superfluous reduplication of the official health paperwork. Its unique features consisted in the fact that it contained not only the nosological units but also some detailed histological findings, especially from the necroptic materials. Professor Vortel personally examined all of them, even those from the most experienced dissection performers.

In the second half of the fifties the spectrum of bioptic investigations expanded. The liver biopsies were introduced. Newly performed suction biopsies of the stomach mucosa required the introduction of enzymatic histochemistry, in which a very important part was played by Dr. František Langr, by that time still a medical student only. However, the volume of routine duties did not allow to go on with these histochemistry tests. From the same reason even the electron microscope had not been brought into permanent use, though since the year 1952 our department had one of the first electron microscopes produced by Tesla Brno and experimentally obtained some very successful pictures.

Sometimes even seemingly common requests exceeded the resources of our institute. Following an improvident mention from our part that even cytological investigations of airways secretion are possible within the framework of oncological screening, during 2 weeks all the laboratory tables were covered with hundreds of sputum samples, demanding this oncological investigation. The cytological investigations had to be purposefully totally abandoned so that the routine and indispensable diagnostics of histological samples could ever go on. Neither the next attempt to operate the electron microscope lab when we obtained a new table electron microscope in the year 1965 was lasting for sufficiently long a time, in spite of a promising start when a complicated immunoelectronmicroscopic method was introduced for determining the gastrin levels. The outstanding advancement of a laboratory dates back from as late as early seventies when after personal transfers dictated by communist leader, a well experienced electronmicroscopic worker Dr. Špaček from the Normal Histology Department who was prohibited to teach, found a refuge in our institute. Here he created a remarkably worthwhile and comprehensive work from the sphere of three-dimensional reconstruction of synaptic structures of central nervous system. Also the introduction of immunofluorescent detection of organ autoantibodies by professor Nožička goes back to the late sixties.

The turn of years 1967 and 1968 brought the completion of the vast extension to our institute. At the same time the up to then divided biopsy laboratories were united.

Besides several other arguments also a large incidence of tuberculosis among the institute workers helped in enforcing the extension construction. Especially professor Fingerland and professor Vortel put it through in the offices. The deputy chief doctor Kopečný and the chief technician František Pospíšil became exceptionally involved as organizers during the construction works.

The personal transfers of the post occupational era did not bring to our institute only the personal benefits as in the case of Dr Špaček, but also substantial and irreplaceable losses. As ordered by nomenclature bodies professor Vortel (generally considered to be the future head of our institute) had to leave his position.

Professor Vortel's departure was extra painful not only because he was an extraordinarily talented pathologist but also a considerable organizer of the institute life. The fact that all the preparations, paraffine blocks, slides and even the residues still have their own places and are able to be found out any time should be credited to his person. Professor Fingerland retired after he reached the age of seventy but his designated successor professor Vortel, as explained above, had been eliminated from the faculty. In this situation associate professor Herout who returned from Kuwait was appointed as the chairman of the department of pathology. Activities of professor Herout (together with doctor Kubeš) are connected with an expansion of nephropathology. An increased concern was stimulated by the bloom of kidney transplantation programme and by demands of nephrologists for electronmicroscopic investigations of kidney biopsies, which were pioneered by associate professor Erben from the 1st internal department.

During the whole seventies and eighties there went on the running of an auxiliary bacteriological lab used namely for necropsy diagnostics. The assistance of fully qualified microbiologist doctor Vondráčková was rather appreciated. Nevertheless, the ever lasting lack of interest of medical doctors in our branch of activity represented the permanent source of difficulties.

Quite on the contrary, thanks to the understanding of the new head of institute, professor Fingerland was still able to attend our place. Till his age of ninety he regularly biked in every morning and never missed a single institution seminar.

By the end of seventies there appeared the requirement for arranging some special cytological duties, especially fine needle aspiration cytology from the thyroid gland and pancreas. The care of this cytological service was taken by doctor Kerekes who had already proven his abilities and initiative in the sphere of haematopathology where he had introduced histological investigation of drilling biopsies of bone marrow, using nondecalcinated sections after previous embedding into the hard resin and cutting them by Jung's microtome for hard materials. Without the benefit of consistent training in other departments Doctor Kerekes himself (mainly by comparing the print prepares from materials processed later on histologically) achieved a qualification for this sphere.

After the Summer holidays 1990 professor Herout retired and as a result of the public competition professor Šteiner was appointed to the head of our institute.

Nowadays there are 17 medical doctors working in our institute (3 of them being professors and 1 associate professor). Our department uses the services of qualified specialists in cardiopathology, pneumopathology, ultrastructural pathology of central nervous system, immunopathology, hepatopathology, nephropathology, neonatal pathology and in immunohistological techniques. Our institute is ready to provide all the pathological anatomical services needed by contemporary medical branches.

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