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MAINTENANCE OF HUMAN-FED LIVE LICE IN THE LABORATORY AND PRODUCTION OF WEIGL'S EXANTHEMATOUS TYPHUS VACCINE

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ABSTRACT

Scientific details and history of the adaptation of the sucking insects, lice, to serve as the cultivated laboratory animals are described, including specific medical applications of this insect during the period before and during the Second World War (WW2). Using lice, Professor Rudolf Stefan Weigl (1883-1957) has developed during Nineteen Twenties and Thirties the first effective vaccine against exanthematous (epidemic) typhus in his Institute at the University of Jan Kazimierz (UKJ) in Lwów, Poland. The production of this vaccine was based on propagation of Rickettsia prowazekii, the microbial typhus agent, in the Weigl's strain of clothes lice, Pediculus vestimenti. The procedure of 1939 - 1945 consisted of:

- (i) feeding of healthy lice with sucked blood, when kept in special cages placed on the skin of human 'feeders',
- (ii) infection of lice and propagation of R. prowazekii in the midgut (stomach) cells,
- (iii) the dissection of louse midgut, and
- (iv) the final preparation of the phenolized vaccine.

Furthermore, the significance of Weigl's vaccine is discussed at the time just before and during WW2, as well as the tragic fate of Weigl's scientific heritage and his Institute, all that in relation to the humanitarian, political and historical ramifications of the very unique and trying period in the history of Central and Eastern Europe, including Lwów and Poland, during and after WW2.

INTRODUCTION

Before the era of vaccination and effective insect control, the louse-transmitted typhus was one of the major infectious diseases and a cause of disastrous epidemics. The first successful anti-typhus vaccine was developed by Prof. Rudolf Stefan Weigl (1883-1957) at the University of Jan Kazimierz (UKJ) in Lwów, Poland (Weigl, 1920, 1930a,b, 1947). This vaccine was produced on large scale in Weigl's laboratory before World War II (WW2) in Lwów, Poland, and used in China (Rutten, 1936,1943), Ethiopia (Mariani, 1939) and other countries. Production continued during the WW2, when Lwów was occupied firstly by Stalin's USSR, in 1939 - 1941, and then by Hitler's Germany in 1941-1944. In early Spring of 1944, Prof. Weigl was forced to leave Lwów and most of his Institute was forcibly evacuated to Germany by the retreating Nazi army or destroyed when Lwów was re-occupied by Stalin's USSR, soon afterwards annexed, and then ethnically cleansed (with USA as an accomplice of its Soviet
ally).

After suffering waves of occupations, by Soviet Union (1939 - 1941), by Nazi Germany (1941 - 1944), and re-occupation by USSR in 1944, the Polish population of Lwów (at the beginning of WW2, the Polish citizenry of Lwów consisted of about 70% Roman-catholics, 20% Mosaic (Jewish) and 10% Armenian-catholic, Greek-catholic, Lutheran and others), if not liquidated earlier or deported to Siberia, was forcibly evacuated to the Western Poland, mainly Silesia.

This forced evacuation followed by annexation of Lwów by Soviet Union was the result of the USA-approved Yalta and Potsdam agreements, an abominable act of "ethnic cleansing" that the Polish citizenry of Lwów considered as a blatant act of treason committed by USA and Great Britain against Poland, their always faithful wartime ally. Altogether, Poland lost over six millions citizens killed by Nazis and one million by Stalin's regime as result of the WW2, and about two millions deprived of the opportunity to return home at the end of WW2. Moreover, and despite of incorporation of some East German territories, the area of the pre-WW2 Poland was reduced by nearly 20%.

As described above, Prof. Weigl had to leave his beloved city of Lwów, and he then moved to Kroszienko nad Dunajcem, where he established a small laboratory, then to Kraków (Cracow) as Professor of General Microbiology at the Jagiellonian University (1945-1948), and finally to the University of Poznan (1948-1951). After retirement, he died in Zakopane, Poland, in 1957, reaching 74 years of age (Krynski, 1967a,b, 1997; Nespiak and Ojrzyński, 1994; Stuchly, 1994).

Weigl's accomplishments and procedures for adapting an insect, louse, as an experimental laboratory animal, and applying it in the production an anti-typhus vaccine are described below.

**LICE AND VACCINE PRODUCTION**

To produce Weigl's anti-typhus vaccine, the typhus-causing microorganisms, Rickettsia prowazekii, were propagated in the midgut (stomach) cells of live lice and were killed by 0.5% phenol. The individual stages of the vaccine production, as based on my recollections in conjunction with a detailed and scholarly account in the monograph of Krynski et al. (1974), were as follows:

1. **Production of healthy lice**
   1. **Louse strains**

   The major accomplishment of Prof. R. Weigl was the introduction of an insect, louse, as the experimental animal, for the purpose of propagation of R. prowazekii. He has 'engineered' a special strain of lice, named Pediculus vestimenti, which was easy to breed, and was well adapted to the production of typhus vaccine. The strain was a Caucasus-African cross between lice isolated from the WW1 Russian prisoners (captured by Austrians) and Ethiopian lice received in 1939 by Weigl from the Laboratory for Rickettsiosis in Addis Ababa. This cross was designated as "Weigl strain" (Krynski et al. 1974; Mariani, 1939). Reasons for cultivating R. prowazekii in lice were manifold:
   - (i) At that time no suitable artificial media existed for cultivation of R. prowazekii,
   - (ii) Subsequently proposed propagation of R. prowazekii in hen eggs resulted in the inadvertent selection of progressively less immunogenic variants of R. prowazekii, and
   - (iii) Cultivation and passageing of R. prowazekii in the midgut of Weigl's strain of lice (Pediculus vestimenti or Pediculus humanus corporis) was resulting in the most potent and reliable anti-typhus vaccine (Krynski et al. 1974).

