67 YEARS AT THE BENCH

Memoirs of an inquisitive Academician

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SUBMITTED FOR PUBLICATION JUNE 2016

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Isaac Ginsburg [1*], Erez Koren [1, 2], James Varani [3] and Ron Kohen [2]

C V

1927	Born Tel-Aviv Israel 1927.
1945	High School certificate Gymnasia Hertzelia, Tel- Aviv.
1945 -1946	Member of the Defence forces, Jewish Settlement Police.
1948- 1950	Military service IDF, Signal Corps.
1950- 1953	Studies at Hebrew University, Microbiology (Major)
1954	M.Sc. in Microbiology, Hebrew University. "Effect of cationic peptides on fibrinolysis".
1958	Ph.D. in Microbiology Hebrew University: Mechanisms of action of Streptolysin S from group, A hemolytic streptococci.
1963	Lecturer.
1966	Senior Lecturer.
1970	Associate Professor.
1974	Professor .
1996	Emeritus.
1996 - 2019	Continuing research and partial teaching activities at the Institute of Dental Research, Faculty of Dental Medicine Hebrew University.

ACADEMIC POSITIONS 1-

1966 - 1975	Chairman Department of Oral Biology, Faculty of Dental Medicine.
1976 - 1979	Dean, Faculty of Dental Medicine, Hebrew University - Hadassah.

<u>RESEARCH IN OTHER UNIVERSITIES</u> :

Post Doctoral fellow, University of Pennsylvania , Children's Hospital, Philadelphia, Pa, USA, 1959-1962 .

Visiting Professor Department of Microbiology University of Minnesota, USA. 1988

Visiting Professor, National Jewish Hospital, Denver Colorado, USA.

Visiting Professor Merc Sharp and Dome, USA 1980

Visiting Professor National Institutes of Health

Bethesda, Md, USA. 1970 - 1971

Visiting Professor, Clinical Research Center Harrow, London, UK 1973.

Visiting Professor, St. Mary'S Medical School, London, UK 1975.

Viiting Professor Texas Medical School, San Antonio, USA

Visiting Professor Robert Koch Institute, Berlin Germany.

Visiting Professor, Department of Pathology. The University of Michigan, Ann Arbor, Mi, USA. 1986- 1996, a total of 4.5 years.

Visiting Professor Department of Pathology Otago University Christchurch New Zealand 1996 School of Medicine and Childrens Hospita, Adelaid, Australia.

AREAS OF RESEARCH: IN THE PAST

1) The role of microbial toxins, and cell wall components in the pathogenesis of tissue injury and granulomatous Inflammation formation.
2) Mechanisms of bacteriolysis induced by polycations and by antibiotics and its inhibition by polysulfates .
3) Role of cationic polyelectrolytes in phagocyte activation.
4) Activation of the respiratory burst in phagocytes.
5) Mechanisms of synergistic cell and tissue injury induced by cocktails of pro inflammatory agonists and by cytokines.
6) The role of phospholipase A_2 and its inhibitors in cell injury.
7. Lipoteichoic acid: role in biology and pathology

CURRENT INTERESTS

1) Role of Tibetan plant extracts as potent anti – inflammatory anti oxidants and anti cytokines, and in animal models of type I diabetes, and experimental

allergic encephalomyelitis.

2) Anti oxidant capacities of body fluids assessed by a novel chemiluminescence technique : Relation to inflammation, infection and post - infectious sequelae
3) Protection of plasma antioxidants by anti oxidants from plant and fruit origins.
4) The role of lipoteichoic acid in inflammation and infection .
5) polycations enhance binding of polyphenols to microbial cells to increase their anti oxidative capacities
6) Binding of polyphenols to RBC, platelets and lymphocytes enhance their anti oxidative properties
7) bacterial catalases and microbicides.
8) Salivary anti oxidants and solubilization of polyphenols.
9) Chlorhexidine enhances the oxidant scavenging abilities of catalase-rich commensals: Role of culture media, salivary proteins, polyphenols from nutrients and red blood cells.
10) Mixed biofilms and sensitivity to oxidants
11) protection by catalase epithpositive red blood cells and by commensals against H2O2 toxicity to catalae negative anaeribes
12. The role of catinic polyelectrolytes and nuclear histone as cidal agents for endothelial and epithelial cells related to sepsis.
13) Role of histones in sepsis and I auoimmune disorders
14) Biofilm and role in oral infecrtions
15) a new looik at the Pathogensis of auto mmune disoders : major role of hlistones .

MEMBERSHIP IN EDITORIAL BOARDS OF JOURNALS:

Inflammation 1980 - 2002 Kluwer Academic. Plenum Publishers.

Inflammopharmacology VSP International Science, The Netherlands, 1999 to present day.

PUBLICATIONS since retirement in 1996

1996 – 2019 (79 articles were published)

Since 1952 about **213** publications in various scientific Journals were published but, several additional papers were **not** included in PubMed but apeared in Google (see end of article).

introduction :

Today In 2109, I live in a sheltered home Nofay Yerusalaim in Beit ve Gan, Jerusalem and have wonderful aid marShane Dungca whom I consider as a devoted caring son and learnt from him since 2017, all about noodles and rice !!!

I was born in Tel Aviv 26.7.1927 in Rechov Ram bam 24, where I spent the first 5 years and then moved to Haifa and again returned to Tel aviv in 1940. Although quite late, at the age of 92, I thought it would be interesting and important especially for my children and grandchildren to receive more detailed summary of my academic work at Hebrew University and to tell in a simple language, why I was always busy in the laboratory and what " made Jhonny run ".Today, in 2019, am still very active collaborating with several laboratories (**see details below**) instructing Dental students to receive an MSc degree/ in Dental orthodontis and 4 monthe ago I decided to close down my laboratoty : "enough is enough" . I am now very busy compiling reviews on ato immune disorders (see later) all involving poly cations, oxidants and inflammation .

I am presenting my story not always according to years but mainly by subjects and interesting events that had occurred during the long years of work. Of the more than 230 papers published and cited by Pub Med-US National Library of Medicne (NIH) an Google I have included in this report only key papers and the complete list of publications can be be down loaded by going into Google or searching in PubMed under the name Ginsburg I.

I hope that my late wife Ruth (unfortunately passed away 15 years ago) and my 3 children understood, why I have been at home so little and available as a real attentive father. Unfortunately Academic life is very competitive and a wonderful cartoon published years ago showed a poor scientist standing in front of firing squad explaining that he did not publish enough !!!. This cartoon is so realistic today and also so true in our competitive modern lives.

