LICE, LIFE, AND LEAFHOPPERS: HOW WEIGL'S VACCINE CREATION INFLUENCED MY VIROLOGY RESEARCH

Karl Maramorosch
Rutgers-The State University of New Jersey, 93 Lipman Drive
New Brunswick, NJ 08901
e-mail: karlmaramorosch@yahoo.com

This paper is dedicated to the memory of my brother Alfred who worked as a physician in the ghetto of Kolomyya. On September 1, 1942 my father was shipped with 8 000 Jews to be gassed in Belzec. Two weeks later my brother was arrested and his dead body returned to the ghetto a few hours later. On October 14 my mother was shipped to the Belzec extermination camp with the remaining 7 000 ghetto inhabitants. My wife and I survived the holocaust in refugee camps in Romania, but 138 closest relatives in Nazi occupied Europe perished in Treblinka, Belzec, and Auschwitz.

In 1928, when I was 13 years old, my brother Alfred, who was a medical student in Lviv, told me that his biology professor, Rudolf Weigl, developed during World War I the first vaccine against exanthematic typhus. Weigl, who was a young officer in the Austrian army, got the brilliant idea how to prepare a vaccine to protect people from the deadly disease that killed thousands of soldiers and civilians. Weigl infected healthy body lice individually, giving them enemas containing typhus rickettsiae. The inoculated lice were maintained for 5 days in batches of 140, in cages carried on the bodies of Weigl's assistants, because they only fed on human blood. After 5 days the typhus-carrying lice were dissected and from 140 intestines, crushed in a glass micro mortar with a few drops of phenol solution, a single dose of the protective vaccine was produced. I listened fascinated to my brother's description of Weigl's procedure and I decided to study medicine, become a researcher, and do similar experiments when I grow up. Twenty years after hearing about Weigl I got the opportunity to follow his steps, not with human lice but with leafhoppers and plant pathogens. Daily contacts with scientists at Cold Spring Harbor, who years later became Wolf Price and Nobel Prize winners greatly influenced me and helped in my own scientific career.

Keywords: leafhoppers, virus taxonomy, Weigl conferences, Cold Spring Harbor.

CHILDHOOD IN POLAND. In the summer of 1914, shortly after World War I started, the tsarist army approached the farm in Soroki near Gwozdziec in the Kolomyya region, where my parents and their two children were living. My parents escaped to the capital city of the Austro-Hungarian monarchy, Vienna, where I was born in 1915. My father was a graduate of BOKU, the Vienna Agricultural University, and my mother, born in Zagreb, Croatia, was an accomplished pianist and a linguist, fluent in German, English, French, Italian and Serbo-Croatian. My two siblings, the 6 year older Alfred and 5 year older Karla
Bronia spoke Polish with my father and German with my mother. I grew into this system, only realizing that this bilingual system required writing not one, but two letters in two languages to my parents when I started my studies of agriculture at the Warsaw SGGW, the University of Biological Studies. My third language was Ukrainian, which was spoken by all peasants in Soroki, where our estate was located. I also had Ukrainian during the last 4 years of high school in Kolomyya, learning the Cyrillic script.

I was 13 years old when my brother (Fig.1) came home from Lviv, where he was studying medicine and where his biology professor, Rudolf Weigl, described how he created the first, and until World War II the only vaccine protecting against exanthematic typhus. I was completely fascinated, hearing how professor Weigl was giving enemas to individual lice, infecting them with *Rickettsia prowazekii*. My brother described how Weigl inserted glass micropipettes into the anal opening of lice and how he maintained the inoculated insects on human volunteers for several days. Subsequently he removed the intestines from batches of 140 inoculated lice, crushed their intestines in a tiny glass micro mortar with a few drops of formalin, and obtained a single dose of his vaccine. Later I found out that this was the only existing available vaccine against trench fever until the end of World War II in Europe. The information about the currently used vaccine, produced first by Harold Cox in embryo-nated chick eggs, was published in the United States but the publication did not reach Europe because of Pearl Harbor and the entry of the United States into the war.

My brother’s description of Weigl’s work was spell binding and I decided to become a medical researcher and try experiments similar to those carried out by the developer of the typhus vaccine. I received my baccalaureate degree at the top of my class and applied to the same medical school in Lviv, which my brother graduated from. I was not accepted because of *numerus clausus*, as only 10 Jewish students were accepted every year and I was not one of the lucky 10. I lost one year, stayed at home, and finished the 12th year in the piano conservatory in Stanislaw (Ivanofrankivsk). I practiced daily at least 8 hours, but by the end of the year decided that I would not become a concert pianist to compete with Arthur Rubinstein, and only become a piano teacher, which did not appeal to me. I decided to apply to the Warsaw SGGW, the University of Biological Sciences, where I was accepted in 1934. After 4 years, in 1938, I received the degree of Agricultural Engineer, the equivalent of an MS degree.