2. **Hatching of louse eggs**

Lice were depositing eggs on small squares of woolen fabric (see below). Each square was then held by a partial constriction near the middle of a cotton-plugged glass vial (hatching tube) and incubated at 32ºC. The healthy lice larvae, which hatched after 3 to 8 days, were dropping to the bottom of the hatching tube and the crop of 400 - 800 larvae formed a pulsating yellowish ball of about 5 - 8 mm diameter. Larvae were transferred to the flat (of about 4 x 7 cm size) cages (Sikora, 1915, 1917, 1924) only about 5 mm deep and made of wood (for a drawing, see Krynski et al., 1974). One wall of these cages consisted of special screen adapted by Weigl, with his usual ingenuity, from screens routinely used for flour sifting in the Polish flour mill industry. Lice were able only to stick out only their heads through this screen, but were not able to escape. About 800 larvae were placed into each cage that contained a loose small square of

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woolen fabric (for depositing eggs), which during WW2 was made of discarded army uniforms, Russian or German. The cages were sealed with paraffin as to prevent escape of any tiny larvae.

(c) Feeding of lice on human legs

Depending on the size of calves or thighs, about 7 - 11 cages were placed screen-down on the leg of the 'feeder'. The screen walls of the cages were pressed against the skin, while cages were held under a wide garter-like elastic band. Sticking out their heads through the screen wall, the lice were able to pierce the skin and suck blood for about 45 minutes, once a day, for about 12 days. The resulting 1 Y 2 inch reddish marks on the skin were washed with 60% alcohol, which contained HgCl2 as the disinfector. Feeders could tolerate well the moderate discomfort and loss of blood (Finkel, 1932). Between WW1 and WW2, Weigl has tried to adapt pigs as large-scale louse feeders, but he did not succeed to make it practical (Weigl, 1920, 1930a,b, and personal communication).

Men usually were attaching the cages to their calves, while women placed the cages on their thighs (as to hide the reddish marks under their skirts). After the 30 - 45 minutes feeding session, the louse gut as well as the entire body was swelling like a balloon, since each louse was ingesting the amount of blood equal to its entire body weight. Lice became rather dark colored (not red) with very shiny abdomens.

(d) Handling of caged lice

Cages were placed 'screen-down' in special holding boxes, as to keep lice dry and clean, while their feces, when drying out, did sift through the screen and collected at the bottom of the holding box. The boxes with cages were kept in 320C incubators. Lice were periodically transferred (first time after six days, then more frequently) to the cleaned and heat-sterilized cages, each containing a square of woolen fabric for depositing eggs (see above). These transfers were necessary both for collecting eggs, and for removing any dead or unhealthy appearing lice and louse debris, including feces and products of molting. All operations were carried out under highly aseptic conditions, assuring that the lice colony remained healthy and free of any viruses, bacteria or other parasites. However, microbial epizootic infections did sometimes occur, requiring destruction of the contents of such infected cages (Krynski, 1967c; Krynski et al. 1974).

The healthy 12-day old lice were produced by "breeding units" (hodowle), each composed of the supervisor (kierownik hodowli) and 12-15 feeders (karmiciele), i.e., persons who fed the lice. The author of this chapter served as a supervisor of such an unit, and also as a feeder, between 1941 and 1944. Most of the feeders in my breeding unit were Professors of UJK (see the section on the Humanitarian, Historical and Political Ramifications, below). The entire operation was supervised by Dr. Zofia Pokorny.

(2) Infection of healthy lice with *R. prowazekii*

The next step in the vaccine preparation was the infection of lice with *R. prowazekii* at a concentration corresponding to one *Rickettsiae* -infected and fully homogenized louse midgut per ml (106 to 107 infectious units per ml). Cages, each containing 300 - 400 of 12-day old healthy lice, were transferred to the completely separate "injection units", where the specially trained "injectors" (strzykacze) were infecting lice manually with *R. prowazekii*, and then feeding the infected and caged lice for another 5 days. The entire injection operation was highly engineered, as to make it efficient (for details see Krynski et al.,1974).

As the first step, 20 to 50 lice were immobilized in a special device (Weigl's clamp) where each louse was held by a very gentle spring clamp, while the anal segment of their abdomen was exposed and easily accessible. These clamp devices were then placed under a 32Y binocular microscope, and each louse was anally injected with a suspension of *R. prowazekii*. A 0.05 - 0.1 mm glass microcapillary (with its end cut on angle and fire polished, as to avoid injury to louse anus and intestine) served as an injection device. Propagation of *Rickettsiae* was taking place in cells which lined the louse midgut, which is straight, strong and elastic; the anal part of gut (ampulla recti) is lined with very tough chitin and thus is resistant to injury by the glass capillary. Injection was mediated by a pulse of weak positive pressure, controlled by reducing valves, electrically actuated solenoid valves, and finally by the operator (injector) who was using a foot-operated pedal. Each injection cycle consisted of the insertion of the capillary into the louse anus, briefly depressing the pedal to effect the injection, and withdrawal of the capillary; the entire cycle required about one second. Including all the auxiliary operations, a team of two highly skilled operators (one immobilizing and the second injecting the lice) was able to inject up to 2000 lice per hour.
Each batch of about 500 injected lice was then loaded into a cage, and fed for 5 more days by sucking blood of the intensively vaccinated 'injectors'. Feeding of the infected lice attested to the efficacy of Weigl's vaccine, since no serious typhus cases occurred among the injectors. When population of R. prozakeri reaches 107 per cell, the gut cells began to burst, resulting in leakage of the undigested human blood into the louse abdomen; thus in the final stage of the rickettsial infection, the lice bodies were turning bright red.