My late wife Ruth ,was a brilliant teacher, an expert in mathematics and an inventor of her own techniques how to teach this difficult subject. She was also a very gifted violin player (won prizes), deeply understood literature and poetry and had many more potentials which unfortunately, she never fully developed. However, she saw to it that we go together to Yad Ben Zvi to listen to interesting lectures which enriched our knowledge, to look at the world and Jewish history, religion, philosophy and tradition. I also used to accompany her in trips by Yad Ben Zvi to various historical places. Speaking of music, it was very funny but also sad, that she had to stop playing the violin because Amir, our older son, could not stand the noise of the violin and it is a pity that she gave up music.

More details and memoirs dealing with our family life is written in Hebrew in a longer text about 120 pages (**Edited by my Sister Shifra Lancet and by her daughter Nava Carmel**).

Before starting the long story of my work at Hebrew University, I would like to thank my family, all the Deans of the Denta School after I had restired, for allowing me to continue working full time, especially in view of the urgent need for a laboratory space for newcomers. My deepest thanks are to the late Dr. S.M Robbins of Cleveland Ohio, USA

for his generous donation of an endowment fund from which, 4 time a year, I receive interest which supports my studies (for the full story about Sam Robbins see pages (**47 -50**).

Early History: What stimulated me to become interested in biological sciences ?

My first encounter with " Science " started already at a very young age. As a child, I was always playing with complex structures, built from laundry pins, hid metal soldiers, tanks and artillery and also constructed huge housed from books. I was aware that a successful war needs a **collaboration/ synergism** among many forces acting together, which was the concept for many of our research projects. I also often played cards with my mother and also invented a " new " complex game of cards ". The usual War game we often played was too simple and boring. This involved one single card fighting against one card. Instead, I suggested to use a line of 5 - 7 cards against 5 - 7 cards and later on, was not only against an identical card, say, a Prince against a Prince but, also a card with a heart symbol bearing number 3, could fight against a card 7 heart. This necessitated 2 - 3 decks of cards which made the game very complex and interesting. This probably led me later on to develop the research concept that in any biological situation you study in the laboratory, you have to take into account that you are dealing with the effect of a mixture of agents (bacteria, red blood cells, plasma, enzymes, hornones, drugs etc.) but not only with single agents. This means that most probably there is always a synergy among many factors working together. Therefore, in many of our research projects we always mixed agents as " cocktails ". One example is a summarizing review article we wrote

in 1995 under the title:

Isaac Ginsburg and Ron Kohen. Cell damage in inflammatory and infectious sites might involve a coordinated "cross-talk" among oxidants, microbial haemolysins and ampiphiles, proteins, phospholipases, fatty acids, proteinases and cytokines (an overview) . Free radical research 1995;22,489-517.

and an additional paper in 1999 - **Ginsburg I. Multi-drug strategies arenecessary to inhibit the synergistic mechanism causing tissue damage and organ failure in post infectious sequelae. Inflammopharmacology. 1999;7207-17.**

Both publications indicated and also may explain why practically all the 150 clinical trials Trials performed on sepsis and septic shock (**in hebrew elech dam**) at a cost of billions of sollars spent, have failed to prolong patients lives suffering and dying of septic shock if only a ***SINGLE*** drug a had been used, at a time , but not using clocktails !!. There might be an explanation why this was done this way.

Sepsis is a condition which dvelopes when bacteria invade tissues and mainly the blood stream either activating exaggerated immunological respones or do the opposite. Only in the USA, 750,000 people come down every year with sepsis and 250,000 wil die due to organs failure **(SEE STUDIES BELOW 119 -125**).

Only very recently, a dramatic paper came up in PNAS which had studied the changes in the apperance of genes related to indection and inflammation during sepsis in mouse models as compared to humans. It was to learn that the results showed **absolutely opposite directions.** If a gene in a mouse model went up, it dropped in humans. This clearly suggested that the mouse model on which all the experimental models in the laboratory had been based,

may not be relevant) to test the human disorder and that billions of dollars had been thrown out due to a wrong slection of an appropriate laboratory animal. This will definitely raisead a big " scandal " due the a severe error made all these years.

See pages 119-125 for the debate on the possible role of histones releasd from white blood cells as agents killing patients suffering of sepsis .

During my early life, I have suffered f every child's disease you can think about and even managed to have whooping cough (**Shaelet**) at the age of 13 and severe measles rubella at the age of 15 which is very unusual. Also, until the age of 10, I had suffered of terrible allergies to meat and other foods which today. miraculously disappeared. However, especially annoying, were very painful " Boils" (**ferunkels**) caused by Staphylococci which had developed on the skin of my hands and legs that in the absence of antibiotics, caused serious pain and discomfort. In the early 1930ties and in the absence of antibiotics (penicillin, the first antibiotic was discovered by Fleminmg only in 1945), one of the methods commonly used by Doctors to treat chronic infections, was to try to increase their non – specific immunity by taking your own blood and injecting it into the muscle of the behind**.** This was hoped to help stimulate the production of antibodies against bacteria. I am not sure it really helped !!!

During the second world war (1940 -1945) and because of the constant bombardments on Haifa by Italian planes comming from Greece, our family moved for a while to Jerusalem to live with Zehava Berman (Berman's Bakery), a close relative of my mothe. However, it

was a terrible tragedy for all of us that in one of those bombardments on Tel Aviv, in 1941, a bomb hit the synagogue where my grandfather Aaron was studying and killed him and 3 of his friends. This was probably the main reason why I became an **atheist**. Can you blame me ?

During our stay in Jerusalem, I joined a summer camp which took us on a trip to Jericho where I must have been bitten by a sand fly transmitting what is known as Leismaniasis parasite (**Leishmania tropica)- shoshanat yericho**. The skin wound induced by the parasite in my leg was large, chronic and painful and it took nearly a year walking with a big bandage, to get rid of the terrible infection which also left a large scar on my leg. You can still see in Israel similar scars on the faces of Jews emigrated rom Iraq where the disease is very common.