On May 24, 1935, I went with a group of SGGW students to Pulawy, the oldest Polish experiment station. The trip, during the 3-day holiday celebrated in Poland, Whitsuntide, was on the deck of an old boat on the Vistula River. We arrived early morning and walked through the ancient park of the Czartoryski estate towards the main station building. Across came a very nicely dressed girl, with a book in her hand. She paid no attention to the 20 students but when she passed me, at the very end of the group, she glanced for a fraction of a second at me. Her shiny black eyes struck me and a colleague noticed the shock that I experienced. He told me that he saw the same young lady in Warsaw in the company of the chemistry student who joined our group, and he offered to help me meet her that afternoon. During the following three years I was “going steady” with Irene Ludwinowska and after I graduated in 1938, we got married.
WORLD WAR II. We returned to the family estate in Soroki where I worked till September 17, 1939. We were far from the war activities in the western part of Poland and only once saw a few planes and heard the thunder of bombing. Five German planes bombarded the bridge in Zaleszczycyki, between Poland and Romania, but the bridge was not hit and the bombs fell on the Romanian side of the river. On September 17 we heard the announcement by Molotov, that the Soviet army was entering Poland to free the Ukrainian and Byelorussian peasants from the yoke of the Polish land owners. At noon 2 cars with Polish officers arrived and they asked where the Romanian border can be crossed. We thought that they were fighting, but they told us that they were fleeing the country. Our estate was 14 kilometers from the Romanian border and I decided to escape across the nearby border to Romania. The nearest route was already occupied by Soviet tanks and we proceeded to the town of Kuty on the Czeremosz River, to cross the bridge linking Poland and Romania. However, Polish authorities prohibited civilians from crossing the bridge, permitting only uniformed armed forces to flee. We were in the car of the Polish major, Karol Krzyzanowski. He ordered his sergeant to remove from the trunk the major’s overcoat which I put on. Then he removed his cap and placed it on my head. A moment later we reached the bridge, where the car was stopped and its passengers observed by a Polish officer. He dictated to his colleague: two majors, one sergeant…and who is she in the back? Major Krzyzanowski replied: This is my wife. Proceed! The officer saluted and for a moment I was a very young major, 24 years old, and my wife was Mrs. Krzyzanowski. On the Romanian side, in Wyznitsa, we found ourselves in an endless column of vehicles, very slowly moving through Romanian villages. Rumors were spreading that we will be interned in refugee camps and that all our possessions, including shoes and clothing, taken away. It was around midnight when high up on a hill I noticed lights in a large house. We decided to leave the long column of military vehicles, said good bye to the kindly major who helped us to cross into Romania, and walked up the hill to the mansion. It was the residence of a gentleman farmer, Mr. Orenski, who was a conductor and composer, as we found out later. There were already 200 Polish refugees in his barn, but the lady who greeted us thought that we were relatives of her father in -law and received us very friendly. In the morning the owner of the estate told us that the rumors of interment in refugee camps were correct and that, if we would have continued on the main road, we would have been placed in such a camp. He directed us to a road leading to Chernivtse. Proceeding on the country road, we were stopped after an hour by a small group of Romanian solders and placed in a bus that took us to Chernivtse. The town was swarming with Polish refugees. All were sent to civilian refugee camps. My wife and I were interned in the town of Braila for the first year, and afterwards moved to Craiova, where we remained for the next 3 years. In Braila I learned how to make shoes on string soles and for the following 3 years I was a shoe maker, producing one pair of ladies’ shoes per day. I also earned some money teaching piano. The refugee camps were disbanded when on August 24, 1944 the Soviet army occupied the country and expelled the Germans. We moved from Craiova to Bucharest, where I was accepted as a graduate student, to work on my Ph.D. thesis. With the help of the American attaché we were able to leave Romania for Sweden the end of 1946. On the basis of my agricultural engineer degree we received a first preference emigration visa to the United States. On April 24, 1947 I was hired as a technician by L. M. Black at the Brooklyn Botanic Garden. Two years later I received my Ph.D. degree at Columbia University and was hired by L. O. Kunkel as his assistant at the Rockefeller Institute for Medical Research in New York City, soon to be changed into the Rockefeller University.
LEAFHOPPER INJECTIONS. My childhood dream to follow Weigl’s lice experiments finally became a reality, although not with lice nor with enemy of tiny insects. Working with a plant virus, the wound tumor virus, I prepared very thin glass needles that I connected with metal needles, and injected extracts from diseased plants, or from viruliferous leafhopper vectors, into healthy leafhoppers (Fig. 2). At the Rockefeller University an adjustable insect holder was constructed, based on the lice holders in Weigl’s laboratory. Virus transmission was successful and I published the results in Science in 1949. The mechanical virus transmission permitted the first titration of the wound tumor virus. It also provided evidence for the multiplication of several plant-pathogenic viruses in their specific leafhopper vectors. I became actively involved in virus nomenclature and classification. The finding that little or no harm was observed in the virus carrying insects could suggest that these viruses originated as insect viruses and over long periods of evolution became harmless to their animal hosts, while their newer plant hosts were severely affected and often killed. Should these viruses be considered as plant, or as insect viruses? The affinity of vector-borne viruses to certain plant or animal hosts should not be used as a classifying criterion. I became a member of the International Committee for Virus Nomenclature (ICVN), but the controversial fights between plant and animal virologists continued and I lost interest in the fights and decided to devote my time to laboratory research and field work.