Weigl's methods for large-scale breeding and infecting insects were quite a unique achievement, thus having an impact on other branches of experimental entomology. E.g., experiments were carried on the infection of lice by Yersinia and Pasteurella, and on testing the efficacy of antibiotics on the rickettsial infection (Beda, 1974; Beda and Krynski, 1972; Krynski and Beda, 1964; Krynski et al., 1966); analogous methods were used for infecting ticks (Beda, 1974). Moreover, the Editor of this Manual, Prof. Karl Maramorosch, had adapted some of these methods for cultivation of leafhoppers and for the construction of the "leafhopper clamp and injection" device (Maramorosch and Jernberg, 1970).

(3) Dissection of lice and preparation of the vaccine

The entire production of vaccine at Weigl's Institute in Nineteen Forties was supervised by Dr. J. Starzyk (Starzyk, 1938). As the first step, cages with the infected lice (about 5 days after injecting with Rickettsiae) were inspected and cleaned of feces and debris. Those lice with adequate crop of Rickettsiae, i.e., those that turned red, were placed into jars filled with 0.5% phenol, earmarked for vaccine production, and consequently transferred to the "dissector's stations". "Dissectors" (preparatorzy) were harvesting louse guts that were heavily infected with Rickettsiae. My younger brother, Stanislaw Szybalski worked as a dissector, and helped to refresh my memory about some details described here. Many instruments are depicted in the review of Krynski et al. (1974). Using a fine scalpel and under the 16X binocular microscope, an incision was made between the thorax and abdomen of louse, and the Rickettsia-infected gut was pulled out using the needle and the tip of the scalpel. Dissection of about 300 lice per hour per dissector was the norm. Both ends of the gut were cut off, and the infected midguts were then transferred to the jars containing 0.5% phenol solution and subsequently ground to a fine suspension in the Weigl's mortar. Debris were then removed at 1000 rpm, the suspension sedimented at 6000 rpm, and finally resuspended in 0.5% phenol buffer. The vaccine was prepared at three different strengths and was composed of the vaccination fluid (buffer) containing fine emulsion of phenol-killed R. prowazekii, equivalents to 15, 30 and 45 louse guts. These three strengths of vaccine were packaged and distributed in sealed glass ampoules. At the time of my tenure at the Weigl's Institute, the entire course of vaccination consisted of three injections, administered at the increasing strength at one week intervals and amounting to a total of 90 infected louse guts.

The vaccine was safe with exception of sometimes serious allergic reactions, especially observed among the Weigl's Institute employees who often became sensitized to louse byproducts. Personally, I have developed asthmatic reaction to louse feces dust; moreover, I had a very strong anaphylactic shock after the third round of vaccination.

SIGNIFICANCE OF WEIGL'S VACCINE

At the time of its development, the Weigl's vaccine was the first and only remedy against the dreaded typhus epidemics. Although at present, typhus does not appear to be of any major epidemiological importance, this disease was responsible for major epidemics and death of millions, up to the second half of the 20-th century. The extensive epidemiological, clinical and historical accounts could be found in the chapter of Snyder (1948). Only a few salient features are mentioned here. The first description of typhus is of year 1083 from a convent near Salerno, Italy, and similar accounts could also be found in the famous work of Fracastorius, "De Contagione", published in 1546. As described by Professor Stefan Krynski, a collaborator of Prof. Weigl, in several of his articles in 1995-1998 in the Gazeta AMG Gdansk (see especially, Krynski, 1997), and kindly sent to me by Prof. Janusz Limon of the Medical Academy of Gdansk, the most severe epidemics of typhus usually accompanied the major wars and famines. One of the biggest epidemics was during Napoleon's retreat from Moscow in 1812, when more French soldiers died of typhus than from Russian bullets. One could say that lice have defeated Napoleon's armies; moreover, their retreat has spread the infected lice and typhus through the entire continent of Europe. Especially severe epidemics affected the Byelorussian and Lithuanian areas of the former Polish-Lithuanian Kingdom. Typhus has contributed also to the defeat of Napoleon III in the war with Prussia, when the fortress of Metz had to surrender because of the epidemics. During the WWI, typhus epidemics have killed 150,000 Serbs in

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1915.
Therefore, the development of the first highly effective anti-typhus vaccine by Weigl was of such a major epidemiological importance, although his discoveries were barely noticed in the Western medical literature (Snyder, 1944; Angstein, 1947). A total of 5 - 6 millions of individuals were vaccinated against typhus during German occupation in the eastern zone of war operations.

Weigl, although having Austrian parents and being born in Presov, in the Moravian part of the Austro-Hungarian empire, moved with his mother and stepfather, a high school principal, to Jaslo, Tarnopol, Stryj and later to Lwów, where he attended Polish schools, and became by his own choice a very patriotic Pole (A Monograph, 1994; Kryński, 1967a,b, 1997). His son Wiktor was my close high school friend; Wiktor's mother, Zofia née Kulikowska, the first wife of Prof. Weigl, was always very kind to me, as she was to all the Wiktor's friends.