When I was 16 and under the impression of all the childe's diseases I had suffered, I looked one day in Tel Aviv at the window of a book store on Allenby street which sold medical books and asked for a book on Microbiology. At that time, I have already known about the very interesting book by Paul de Kruif from Michigan,USA **: Microbes Hunters**)- 1928 . The owner of the shop recommended a book on **Bacteriology** by Prof. Bigger, used by medical students in England. I went home and told my father I wanted to buy the book since I thought I would like to become a microbiologist in order to understand **why** I had suffered so many infections. To my joy, he openly agreed. So, I started reading the book very slowly, learnt a lot of English (***always using an English - English dictionary !!!)*** and this was my very early step to later on chose Microbiology as a profession.

After 8 years in high school in Haifa (which I hated), we moved

back to Tel Aviv in 1941. This is where I got my new name **Guli, (Guliver)** because I must have been the tallest student in class and also because in Haifa, there was a fashion among young children to ware a wide black belt, very similar to what they saw in the movie Gullivers Traveles

I graduated from Gymnasia Herzelia high school in 1945 in the Agricultural class where I was exposed not only to basic sciences but also to the physiology of plants, to practical work how to grow vines for the production of wines, vegetables, chickens, cows and also studied Earth studies which was mainly chemistry. I never thoughtthat 60 years later, I shall return to deal with red wine and its anti oxidant activities *(**see section on polyphenols pages 93-100**).*

I admit that I was never the best student in class, got an average of 75 which in those days, was sufficient to be accepted at the University. In a way, I was following my father's footsteps. He went to Mikve Israel, the first agricultural school established by Baron Rothschild where teaching was in French. As a brilliant student, he was chosen by the school to go to France to become an Agronom but, it never happed because of lack of money that year. So, he went in another direction and finally became the chief financial administrator of PICA (Palestine Jewish Colonization Association). It was established in 1924 and played a major role in supporting the Yishuv. The Jewish Colonization Association (ICA) was founded by a Bavarian philanthropist, Baron Maurice de Hirsch in 1891 to help Jews from Russia and Romania to settle in Argentina !!!. The Baron died in 1896 and thereafter, ICA began to assist the jewish **Palestinian** colonizers. In 1899, Edmond James de Rothschild transferred ICA to his colonies

in Palestine plus fifteen million francs, which were used to re organize the Palestine Jewish Colonization Association in 1924. When the State of Israel was founded in 1948, ICA was transferred to Jewish Nathional Fund. Today, only a few people in Israel know that our country owns thousands of **dunams** of land in the Choran in south Syria which were bought by Baron Rothschild. Before my father met my mother Zehava, he served 7 years as aguard in the Choran area (now Syria) riding on a horse with a gun and a sword and looked like a typical beduin fighter. He probably married my mother by mail!!! but also used to come home but not too often.

At the age of 14, I joined the Hagana(Defence underground), swore on a pistol and remained loyal to our efforts to build a new State.

In 1945, upon graduating from high school and being a Hagana member and by advise of my Hagana instructors, I joined the Jewish Settlement Police which was actually "Hagana" in British uniform and earned a "fortune" of 6 English pounds a month which in those days, was an average salary of a worker.

I was stationed in Gedera, in **Unit number 3** we wore beqautiful Australian helmets on which a white triangle was stuck representing the area in the country. I lived a whole year in a water tower which was made into a police station. I was responsible for 22 British rifles, , pistols etc. which made me a "fighter" who new how to use a gun.

This was very practical when the war of liberation started in 1947. These rifles were taken every evening by 13 officers who were supposed to go on a night patrol!!! but, actually they never performed their duties just went home to sleep (Isra blof !!!). Then, they brought the rifled back for so called cleaning. I was always afraid that

the **Brit** as we called the British officer in charge, might come over from Rehovot to check on the station. However, the hagana made sure that Taylor, the officer, will receive large quantities of fruits, vegetables poultry and wines to keep him " busy ". Since I actually had nothing to do except to clean the rifles every day, I enrolled in the British Council and got free text books in biology and chemistry, and managed to learn more English and even some Latin. This archaic language is used today only in prayers by Christians but was also thought to be important in science since most of the names given to animals, bacteria and diseases were in Latin. These studies were actually intended to help a dream to study Medicine. However, since at that time, Palestine did not have a Medical School, you had to go abroad. This was not practical since my older sister, Shifra, was already in Switzerland accompanying her husband, Moshe, who studied Medicine in Geneva. Therefore, sending me abroad was a huge expense, out of reach of my parents. So, I decided to follow my original plans and enrolled as student at the Hebrew University in Jerusalem hoping to study Microbiology, the closest to Medicine. My luck was that 1946 was the year when the University had resumed microbiology studies as a mjor subject after it had been dropped out for years since there were no jobs available for this profession. The main Microbiological Institute building (Beit Rattenoff) was on Mount Scopus near Hadassah Hospital but, studies in the first years were in the main University campus on Mount Scopus. We were sad not to see or learnt about bacteria and diseases until later, in the third year.

First/second year at Hebrew University and the beginning of the war of liberation (mil che met ha shi ch rur)

I was accepted as a student in October 1946 and joined 13 students several of whom had been discharged from the British army after world war 2 had ended in 1945 and were older than most of us. This was not the best situation because they said: no games, we had lost 5 years and we now want to study, no social activities !!

After completing successfully the first year studying biology, zoology, botany, geology, chemistry, mathematics and physics with relatively good grades (80), I started 1947 with high hopes. However, on November 29 1947, at 1030 and in the middle of an Organic Chemistry class under Prof. Moshe Weitzmann (the Brother of Chaim Weitzmann our first president) - the door of the lecture room burst open and a head of a young man shouted: All Hagana members should immediately assemble downtown. We immediately ran toward Jaffa gate with long sticks in our hands the - secret weapon| of the hagana, where Arab mobs had attacked the Jewish commercial center. We tried to defend the site, but the Arabs, under British protection, had already set fire to the center. **The War of Independence had started.!! and good bye studies !!!**

Before going on with my story, I would like to tell a little episode so characteristic of our internal political status when Hagana, Etzel and Lechi, the 3 underground movements, were fighting each other. One day, during my first year I was approached by a Hagana member who asked me whether I would agree to spy on students who supported Etzel and Lechi. Being a devoted member of Hagana, I first agreed to look around the lecture room, try to remember faces and learn the names of those students thought to support the Hagana rivals. After a week, I had decided that I was ***not*** going to spy on " enemies" of Hagana and that he should find somebody else to do the job. This was

the beginning, or the continuation of the long struggle between the Left and Right which is continuing to this very *day*.