During the 1950s I spent eight summers at the Cold Spring Harbor Laboratories on Long Island. Barbara McClintock permitted me to use her greenhouses while she was working outdoors with corn, Zea mays. My informal chatting with scientists in Cold Spring Harbor, who years later became Wolf Price and Nobel Prize winners, greatly influenced my own scientific career. I carried out an experiment in which I injected antibiotics into the abdomens of leafhoppers, exposed to plant pathogens, at that time believed to be viruses, but 10 years later found in Japan to be phytoplasmas and spiroplasmas. The two plant diseases which I worked with were aster yellows and corn stunt. I used penicillin, streptomycin, and tetracycline, convinced that the two plant diseases were caused by viruses. Penicillin and streptomycin injections did not prevent transmissions, but tetracycline-injected leafhoppers failed to infect the exposed seedlings. Knowing that tetracycline had no effect on viruses, I did not believe the results of the tests and assumed that the failed transmission was due to the heat in the greenhouses. I did not repeat the experiment after I returned to the Rockefeller greenhouses and I published the results and my wrong conclusion in the Transactions of the New York Academy in 1954. Had I repeated the tests, I would have found that not the summer heat, but tetracycline interfered with the presumptive viruses. Ten years later, my Japanese colleagues in Tokyo discovered the phytoplasma nature of the aster yellows and mulberry dwarf disease.
The profound influence of Rudolf Weigl on my own research led to the establishment of my scientific career. Among numerous honors and awards, the Wolf Foundation award in agriculture in 1980, often considered the Agriculture Nobel Prize, was based on my work with leafhoppers, injected with viruses, phytoplasmas and spiroplasmas. It was based on my early exposure to Weigl’s experiments. Although I personally did not know him, his work, related to me by my late brother in my childhood, inspired me and resulted in my successful scientific career.

1. Maramorosch K. Multiplication of aster-yellows virus in its vector. Nature, 1952; 169: 194–195.
2. Maramorosch K. Multiplication of plant viruses in insect vectors. Adv. Virus Res., 1955; 3: 221–249.
3. Maramorosch K., Jernberg N. An adjustable multiple insect holder for microinjection. J. Econ. Entomic, 1970; 63: 1216–1218.
4. Maramorosch K., Brakke, M.K., Black L.M. Mechanical transmission of a plant tumor virus to an insect vector. Science, 1949; 110: m162–163.
5. Maramorosch K. Plant pathogenic viruses in insect vectors. Curr. Opin. Microbiol, 1968; 42: 94–107.

VOШІ, ЖИТТЯ І ЦИКАДКИ: ЯК СТВОРЕННЯ ВАКЦINI ВЕЙГЛЯ ВПЛИNUЛО НА МОЇ ВІРУСОЛОГIЧНI ДОСлiДЖЕННЯ

Карл Мараморош
Унiверситет Рутгерс штату Нью Джерсi
Лiпман Драйв, 93, Нью Брунсвiк, Нью Джерсi 08901, США

Ця стаття присвячена пам’яті моєго брата Альфреда, який працював лікарем у гетто міста Коломії. Мiй батько 1 вересня 1942 року був відправленний разом із 8 000 євреїв до газової камери у містечку Бельц. Через два тижднi мiй брат був заарештований і кiлькома годинами пiзнiше знайдений мертвим у гетто. Моя матi 14 жовтня була вiдправлена у концентрацiйний табор мiстечка Бельц iз решtoю 7 000 мешканцiв гетто. Моя дружина i я пережили голокост у таборi для бiженцiв у Румунiї, а 138 моїх найближчих родичiв були знищенi нацистами в окупованiй Європi у таборах Треблiнки, Бельц i Аушviц.