It has been established in 1909 by Charles Nicolle of The Pasteur Institute, Paris, that louse was the vector for typhus. It has been proven in 1916 by H. da Rocha-Lima that the infectious agent carried by lice is Rickettsia prowazekii [named after the American and Czech investigators, H. T. Ricketts and S. von Prowazek (born as Stanislav Provazek), both of whom died of typhus when studying Rickettsiae as the etiologic agent]. Weigl was destined to make next critical contribution to the field; being a zoologist, parasitologist, comparative anatomist, histologist, and entomologist, and also a professor of biology at the University of Lwów, Poland, he became an expert in the biology and pathology of lice. In the early Twenties, feeding infected lice and testing typhus vaccine were all done with guinea pigs (Weigl, 1920), especially since Weigl, who was not a physician, was reluctant to perform human experiments. The latter were done without prior Weigl's knowledge, by a dedicated couple of technicians, Michal and Rozalia Martynowicz, who in this manner tried, however surreptitiously, to help their beloved Professor. Michal was a typhus survivor, and thus immune, whereas his wife Rozalia was never exposed to typhus. Without notifying his boss Prof. Weigl, Michal has vaccinated his wife Rozalia with Weigl's vaccine, and then she agreed to feed the R. prowazekii-infected lice. Only after both were sure that Rozalia did not contract typhus, they notified Prof. Weigl about their results. This experiment became crucial for Weigl's decision to perform large-scale vaccination trials in the regions of endemic typhus (Chodžko, 1933; Radło, 1937). The very successful results were widely reported and praised in the Polish and World Media. Especially successful was the vaccination campaign in China carried out by the Belgian missionaries (Rutten, 1936, 1943). As cited from the report of Father Rutten: "Typhus was one of the greatest human enemies killing at that time more victims then all other epidemics combined. Of 130 Fathers active in China, 70% died of typhus in the years of 1908 to 1931." Father Rutten wrote further, that: "When news reached us, that a Polish Professor has developed a vaccine, we were first very skeptical, since many 'cures' were proposed or sold to us before, but all of these failed. Anyway, we decided to try the Polish vaccine and the results turned out to be dramatic. During the past 7 years, since we started using the Weigl's vaccine, not a single of our missionaries or of the vaccinated Chinese patients died of typhus. Your Polish vaccine saved lives not only of missionaries but also of many thousands of Chinese".

Praises were heaped upon Prof. Weigl, including a kind statement by Charles Nicolle, a Nobel laureate, who established that louse is the typhus' vector; he wrote: "The 'war' with typhus did not appear promising, until it was joined by the brilliant Polish scientist, Professor Rudolf Weigl from Lwów, Poland. Weigl taught us how to cultivate the typhus agent in lice, and moreover, has developed a superior vaccine, which saved many thousands of lives. As person, Weigl deserves highest recognition, as a brilliant intellect, untiring worker and a fanatic of science" (Kryński, 1997).

Unfortunately, the well deserved Nobel prize has eluded Weigl because of the politics and WW2. However, he received many honors, including the Order of St. Gregory from the Pope Pius XI, the Leopold Order from the Belgian King, the Order of Polonia Restituta, and in addition he was elected to the Polish Academy (PAU or Polska Akademia Umiejetnosci) and many foreign Academies, and he became an honorary member of several scientific societies (A Monograph, 1994; Kryński, 1967a,b, 1997). Weigl's typhus research was continued for a few more decades by his collaborators and students, among them Anna Herzig-Weigl (Herzig, 1939), the second wife of R. Weigl, Stefan Kryński, Stanisława Woyciechowska (see Kryński,1987), Henryk Mosing (Mosing, 1947), Zbigniew Stuchly, and Albina Kuchta (A Monograph,1994; Kryński, 1967a), but at present it is mainly of a historical interest.

HUMANITARIAN, HISTORICAL AND POLITICAL RAMIFICATIONS

(1) The period before WW2
Most of the methods for the cultivation of lice and production of the Weigl vaccine were developed before WW2 in the Department of Biology of the UJK in Lwów, in the free and independent Poland (see
Introduction. Lwów was a very Polish city, with several Universities or Schools of Higher Learning, best characterized as a counterpart of Boston in USA or Cambridge in England. It had rich Western and international traditions, as reflected by its many names (Leopolis in Latin; Leopoli in Italian; Lemberg in German; Leopol in French; Lwów in Polish; Lvov or Lwow in Russian; Lviv or Lwiw in Ukrainian, the Russian and Ukrainian names depending on the transliteration). Lwów was a city with about 600 years of Polish history and tradition and the extremely patriotic Polish citizenry. At the eve of WW2 in 1939, its population of about 350,000 was a rather homogeneous 'melt' with various ethnic and religious backgrounds: predominantly (about 70%) Roman-Catholic, about 20% Mosaic (integrated and orthodox Jewish), the remaining about 10% Armenian-catholic (of Armenian origin), Greek-catholic (predominantly of Red Rutenian or Ukrainian origin), Lutheran (mainly Austrian or German) and others.

(2) The period during and just after WW2

WW2 started on September 1, 1939, and as results Lwów became occupied first by the Soviet and later by the Nazi's Armies. However, Weigl's Typhus Institute became a 'safe haven' for the intellectuals in 1939 - 1944, during both occupations. Otherwise, during this period, Lwowian universities have lost (killed or deported, mainly to Siberia) over 40% of Professors, with highest loss of about 90% at the Medical School (Albert, 1989a).