My involvement in the long war of liberation, fighting in north of Jerusalem, Kfar Etzion and later on, on Mount Scopus - Har a zofim is described in more detail in my memoirs (zi ch ro not) in Hebrew

M.Sc Master's thesis

It took nearly 2 years of war to go back to finish school and upon completing 4 years, I was required to perform a thesis for the M.Sc degree **(at that time there was no B.A path).** In the early nineteen fifties the laboratory technologies were very primitive, no computers, no automatic plate readers (studies on enzymes and genes) and no plastic dishes or test tubes, only glass. Every information had to be relied upon the library which not always had the journals needed so, you had to order a reprint from abroad which took weeks. Typing was on a mechanical typewriter and every mistake you made cost you, since you had to retype the whole page. Graphs needed aspecial artists who took a lot of money. Nevertheless, we did a good job and used more our heads and imagination and the level of research and discoveries of new phenomana were excellent.

Regarding my MSc thesis **in 1952 ,** I fell in love with Streptococci for 2 main reasons. First, it was a very interesting microbe capable of causing so many diseases such as rheumatic fever arthritis, nephritis most probably by producing many enzymes and toxins ***working together*** (**synergism)** ?.This allowed the bacteria to spread out by destroying the connective tissue which enabled them to cause cell damage in remote areas. The second reason might have been due to

the sad story that my late cousin Rivka Forer (Shukie's sister), died of post - streptococcal rheumatic fever at the age of 17, and I was curious to learn how and why the disease had killed her.

Looking around for a tutor, I met Dr. Andre De Vries, a Dutch hematologist at Hadassah Hospital who was a new immigrant but, had to work 7 years as a cow keeper in a kibutz because there were no open positions available in hospitals.

In Hadassah Hospital, he also worked together with Efraim Katchalski (KAZIR) from the Weizmann Institute who later on became President of Israel.

My thesis involved studying the mechanisms by which long chains of amino acids (called polymers) with a positive charges, inhibited the normal lysis of blood clots which, if happens in vivo during sepsis and disseminated intravascular coagulopathie, may induce massive hemorrhages. At that time, the Weitzmann people had offered a new theory to explain the structure of proteins. They suggested that contrary to what had been thought before, proteins are actually made of straight lines of amino acids called **Polymers.**

I shall try to tell in a few simple words about the study performed and hope the general picture will be understood. Work in the laboratory was lively and exciting. It was divided among several students using a weekly donation by each students, of 30 mL of blood.

One student studied the effects of the **polymers** on blood clotting (coagulation), another studied their effects on blood vessles and on microorganisms, and my work was to test the effect of the polymers on the lysis (**fibrinolysis**) of blood clots where fibrin is the main protein involved in the formation of a clot. This project was also connected with microbiology since hemolytic Streptococci which cause

rheumatic fever**,** produce a substance, streptokinase, which activates plasminogrn to plasmin, a protease, which lyses blood clots and therefore, allows the bacteria to spread in tissues by passing through the walls of the blood vessels. We got very nice results showing that the positively - charged polymers, poly lysine and poly arginine , actually histone mimics, inhibited fibrinolysis and published the results in the very prestigious Journal, **Science.** This was my first publication **!!!**

Ginsburg I, De Vries A and Katchalski E. The action of some water-soluble poly-alpha-amino acids on fibrinolysis. Science. 1952: 116: 15-6. in 1955, we also published the results

De Vries, Shafrir E, Biezunski N, Ginsburg I and Katchalski E. Effect of synthetic basic polyamino acids on the clotting of fibrinogen and on fibrinolysis. Rev Belg Pathol Med Exp. 1955 ;24,112 - 116.

Ginsburg I, De Vries A Shafrir E. Studies on the action of poly lysine on the fibrinolytic reaction. Bull Research Council of Israel 1954:4,51-56. *These publicatond were never cited in the literature* !!! and are , therefore lost. !!!

During my work in the laboratory, De Vries introduced me to Dr. Chanoch Melvitsky, the first surgeon in Israel who had performed open heart operations to replace or correct heart valves damaged by rheumatic fever. Unfortunately, Melvitsky was later on killed in 1955 when an El Al plain was shot down over Bulgaria. (***see Wikipedia***).

My direct involvement with heart surgery was because several publications on open heart operations reported from France, described a sudden death of patients due to the activation of fibrinolysis, causing uncontrollable massive bleeding and death. My task was to obtain fresh blood during open heart surgery and to look for any signs of change in blood coagulation or dissolution. However, in 10 operations

I had attended, we never detected any tendency for the blood **not to** coagulate. Nevertheless, this study, with negative interesting results, was also published :

Milwisky H, Ginsburg I, De Vries. Studies on fibrinolytic enzymes in patients undergoing thoracic surgery. J Thorac Surg. 1955,29: 604-607

However, at that time, it was already known that patients suffering of mental depression and who were treated by **electro shock**, showed a significant inprovement in their condition but, the mechanisms involved are still not fully known to this day. However one thing was sure, electro shock, induced a very rapid **fibrinolysis** !!!. Therefore, it was of interest to study the enzyme generated by shock and to compare its action to the enzyme activated by streptococci. I went to the psychiatric clinic, stood by when they performed shock on patients (a terrible scene to look at), got blood and ran to the laboratory to analyze the samples. Indeed, electro shocked blood, did not coagulate and I also found that both enzymes were probably identical. This ended my M.Sc thesis.

However, since that day I have been thinking a lot about depression and why patients feel much better after shock treatment which also induced fibrinolysis !!!. If I am not mistaken, depression may probably be caused by small peptides generated by brain which affect electrical pulses which go though nerves. In recent years, studies have suggested that those small peptides systems are modulators of the behavioral states found in mood and anxiety disorders. So, I thought may be what, if the enzyme in blood **which destroys fibrin - a perotein)** during shock can also destroy these peptides and it takes some time until they are reformed again?. This might perhaps explain

why electro shocked patients feel much better after treatment. I tried to sell this "idea" to psychiatrists and pharmacologists but as of today, with no luck. I may still try again soon, what a dream. !!!

Work at the Department of Epidemiology, Ministry of Health and meeting David Aminoff.