У 1928 роцi, коли я мав 13 рокiв, мiй брат Альфред, який був студентом медицини у Львовi, розповiв менi, що його професор бiологiї Рудольф Вейгль створив пiд час Першої свiтової вiйни першу вакцину протi сильного тифу. Вейгль, який був молодим офiцером у австрiйськiй армiї, спала на думку блискуча ідея – виготовити вакчину, яка би врятувала людство вiд смертельної хвороби, що вбиває тисячi солдатiв i цивiльних громадян. Вейгль вводив здоровим вошам iндивiдуально їхнiх ворогiв – тифознi рикетсiї. Інфiкованi вошки утримувалися протягом 5 дiб по 140 особин у спецiальнiх коробочках, якi фiксовалися на тiлi асистентiв Вейглi, бо вошами були необхiдна свiжа людська кров. Пiсля цього вошей-переносникiв тифу пiддавали препаратуванню, внаслiдок чого одержували 140 кишечникiв цих вошей, якi розтирали зi скляними мiкрочастинками у кiлькох краплях розчину фенолу, що становило одну дозу захисної вакчини. Я слухав iз захопленням оповiдання брата про процедуру, розроблену Вейглем, i вирiшив для себе вивчати медицину, стати досiдником i здiйснювати подiбнi експерименти, коли виросту. Через 20 роки пiсля початку про Вейглa я одержав можливiсть пiти його шляхом, але не з людськими вошами, а з цикадками та патогенами рослин. Щоденнi контакти з науковцями
лаборатории у Колд Спринг Гарбор, які роками пізніше одержали Премію Вольфа і Нобелівську Премію, сильно вплинули на мене і допомогли у моїй власній науковій кар'єрі. Ніні Вейглівські наукові конференції проводяться кожні 2 роки, почергово змінюючи місце проведення у Польщі та в Україні.

**Ключові слова:** Вейглівські конференції, цикадки, таксономія вірусів, Колд Спринг Гарбор.

ВШИ, ЖИЗНЬ И ЦИКАДКИ: КАК СОЗДАНИЕ ВАКЦИНЫ ВЕЙГЛЯ ПОВЛИЯЛО НА МОИ ВИРУСОЛОГИЧЕСКИЕ ИССЛЕДОВАНИЯ

Карл Мaramoroш

Университет Рутгерс штата Нью Джерси
Лилеман Драйв, 93, Нью Брунсвик, Нью Джерси 08901, США

Эта статья посвящена памяти моего брата Альфреда, который работал врачом в гетто города Коломыя. Моего отца 1 сентября 1942 года отправили вместе с 8 000 евреев в газовую камеру в городе Белжец. Через две недели моего брата арестовали и несколькими часами позже его нашли мертвым в гетто. Мою матери 14 октября отправили в концентрационный лагерь в Белжец вместе с 7 000 жителей гетто. Моя жена и я пережили голод в лагере для беженцев в Румынии, а 138 моих ближайших родственников были уничтожены нацистами в оккупированной Европе в лагерях Треблинки, Белжец и Аушвице.

В 1928 году, когда мне было 13 лет, мой брат Альфред, который был студентом медицины во Львове, рассказал мне, что его профессор биологии Рудольф Вейгль создал во время Первой мировой войны первую вакцину против сыпного тифа. Вейглю, который был молодым офицером в австрийской армии, пришла в голову блестящая идея – изготовить вакцину, которая бы спасла человечество от смертельной болезни, убивающей тысячи солдат и гражданских лиц. Вейгль вводил здоровым вшам индивидуально их врагов – тифозные рикетсии. Инфицированных вшей держали в течение 5 суток по 140 особей в специальных коробочках, которые фиксировали на теле ассистентов Вейгля, поскольку вшам была необходима свежая человеческая кровь. После этого вшей-переносчиков тифа препаратировали и получали 140 кишечников этих вшей, которые растирали со стеклянными микрочастицами в нескольких каплях раствора фенола, что составляло одну дозу защитной вакцины. Я с восторгом слушал рассказы брата о процедуре, разработанной Вейглем, и решил для себя изучать медицину, стать исследователем и осуществлять похожие эксперименты, когда вырасту. Через 20 лет после услышанного о Вейгле я получил возможность пойти его путем, но не с человеческими вшами, а с цикадками и патогенами растений. Ежедневные контакты с учеными лаборатории в Колд Спринг Гарборе, которые позже стали лауреатами Премии Вольфа и Нобелевской Премии, сильно повлияли на меня и помогли в личной научной карьере. Сейчас Вейглевские научные конференции проводят каждые два года, поочередно меняя место проведения в Польше и в Украине.

**Ключевые слова:** Вейглевские конференции, цикадки, таксономия вирусов, Колд Спринг Гарбор.

Одержано: 09.11.2013