(a) The period of Soviet occupation 1939 - 1941: During the USSR occupation period of 1939 - 1941, Weigl did his utmost to protect the Institute employees from the systematic and inhumanely cruel deportation to Soviet gulags in the North (for men), and to Southern Siberia (for women and children, who were arrested at night and exiled by trucks and trains to very primitive kolkhoz'es situated mainly in the wilderness of Kazhakstan), where the death rate approached 40% per year, and where I lost many of my friends and colleagues (see also, Kopanski, 1997).

About the time when the Soviet secret police (NKVD or KGB) organized their first cruel deportation of Polish population of Lvow to Siberian, the first secretary of the Ukrainian Communist Party, Nikita Khrushchev, has visited Weigl at his Institute and offered him the title of Academician and a directorship of an Academy Institute in Moscow. Weigl has politely rejected it, which fortunately did not cause any negative consequences; instead Weigl has received promises of additional buildings for his Institute in Lwov (Nesplak and Ojrzyński, 1994), and of an exemption for his employees from deportations to Siberia. I learned about the details of the Khrushchev's visit from my father, who spoke fluent Russian, because long before WW1 he lived and studied in the Russian-occupied provinces of Poland, including Warsaw. My father, Stefan Szybalski, a pre-WW1 graduate of the University of Toulouse, France, was asked by his friend Prof. Weigl (who was unfamiliar with Russian language, similarly as nearly the entire population of Lwów, including myself) to help him in dealings with Russian visitors and the Soviet Russian administration, including Khrushchev and the NKVD (who supervised arrests and deportations), during the 1939-41 period of the Soviet occupation of Lwów. It is a pity that Stefan Szybalski has not written up his memoirs of this period, since many famous Russian professors and Academicians, who were 'starved' of the contacts with the Western world made a pilgrimage to Weigl's Institute in Lwów, considered by Russian as a Vienna-like Western European city, though just occupied then by Soviets. Posing as biologist in a laboratory attire, my father played deftly a role of Weigl's translator. Frequently, after a few glasses of vodka, the Russian visitors could not resist temptation of describing their hardships and then share their personal tales of terror related to life in USSR and to Stalin's terror; one of the Russian visitors, after getting drunk, gave the following very characteristic and helpful advice: "Do not ever join the Communist Party and do not steal excessively". He then elaborated: "If you are not a Party member, they will always court you to join, but once you join and then are kicked out of the Party this is your end. If you steal too much this will lead to your demise, but if you do not steal at all, you will starve; thus remember to steal only in moderation, just enough to survive!"

Through his influence, and often helped by my father, Weigl was able to help in securing the release and return to Lwów of several of the Siberian deportees. Among those was Stefania Skwarzynska (at that time at UKJ, and after WW2 the Professor of Theory of Literature at University of Łódź, Poland, and a member of PAN), who was deported to Kazhakstan, because of her "guilt" of having a husband, who was a pre-WW2 colonel of Polish Army and at that time a war prisoner in the German 'Oflag'. Stefania was helped by her UKJ major professor, Juliusz Kleiner, and by Weigl to return from Kazhakstan in Siberia with her mother and two very young daughters (one of her daughters, Maria Olszewska, is at present a Professor of Cytology and Cytocchemistry of Plants at the University of Łódź, Poland, and a member of PAN). Weigl has provided Stefania with a safe employment in his Institute, and while working with her at adjoining desks in 1941-43, I learned plenty about her own and her mother's and children's hunger and misery of the inhuman deportation. She was an enthusiastic and gifted raconteur, and as an accomplished story teller, she described to me some amazing and cruel experiences in the steppes of the Soviet Kazhakstan, hundreds of miles away from the 'civilization' (as represented by the nearest railway station). My family have also been directly threatened by deportation to Siberia at least twice. Our 'passports' were
first confiscated by NKVD and then the dreaded "paragraph 11" was added to them; this meant that we had to be moved to some place located hundreds of miles away from any even smallest town, which 'location' meant the wilderness of Siberia. Again, thanks to Weigl's help, my father was able to nullify this cruel ruling; however, the same happened to us again, but this time our second "paragraph 11" ruling reached us only one day before the Nazi invasion of Soviet Union. Hitler's armies have attacked the Soviet army on June 22, 1941, and entered Lwów on June 29, 1941. During this ghastly last week of the Soviet occupation there were massive arrests and all jails became overfilled with Lwowan citizens. Then in the middle of that week the Soviets have initiated the systematic mass murder of the prisoners.

Just after June 30 when we have entered the first prison, trying to help my friend in finding his father (who was a lawyer and a lice 'feeder' at the Weigl's Institute, and who was arrested a few days earlier), the partially decomposing bodies were stacked four to ten deep on the cell floors. In this prisons, the Soviet policemen murdered about 3500 prisoners before the Soviet retreat (Kopanski, 1997). During the next few days of the unusually hot weather, we searched other prisons, finally finding the body of my friend's father among the progressively more viscous mass of the victims of this Soviet atrocity of ethnic cleansing.

(b) The period of German occupation (1941 - 1944):

During the Nazi occupation of Lwów (1941 - 1944), employment in Weigl's Institute provided some degree of protection from the random arrests and deportation to the Nazi concentration camps; Gestapo seemed to prefer to avoid 'dealings' with persons from whom they might accidentally acquire typhus-infected lice (it was well known that carrying lice was our occupational hazard). Moreover, all employees carried an impressive looking identification card ("Ausweiss") from the "Oberkommando des Heeres" (Office of the Commander-in-Chief of the German Army); this "Ausweiss" was another of Weigl's life-saving "inventions" (as partly "engineered" by my father).