Upon completing my M.Sc thesis, end 1954/55, whie waiting for a position as a Ph.D candidate at the Hebrew University, I was offered a temporary job in the Department of Epidemiology, Ministry of Health in Abu Kabir, south of Tel-Aviv. This was under Emanuel Oleink (**Eilan)**, a close friend of Moshe Lancet my sister's husband who both studied medicine in Geneva Switzerland. The task of the Department was to watch and control outbreaks of epidemics of infectious diseases, especialy among new immigrants. In the laboatory, I had the " honor" of sharing a desk with **Vanda Klingberg**, the wife of the famous spy**,** Marcus Klingberg, who was later on tried for treason for working for the Russians. She was a very charming blond and a very clever investigator. I have the feeling that she was the real spy !!!

My work in the lab was also on Leptosprosis, (**Achberet)** the world's most common disease transmitted to people from animals, mainly from dogs urine which comes in contact with breaks in the or the eyes. Unfortunately, despite obtaining interesting results, no publication came out of this work.

At that time, I was also in**, Reserve** in Chemed-Sientific core and served in Ness Ziona (Biological Institute). Then, one morning during our trip by truck to Nes Ziona, I met David Aminoff, a close

friend from Ann Arbor Michigan, who worked in Chemed. He had originally arrived by invitation of (Davids Shield) Magen David and was asked to establish a plant to produce large amounts of blood products such as albumin and gamma globulin (the fraction which contains antibodies) – for hospital uses. Aminoff, being a world expert on red blood cells and hematology suggested to follow the techniques developed in the blood bank in The Netherlands but, Magen David thought that the American system might be better. So, he packed his belongings and went to the USA. I lost track of him until 1986 !!!. It was when we met again in Ann Arbor, Michigan. Delivering a seminar at the Pathology department **(see chapter on Ann Arbor below pages 81-86).** In the first row, I noticed a smiling face but failed to remember where I had seen it before. After the lecture, he approached me and said in Hebrew don't you remember me, I am David aminoff, we used to take the same transportation to Nes Zioina ?. Since then, I had often seen David in Michigan. He is now 89 years old, an emeritus professor of Biochemistry at the University of Michigan. Saw him and Helen his wife in Jerusalem December 25, 2012. The full story about my research in Ann Arbor (**1986 – 1996**) will be described later on.

My main routine work at the Ministry of Health was epidemiological travelling twice a week to a large refugee camp (used to be a British army camp) in Hadera, crowded with thousands of immigrants mainly from north Africa, Iraq and Kochin in India. Outbreaks of dysentery, typhoid necessitated the isolation of patients to allow to determine the antibiotic sensitivities of the bacteria isolated. Unfortunately, because of

rumors in the camp that hospitalized children " disappeared " (**the big scandal with the Yemenite children 1948-1954**), the families simply hid the sick children from the police and the medical authorities and it was tragic that more than 300 children simply died of typhoid and dysentery, a terrible tragedy which could have been prevented by antibiotics treatment. Later on, the Israeli government led by Yitzhak Shamir established in 1988 a Clarifying Commission headed by Justice - Dr. Moshe Shalgi (our old late friend). This committee received new evidence on 301 children, and determined that in 65 of these cases, their fate was unknown. It also determined that in all other cases, the children simply died. What a sad story !!

Sanitation in the Hadera camp was horrible and a lot of efforts had been made by the Jewish Agecy and by **Wizzo woman** to help the refugees learn simple hygienic rules, such as how ***not to cook*** in the lavatory, and the need to wash your hands etc. This period was very traumatic for me where I learnt about primitive societies, traditions, poverty, ignorance and also some practical Bacteriolgy.

Work in Pathology and starting my Ph.D thesis

Upon completing my work at the Ministry of Health, and still looking for a position as a Ph.D student with no luck (no openings available until later on that year), I joined the Pathology Department under Prof. Henry Ungar, the head of the Department, an expert on liver diseases. Since I was already an " expert" on **fibrinolysis** (my M.Sc thesis), I suggested to him to examine whether inhibition of blood coagulation by certain

drugs, such as coumadin (dicoumarol) used by people who had suffered an heart attack, might affect the healing of wounds created around a surgical gut inserted through the liver. The procedure was very simple. Rats were put to sleep, we opened their abdomen**,** exposed the liver and a surgical thread was passed through the liver. The animals were treated either by Coumadin, trypsin a proteolytic enzyme or bled to lower the amounts of clotting agents**.** The animals were sacrificed 4 - 6 days later and the degree of wound healing around the thread was measured. It was very interesting to find out that if blood coagulation had been prevented, or its activity lowered by bleeding, the liver did not develop a good and strong healing process and we could simply remove the thread. It indicated that for good healing to occur, one needs the proteins involved in the wound healing. Unfortunately, hese reults weres never included in the doo journals..

Ungar H and Ginsburg I . The effect of trypsin on localized inflammation in the liver Bull Res Council Israel 1955; 5B, 139-143

So, the world ad "lost" an **IMPORTANT"** observation !!! which was however later on confirmed by others but who did not mention our original findings. Nobody to be blamed !!!

Work on group A hemolytic Streptococci, a main line of research for many years pages 44-47

With already some experience with streptococci and an " expert **"** in heart surgery" ***(see studies on fibrinolysis from 1952),*** the end of summer 1956 arrived and I was lucky to be accepted as a Ph.D student at the Bacteriology Department,

School of Medicine, Hebrew University under the tutordship of Nathan Grossowich. Uforrunately, I immediately discovered that I had made a teible mistake. Although he was nice man, he was a "pain in the neck", who interfered with everything I wanted to do. I do not believe I learnt anything useful from him and had to do everything with my own hands or to look for advise from others.

I suggested that my thesis be directed to streptococci since in the 50ties, a lot of **rheumatic fever** still occurred because patients did not take penicillin, the best drug to prevent rheumatic fever. Also, and as mentioned before, it was linked with a family tragedy. My late cousin, Rivka Forer died of rheumatic fever. The disease ususally starts as a simple streptococcal soar throut - angina and statistically, **3%** of those patients who do not take antibiotics and who suffer of streptococcal **angina,** will develop Rheumatic Fever. It affects the heart valves and the joints-causing arthritis (**dakeket mifrakin)/** As stated in one of the Pathology books, rheumatic fever "licks the joints and bites the heart".

Again, I was playing with streptococci (**see my M.Sc thesis**). This time, my thesis involved efforts to produce a synthetic medium to grow streptococci. It should be only composed of a mixture of amino acids, vitamins and salts. The advantage of a synthetic medium over complex media, is that you can easily isolate bacterial proteins and toxins with no contamination by proteins from the medium.

This was not so simple and necessitated a lot of combinations and permutations among many amino acids and vitamins. After successfully forming the synthetic medium, **Ginsburg I,**

Grossowicz N. Group A hemolytic streptococci I. A chemically defined medium for growth from small inocula. Proc Soc Exp Biol Med. 1957;96,108-12.

qe focused on the mechanisms of the formation and properties of a very potent streptococcal toxin, named Streptolysin S **(SLS).** It is capable of destroying- hemolyzing red blood cells and can also kill a large variety of other mammalian cells by punching holes in their cell membranes. This Toxin, is believed to play an important role in diseases caused by group A hemolytic streptococci. The toxin is a real menace since it does not induce formation of antibodies and therefore, is allowed to rapidly kill cells un touched.

This **toxin** is a very small, non- immunogenic peptide, which is produced intra cellularly**,** remains bound the bacterial cells, is not released spontaneously during the bacterial growth, but can induce cell damage by a **kiss lof death**, by simply binding to cell targets such as red blood cells, fibroblasts, epithelial and cancer cells etc. It can however be released from the cells by plasma and by albumin and can thus be transported to remote tissues.

Examples on the toxic effect of **SLS** on cancer cells in culture were published in 1959 / 1960

Ginsburg I. Action of Streptococcal Haemolysins and Proteolytic Enzymes on Ehrlich Ascites Tumour Cells. Br J Exp Pathol. 1959 :40,417– 423.

Ginsburg I and Grossowicz N. Effect of streptococcal haemolysins on Ehrlich ascites tumour cells. Pathol Bacteriol. 1960;80,111-119.

After nearly 3 years of hard work, and further work in Philadelphia

(see below), we managed to publish several additional key papers on SLS.

Then, in the end of 1959, after completing my thesis, I corresponded with Zvi Harris, a Medical Doctor and an immunologist who was one the first scientists to show that antibodies are produced by lymphocytes **!!!.** However, he also had a clinic for Rheumatic fever patients and a very active group of young researchers all working on streptocococi. He accepted me as a post- Doc wth an annual salary of 5500 $ which was not a big deal.

Our stay in Philadeplphia was very rewarding and also exciting. Zvi and Shus and Zvis parents, grandma and grand father Chayeem Harris, lived near by, helped Ruth to settle down and also gave AAmir and yoram yiram us many toys including 2 green small bycicles for our 2 sons and a monkey a dog name **ugly** and a blond manmed **chrisi. These wonderful animals** are noe in my ber roolm !!!

. Grandmother Harris was a highly – self - educated women and Chayeem was a pharmacist and also a secular Cantor (chazzan) member if Hashomer Hatzaur, rwho build a synagogue in the basement. He put out several records of religious cahantings- **chazanut** (I still have them at home). Ruth found a nice job as a Hebrew teacher but had to travel long distances (phildelphia was a big town with 4 million inhabitants),

My work (1959-1962) was very interesting and I learntg a lot from Zvi about rheumtic fever and how to produce streptococcal antigens for analysis. The laboratory crew was very nice and helpful and we had a good time helping each other. In particular,

the laboratory had a very useful apparatus calle a **chemostat** capable of growing very large amounts of streptococci in a complete synthetic medium in a logarithmic fasion from which you could isolate streptococcal proteins and toxins. It might seem like a joke, but upon return from Philadelphia in 1962, I brought with me dried powder of the streptococcal preparation I had isolated, which was still active to this day!!!

Our work with Grossowicz and with Zvi Harris, led to 4 publications in the prestigious ***Journal of Experimental Medicine***

Ginsburg I , Harris TN, Grossowicz N. Oxygen – stable hemolysins of group A streptococci I. The role of various agents in the production of the hemolysin. J Exp Med. 1963;118:905 -917.

Ginsburg I, Harris TN. Oxygen - stable hemolysins of group A streptococci II Chromatographic and electrophoretic study J Exp Med.1963;118:919-3

Ginsburg I, Bentwich Z, Harris TN. Oxygen- stable hemolysins Of group A streptococci III. The relationship of the cell – bound hemolysin to streptlysin S. J Exp Med. 1965;121:633-45.

Ginsburg I and Harris TN. Oxygen – stable hemolysins of group A beta hemolytic streptococci IV. Studies on the mechanisms of lysis by cell – bound hemolysin of red blood cells and Eherlich ascites tumor cells. J Exp Med. 1965;121:647-56.

One day, Zvi told me that he had invited 2 Japanees Immunogists to spend severl hours in the lab to learn new things. However, he forgot that on the same morning, he had to give 2

lectures at the Univeresity. So, he asked me to stay with them to tell what was going on in the laboratory. At the right time, 2 persons with rich ties and black suites had arrived and sat with me arround the table saying Hii !! several times . From that moment on (took nearly 2 hours) I had described in great detail the topics of research in the lab. Then, when time was over, they stood up, smiled and said: Hi !!! Sorry **NO English** !!!. I later on undestood that their culture did not allow them to admit that they did not understand a word I was saying. I am sure that today, most Japanees speak fluent English.

After Philadelphia (end of 1992), we continued with streptococcal research and published a series of additional papers in collaboration with Mike Sela, Ofek Itzhak my 2 Ph.D students, with several students and with the colleagues Zvi Bentwich (today chairman of the Society for war against AIDS), and also with the pathologist, Alexander Lufer.The following papers were published

Marcus Z, Davies AM, Ginsburg I, Elias N, Heller M, Ginsburg I. Oxygen-stable hemolysins of group A streptococci. V. Effect on rat heart and kidney cells grown in tissue culture. Proc Soc Exp Biol Med.1964; 117:670-5.

Ginsburg I Harris.T N . Oxygen – stable hemolysins of group beta –hemolytic streptococci. Ergeb Mikrobiol (A review) Immunitatsforsch Exp Ther. 1964:38,198-222.

Elias N, Heller M, Ginsburg I, Binding of streptolysin S to red blood cell ghosts and ghost lipids. Am J Pathol. 1967:51, 351-71.

Ginsburg I, Bentwich Z. Effect of cysteine on the

formation of streptolysin S by group A streptococci. Exp Mol Pathol. 1966;5 93-107.

Zeiri N, Bentwich Z, Boss JH, Ginsburg I, Harris TN. Streptococci and Some of their Extracellular Products. Isr J Med Sci. 1966 May-Jun;2:302-9.

However the publication with with Laufer Ginsburg I, and Rosenberg SZ . Cardiac lesions produced in the rabbit by intramyocardial injection of various micro-organisms. Br J Exp Pathol. 1960 41:19-23.