Weigl helped to protect many of the unemployed university professors and their associates by employing them as lice feeders; such employment entitled to special food rations and made them at least partially immune from arrests, deportations and/or death during the Nazi occupation. Some aspects of employment in Weigl's Institute had some elements in common with Spielberg's Hollywood movie "Schindler's List". Since feeding lice occupied the feeders for only one hour per day, and since the University (with exception of the Institute of Technology, renamed by Germans as "Technische Fachkurse") was closed by Nazis, the 'feeders' had the remaining time left for organizing the underground University courses and for other patriotic activities. For instance, I was supervising a 'breeding unit' consisting of feeders who were mostly mathematicians of the famous Lwów school of mathematics, including the world famous professor, Stefan Banach, and others including Jerzy Albrecht, Felix Baranski, (Kazimierz Berg ?), Bronislaw Knaster, Wladyslaw Orlicz, and also other scientists like Tadeusz Baranowski (biochemist), Ludwik Fleck (bacteriologist; Fleck, 1947), Seweryn Krzemieniecki and his wife Helena (both famous bacteriologists), and Krukowski (archeologist). Famous artist Stanislaw Skrowaczewski (with whom I attended piano classes of Florentyna Listowska) was also a lice feeder; he became a composer and a famous conductor of the Minnesota Symphony Orchestra, whom I was meeting many times at his concerts in Madison, WI, in the Sixties or Seventies.

Lwów school of mathematics was also known as "Scottish", not because of any direct relation to Scotland, but because of the name of the Kawiarnia Szkoaka (Scottish Coffee House) where Lwowian mathematician were routinely meeting and solving their theorems on the paper napkins or on disposable table cloth. The first computer language ("Polish" or "reverse Polish", as used by the Hewlett-Packard Company) was also created by this group. It was intellectually so stimulating but also somehow surrealistic, to listen to their long discussions about frontiers in mathematics, including elements of topology and theory of numbers, while they were feeding lice. But I had to watch that in the fervor of their discussion they did not overdose lice, beyond 45 minutes, because our laboratory lice lost their natural instinct to stop feeding, with a disastrous consequence to them, because their guts started to burst due to 'drinking' too much blood. During Nazi occupation of Lwów, 1941-44, Weigl used his fame and the pre-WW2 scientific connections with German biologists to protect all of us from Nazis. To achieve that, however, he also had to play dangerous and potentially questionable humanitarian role by being forced to produce his vaccine for Germans. At the same time, he had to resist a Nazi's offer to become a director of a special Institute to be established for him in Berlin and to become a German citizen called "Reichsdeutsche". Despite great personal risks, he made a brave response in 1941/42 to this offer by a high-ranking German Army emissary (as combined with subtle threats); he said that: "As biologist, I know the phenomenon of death; it is to you to accept me as Polish professor of Polish nationality". He also added that "It might appear questionable for the Germans, to offer honors to a Polish Professor, who by accepting the German offer would have dishonored himself". The high-ranking German officer understood that Professor Weigl was neither to be threatened nor to be bought. About this dramatic exchange Professor Weigl has informed,

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thereafter, my father, who in turn related it to me.
Weigl had a permission to have a radio, and allowed my father to listen to it and spread the political news among his trusted friends during this very bleak and sad time of the Lwowian history; this access to the radio was a blessing, since otherwise there was a death penalty for having a radio. Weigl was very courageous and not afraid to secretly cooperate with Polish Underground (AK or Polish Home Army, about one million strong) during Nazi occupation. Several shipments of Weigl's vaccine were surreptitiously and illegally delivered to the Warsaw ghetto and to other Nazi-imposed Jewish ghettos in other major cities, where typhus epidemics were rampant. My father, who was assisting Prof. Weigl in administration of the Exanthematous Typhus and Virus Research Institute, had more than once transported the vaccine to the Warsaw ghetto; I was helping him with that task. Tomasz Cieszyński [whose father, Dr. Antoni Cieszyński, Professor of Stomatology of UJK, was murdered by Nazi Gestapo in Lwow in July 1941 (Albert, 1989b) as among the 25 massacred Professors of the UJK, Institute of Technology and other Academic Schools of Lwow] has described one of the sessions in Weigl's office that preceded the second of the transports of Weigl's vaccine to the Warsaw ghetto (Cieszyński, 1994). The 'death penalty' threatened all of us engaged in this activity, but this did not deter us, since the everyday life was more than dangerous anyway. As an example, of 120 Polish students (about 10 Roman-Catholics, 10 Greek-Catholics and 100 Mosaic Jewish) who together with me succeeded to pass the Communist-administered entrance examination as to enroll in October/November 1939 at the School of Chemistry of the Lwow Institute of Technology (Politechnika Lwowska, with its name being modified depending on the changing occupations), only 14 could be accounted for by 1944.

(3) Re-occupation by Soviet Union in 1944
Although successfully resisting the "enticing" and personal German offers in 1942/43, Prof. Weigl was not able to resist the unavoidable forced move from Lwow to central Poland. Soviet army re-entered Lwow in July 1944, and Weigl's beloved city was ethnically cleansed by the Soviet administration, as a consequence of the abominable USA-approved Yalta/Potsdam acts (see Introduction), which authorized the forced and cruel deportations. The annexation of Lwow and its ethnic cleansing was secretly planned in advance and signed by Roosevelt, (later Truman), Churchill and Stalin during the Yalta and Potsdam Conferences. This was done treacherously, behind the back of the Polish Government in Exile in London, the staunch WW2 ally of USA and UK. Thus, USA was in fact promoting the second, practically total ethnic cleansing of Eastern Poland by Soviet Union. Such first ethnic cleansing, even a more cruel one, was perpetrated by USSR in the years 1939-41, as a result of the Hitler-Stalin pact of 1939, but involved 'only' about 20% of the Polish population of the Eastern half of Poland, because USSR did not have enough trains and time to deport to Siberia the entire population (Kopanski, 1997).