We also raised an important question regarding the mechanisms by which streptococci cause rheumatuc fever, where in the human heart muscle, you found foci of a peculiar multinucear giant cells first discovered by the German pathologist, Rudolph Aschoff, and which were named after him. Similar cells can also be induced by injecting either cell walls of bacteria or foreign particles which are all highly resistant to biodegradation by enzymes of the host and thetefore, can induce chronic inflammation. Indeed, a simple injection of washed bacteria into the heart muscle of rabbits resulted in the generation of giant cell granuloma which bore some similiarity to the giant cells seen in rheumatic fever. We later on also completed a paper intended to study the reaction of tissues to Streptococcal products in order to shed more light on the possible mechanisms involved in rheumatic fever

Also see Zeiri N, Bentwich Z, Boss JH, Ginsburg I, Harris TN. Organ Lesions Produced In Rabbits by Group A Streptococci and Some of their Extracellular Products Am J Pathol. 1967;51, 351-71.

The focal infection theory

This study also raised an hypothesis that during infections with streptococci in the oral cavity, a toxin is generated which injures the heart muscle. This results in the accumulation of inflammatory cells which had phagoctyosed streptococci which were then transported and deposited in the injured heart muscle resulting in the formation of **giant cells** typical for rheumatic fever. This hypothesis was also confirmed experimentally by first injuring the heart muscle of rabbits by a needle to induced a local inflammation. This was then followed by the intravenous injection of streptococci. We were very surprised and excited to discover that streptococc were found wihin neuropils in the injured heart lesion (Work performed I in the NIHin 1971 See **Ginsburg I, Gallis HA, Cole RM. Group A streptococci: localization in rabbits and guinea pigs following tissue injury. Science. 1969;1661.161-3.**

A similar phenomenon was also observed when we first induced inflammation in the joints of rabbits and then injected intravenously either streptococci or particles of titanium dioxide which in darlk field microscopy, were shining and could also even be counted.

Isaac Ginsburg and Rama Trost. Localization of Group a Streptococci and Particles of Titanium Dioxide in Arthritic Lesions in the Rabbit The Journal of Infectious Diseases 1971:123,. 292-296

These very exciting observations led to support a general hypothesis that any injury to tissues induced by toxic agents or by bacterial cell walls, might result in the transport and deposition of live / dead bacteria, viruses, parasites and other particles in inflammatory sites. Although this hypthesis seems " crazy " it should seriously further be investigated.

STREPTOCOCCI AND A VISIT TO PARAGUE IN 1969

The **Prague Spring** was a period of short liberalization in Czechoslovakia during the era of its domination by the Soviet Union after World War II. It began on 5 January 1968, when reformist Alexander Dubček was elected the First Secretary of the Communist Party of Czechoslovakia, and continued until 21 August 1969 when the Soviet Union and all members of the Warsaw Pact with the exception of Romania, invaded the country to halt the reforms.

During the short Spring days of 1969, I received an invitation by the Czecoslovakian Academy of Sciences to come over for one week to lecture on streptococcal infections. They had a strong group working on the immune resposes to streptococci. My host was Jiri Havlicek, a nice, half - Jewish investiogator. I happily accepted the invitation but was a little aftaid since I shall be flying to a communist country. Inded, I received instructions from our University and by the Ministry of foreign affairs to stay only in Hilton Hotel. I flew to Pargue via London and upon arrival was greeted by 2 persons. One was Jiri Hvlicek and the other guy was his KGB guard who spoke no English. I was warmly gteeted but Jiri told me that he could not find a room in Hilton since Pargue held an Agricultural conference and the city was invaded by Bulgarians, Poles and East Germans and therefre, I shall have to stay in their home. I had a terrible dilemma whether to go home or, to break the law and pay for it if my secrete be revealed. Then, I saw the 2 people taking fast and started to laugh. Dont worry they said, you are in safe hands and besides**,** do you see this blnod man standing near me, he was the pilot who flew to Israel in 1948 bringing all the arms and ammunition given to Israel during your war

of Independence. He visited Tel Aviv several times. I was so excited and decided to stay in their home. The house (belonged to his father) was very large but, was divided by a string (among 3 families. So, Jiri and his wife slept in the kitchen and I was offeed their bedroom, what a hospitality **!!** Jiri was catholic, educated by his father but his mother was Jewish. So I already felt better. Also, his aunt lived in Jerualem, Rambam Street !!!, What a small world. I had a wonderful week talking to people in the laboratory and was also shown all over the historical parts of Pargue. My main lecture delivered on the eve of my departure went on very well and after finishing my presentation, I was handed an envelope. Jiri said to me, this is probably an honorarium. Since Czeck money was worthless outside the country and there was actually nothing to buy in the empty shops except for crème cakes for which Paragues was famous, I proposed to run down town to a shop which sold goods to tourists for dollars. Despite their insistence I bought then salami, coffee, tea and chocolate, etc. However, I still was left with worthless czeck money. After an exciting week I went to the airpott to fly to London. Since I had a pack of money I decided to spend it in the restauranat of the airport. I sat at a table with a young scientist who spoke fluent English and who also went to London. So I asked him politely whether he will agree to be my guest and to have a rich breakfast. We had a wonderful time exchanging views and ideas and finally I gave him the rest of the money and happily flew out of Prague. I took with me a letter from Jiri to his aunt in Jerusalem and when we met in Rmabam street, she told me all about her family history. What a story !!!. For many years, I have been trying to keep in touch with Jiri and looking at PubMed. I realsized that he had stopped publishing in 1992. All my efforts to find

his phone number via the embassy were not successful.

Finally, after spending years working on streptococci, I was invited In **1972** to review the field of Streptococcal research. It came out in 2 parts (**about 500 references)** and as of today, was cited **204 times** !!!

Ginsburg I, Mechanisms of Cell and Tissue Injury Induced by Group A Streptococci: Relation to Post streptococcal Sequelae. The Journal of Infectious Diseases, 1972:126, 419-456,

In 1972, I also contributed a chapter on Streptolysin S in: Microbial Toxins volume 3 Academic Press Press and wrote a large chapter on Streptococccci in a Text book of Microbiology

See section in the end of papers not included in Medline

Much later, in 1999 I also summarized our current knowledge on streptolysin S **(SLS)** dpublished as a review.

Ginsburg I. Is Streptolysin S a virulence factor ?(. Acta Pathologica Microbiologica Immunologica Scadinavica 1999:107,1051.