Moreover, by this act USA was also approving all the atrocities inflicted upon Polish population during and after WW2 by the Soviet Union, although USA could have easily prevented this unnecessary tragedy, simply by refusing to 'ratify' the Hitler-Stalin (Ribbentrop-Molotov) pact of 1939, which divided Poland between Nazi Germany and the Soviet Union; the Hitler-Stalin pact still appears to be in force, as far as the Soviet-imposed ethnic cleansing and annexation of Eastern Poland, including Lwow, and the loss of the pre-WW2 private property are concerned.
Beginning in 1945, and as result of the Stalin-imposed 'ethnic cleansing', the settlers from Soviet Union became almost the entire new population of Lwow, which presently is reported have over 800,000 inhabitants. However a few thousand of survivors and descendants of the pre-WW2 Polish Lwowians still remain in Lwow (presently Lviv) and try to preserve their heritage, including the Roman-catholic Cathedral, a few churches, and some very monumental and old cemeteries.

CONCLUSIONS AND COMMENTS

Weigl's scientific research flourished in Lwow, and led to the development of an effective anti-typhus vaccine in the period between WW1 and WW2, during the total independence of Poland. Weigl's Institute remained very active during most of the WW2, but it became liquidated concurrent with the 1944/45 annexation and ethnic cleansing of Lwow and Eastern Poland by the Soviet Union. This annexation was treacherously approved by Western Allies at the Yalta and Potsdam Conferences. Instead of sponsoring this rape of Lwow and Poland, USA should have never accepted all those totally unreasonable Stalin's demands of the westward USSR expansion at the expense of Poland and other smaller and previously independent countries. Poland, after all, was the first country, which on September 1, 1939 has resisted Hitler's aggression, whereas Stalin's USSR was Hitler's ally between 1939 and 1941. USSR was actively supporting Hitler's war effort by supplying Germany with train loads of war materials and food. On the other hand, a very secret small unit of the Lwowian underground, with which I and at least one other

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'louse feeder' in Weigl's Institute were associated (my close friend and high school colleague, Broniek Wojciechowski), was charged with disrupting the Soviet supplies for Hitler's armies which were attacking Western Europe; our unit had derailed or destroyed several Russian-German transport trains in 1940 and up to June 1941 (using explosives, which were secretly produced by myself as the student at Politechnika Lwowska in the laboratory of the Organic Chemistry Department directed by Professor Edward Sucharda and with his 'blessing'). Derailling Soviet trains had a doubly beneficial role for the Eastern Poland and Allies, since the same trains that carried Soviet supplies for Hitler's armies, were used also for cruel deportation to Siberia of the pre-WW2 citizenry of Lwów and of Eastern Poland (Kopanski, 1997).

During this period of close USSR-Nazi Germany cooperation, the USSR authorities have also confiscated practically all private property in Eastern Poland, including Lwów. These properties were never returned to the rightful owners (like, e.g., Professor Weigl, his family, many University Professors, including those who fed lice, or my family and myself), neither at the end of the WW2, nor even now. Moreover, the over 600 years of Polish history of Lwów and Eastern Poland, including many art galleries, museums, historical and scientific edifices, all that what was the essence of Polish culture, had perished as result of the forced resettlement and ethnic cleansing. This is the sad end to the personal fete of Prof. Weigl and many others like him, who gave so much to the science, medicine, and humanity in general, and as the 'reward' were deprived of their roots and have lost everything, many of them their lives.*

One could add here, at the end, that the generally despised insect, louse, has played a double role during the WW2: it was not only a carrier of a dreaded disease, typhus, but ironically it was also protecting lives of those who worked with this insect and of those who were vaccinated with the product of Weigl's brilliant research effort. --- Moreover, one could also conclude that while Weigl was trying to help humanity by developing his insect cultivation methods and typhus vaccine, the Stalin's USSR (1939 - 1941 and after 1944) and Nazi Germany (1941 -1944) were engaged in murders and cruel deportations, whereas the naive and destructive USA policies of 1943-1950 were responsible for the further human misery in the USSR-dominated, annexed or occupied, but pre-WW2 independent Eastern and Central European countries, and moreover, for a definite possibility of a nuclear holocaust, which almost by miracle was avoided because of Stalin's death. Most of that seems to be now forgotten, and the insect louse plays practically no role in our lives. But the noble deeds, courage and scientific discoveries of Professor Rudolf Weigl and those associated with him should not be forgotten!

FOOTNOTE
* This tragedy could have been easily avoided, if not because of the treacherous and shortsighted US policies at the end of WW2. USA could have easily protected people of Eastern and Central European countries, including citizens of Lwów and of Poland, from the rape by the Soviet imperialism.. This could have been done simply and as a matter of fact, without endangering any American lives, since in 1945 Stalin well understood the overwhelming power of USA and its atomic weapons. Any well motivated and skilled USA statesman or diplomat could have easily 'persuaded' Stalin and his henchmen not to tamper with the pre-WW2 status quo in Eastern Europe, and to desist from any unilateral Soviet annexations or other changes, unless based on USA-supervised elections or plebiscites. After all, the despotic nature of the Soviet regime, including its atrocities, ethnic cleansing of the Eastern Poland and mass murder in Katyn during the first two years of WW2 (when USSR was in an alliance with Hitler), were well-known to the British and American Governments, according to information supplied by their Intelligence services and by Polish Government in Exile. Moreover, Edward Pfeiffer, a homosexual double agent and his British lover Maclean, have passed in 1940 onto the Soviet Union the information about a French plan of sending 15,000 Polish officers from the Soviet detention camps to Syria, where General S. Kopanski was forming a new Polish Carpathian Brigade. Report by these spies certainly had influenced Stalin's decision on "the Katyn liquidation" of the intellectual elite of the Polish army, deceptively captured in 1939 by the Soviet armies (Kopanski, 1997). However, when the Soviet killing fields of Katyn were discovered in 1943 and the finding was disclosed to the shocked world, President Franklin Roosevelt falsely declared it all to be "a Nazi lie and Hitler's plot". Maybe this Roosevelt's lie was justified during the war, but the American tacit approval and lack of any corrective actions during years just after WW2 concerning Stalin-instigated ethnic cleansing, murders and other atrocities, together with the lack of any decisive remedies against development of the atomic weapons by Stalin, was unbelievably shortsighted, if not treasonous!

Neither Stalin and his henchmen nor the oppressed Poles and other citizens of Soviet-dominated parts of Europe were able to understand the American ineptitude at the end of WW2. The general feeling among the Eastern and Central European intellectuals (shared also by the most of the general population) was that USA policies were either guided by politicians totally inept in the art of foreign policy, and ignoring the true American interest, or critically influenced by American traitors, as exemplified by Alger Hiss, President Roosevelt's advisor and a convicted traitor. As the result of these USA policies, the World have just exchanged one dictator, Hitler, for an equally cruel but probably more cunning Stalin.
This monstrous American mistake, so easily avoidable in 1944/5 and even for several more years until USSR has developed its own atomic weapons, set the precedent for the future massive ethnic cleansing and was also destined to end in Stalin-initiated nuclear holocaust, providing that Stalin would have lived for another 5 or 10 years, or would have been succeeded by a similarly fanatic dictator; such grim possibility could not have been excluded at that time. It was a sheer luck for the humanity that Stalin, who progressively become more and more obsessed, died in 1953, before he had a chance to deploy the nuclear weapons. Providing they had an access to such weaponry, dictators like Stalin or Hitler would have not hesitated to use atomic bombs indiscriminately to advance their political plans or fantasies.

ACKNOWLEDGMENTS
Dr. Karl (Karol) Maramorosch, the Editor of this Manual, and originally from the Kolomyja region of pre-WW2 Poland, has encouraged and helped me to prepare this Chapter. The technical part of the louse breeding and vaccine production was based first on my imperfect memory, and then substantially rewritten based mainly on the publications of Stefan Krynski, Professor Emeritus of the Medical University of Gdansk (Krynski, 1967a-c, 1997; Krynski et al., 1994; and others below), who also read a late version of this manuscript, all that thanks to the generous help of Prof. Janusz Limon, Chairman of the Department of Biology and Genetics, Medical University of Gdansk. My memory was also refreshed and the manuscript was corrected by my brother, Stanislaw Szymbalski, as acknowledged in text. Dr. Malina Kuczynska, Professor Emeritus of the Slask Institute of Technology in Gliwice, Poland, was extremely helpful in locating persons connected with the Weigl's Institute, including Dr. Jakub Cieszyński and his father Dr. Tomasz Cieszyński; the latter, a Professor Emeritus of the Medical Academy in Wrocław, Poland, has spent very many hours correcting the latest versions of this manuscript and adding some crucial details (see also Cieszyński, 1994). Furthermore, I was helped by Prof. Jerzy Chmielowski, Chairman of the Department of Biochemistry of the Slask Institute of Technology in Gliwice, Poland, who is trying to erect a memorial plaque on the edifice of the former Weigl's Institute at the corner of Mikolaja and Dlugosza Streets in Lwów, Poland (presently Lviv, Ukraine), by Dr. Liliana Nitecka of midwestern University, Chicago, and by my cousin, Dr. Romana Tuma (nee Bogdanska), one of the "feeders" and presently a retired ophthalmologist in Florida.

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615-618.

FIGURE 1. Prof. Dr. Rudolf Weigl. The winner of the battle with the epidemic (exanthematic) typhus. This postcard represents a rather faithful rendition of Prof. Weigl's facial features, and stresses his major hobby, archery (in which he was very proficient) in an allegoric relationship to the battle with louse-transmitted epidemic typhus. This watercolor painting is from the album of the 23 cartoons painted by Leszek Szczepanski and offered to Professor on his namesday on April 17, 1941 (Aquarelle 38 x 20.5 cm; owned by the Muzeum Narodowe Ziemi Przemyskiej, 37-700 Przemysl, Pl. T. Czackiego 3, Poland).

[Please return this color postcard to:
Dr. W. Szybalski, McArdle Laboratory, University of Wisconsin Medical School, 1400 University Ave., Madison, WI 53706-1599]

FIGURE 2. A photograph (ca. 1941) of Prof. Dr. Rudolf Weigl. Reproduced from a photo-portrait owned by Prof. Stefan Kryniki and received from Prof. Janusz Limon. Copyright © 1998 Wacław Szybalski. All rights reserved.

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