This happened after Infection and Immunity, the prestigious Journal of the American Society for Microbiology (**see ethical scandal with this Society**), had simply rejected my paper claiming that I have actually not added any new information.The editor simply did not read the paper to its end to realize a large number of new observations had been included. **Their loss** !!!

The synergism concept of cell injury

At that time, I already liked the concept that cell damage in infections and inflammation could not be induced by a **single agent**, a virulence

factor(**Chomer alim**), but, necessitated a cross – talk among many factors generated in vivo mostly in inflammatory conditions. These include toxins , oxidants, proteinases, phospholipases, cationic peptides, cytokines and chemokines. Later on, in 1999 this led to the publication: (already mentioned anove)

Isaac Ginsburg; Ron Kohen. Cell damage in inflammatory and infectious sites might involve a coordinated "cross-talk" among oxidants, microbial haemolysins and ampiphiles, cationic proteins, phospholipases, fatty acids, proteinases and cytokines (an overview). Free radical Research 1995;22, 489-517.
And an additional papers :

Isaac Ginsburg, Ron Kohen. Synergistic effects among oxidants, membrane-damaging agents, fatty acids, proteinases, and xenobiotics: killing of epithelial cells and release of arachidonic acid. Inflammation 1995;19 101-18.

Isaac Ginsburg. Multi-drug strategies are necessary to inhibit the synergistic mechanism causing tissue damage and organ failure in post infectious sequelae InflammoPharmacology 1999: 7, 207-21

The suggestion that synergism may be a main mechanism in tissue damage, had emerged from studies on the pathogenetic strategies developed by hemolytic streptococci, and also from the strategies of human neutrophiles **white blood cells** with ability to eat and kill bacteria by phagocytosis. Both live/dead microbial cel wall components, s have the ability to migrate within leukocytes to remote tissue sites and this by by destroying the extracellular matrix (**the substances between cells**), and to kill host cells by a synergism among a multiplicity of agents. This led us to write a **" theoretical paper "** under the titles:

Isaac Ginsburg, Peter A Ward, James Varani. Can we learn

from the pathogenetic strategies of group A hemolytic streptococci how tissues are injured and organs fail in post-infectious sequelae FEMS Immunology & Medical Microbiology 1999;25,325-338 .

It also suggested that might be, from the evolutional point of view, streptococci could perhaps be looked upon as some kind of fore - fathers of leukocytes and this, because their invasive activities are so similar

Isaac Ginsburg . Can hemolytic streptococci be considered "forefathers" of modern phagocytes? Both cell types freely migrate in tissues and destroy host cells by a "synergistic cross-talk" among their secreted agonists. Comp Biochem Physiol C Pharmacol Toxicol Endocrinol. 1994;109,147-58.

(**Also see below studies in Michigan 1986 – 1996).**

At that time, we also strengthened the assumption proposed that the inability to degrade the hard cell walls of bacterial cells might lead to chronic inflammation.

I. GINSBURG. I. Can chronic and self-perpetuating arthritis in the human be caused by arthropatic undegradd microbial cell wall constituents: A working hypotheses Rheumatology and Rehabilitation, 1977, 16, 141 (see also section on Arthritis

Streptococci and ethics

Going back to 1982 and still deeply involved in streptococcal research (**see the list of publications above),** I had to deal with a very serious and unpleasant ethical episode which had lasted more than a year. It involved the **plagiarism (gneva sifrututit)**
by 2 scientists from Chicago university of whole parts of my publicationon streptolysin S (**SLS)** and pubishing them in the Journal

Infection and Immunity - the American Society for Microbiology – ASM main Journal, while failing to cite any of our already published studies. It was very annoying since one of the authors participated with me in a symposium in London a year before, heard my lecture and knew all about my publications. I immediately demanded that the authors apologize publically by including a note in the Journal indicating that citations of my publications had been omitted. However, since these authors agreed only to apologize privately, which of course, was unacceptable, the episode went over a year. Finally, after tens of letters exchanged with the Editorial Board, I was finally allowed to publish a short note on the subject in the Bulletin of the American Society for Microbiology News which regularly published Letters to the Editor. It appeared under the titltlel" **Can the Editorial Boards of journals be "infallible"**? (**bilti toiim** . This letter led to many angry and supporting responses by eminent microbiologist who fully backed my arguments**.** This also raised a small scandal and the Editors of all 5 Journals of ASM, had been summoned to Washington to deal with this " crisis ". Eventually, this story led much later, in **2002,** to an invitation by Eugene GarfieldL to write an **Opinion** in the prestigious Magazine, the **Scientist** Exploring inspiring innovations and ideas under the title: ***Opinion : The Disregard syndrome Is it a menace to honest Science ?. The Scientist 2002, December***

The article actually condemned Journals for closing their eyes to plagiarism and oversights. Some statistics have indicated that about 25 % of scientific publications failed to cite already published work. This is how pioneering investigations are forgotten. **I am so happy that I did not give up and fought it all the way.**

To Sum up, it seems that we have added a new information on the role of streptococcal toxins and their cell walls in tissue injury as related to human disease and stressed the importance of **synergism** as a basic phenomenon to better understand mechanisms of cell damage. However, today, we need to update the huge streptococcal literature which had been added since 1972. This **is** today, beyond my ability.

Work at the Department of Experimental Medicine and Cancer Research at the Medical School.

In 1961, upon returning from Philadelphia, I found a job as an assistant at the Department of Experimental Medicine and Cancer Research under Jack Gross, studying the effects of bacterial toxins on cancer cells.

Working in collaboration with the late Theodor Dishon also at the Department of Experimental Medicine and Cancer Research, we studied a very interesting observation that a certain protein present in all human plasmas, (an antibody ?) could kill mouse cancer cells. This factor was purified by us and we argued that this agent is an "natutal antibody" specific for certain tumor cells which, by coating them, makes them resistant to killing by the hosts immune system. We also found that certain cells from a cancer origin grown in culture when coated by this human factor, became immune to killing by antibodies against the cells, produced in a rabbit. We hypothesized, therefore, that there imight be a possibility that in humans, the failure of the immune system to fight against cancer cells, might be due to the coating of the cancer cells by the patient's **own** plasma factor and that this agent may function as a blocking agent. It sounds fantastic

but I deeply regre that I did not continue studying this exciting idea. Unfortunately, this topic was not found of interest to several groups at our medical school studying the immunology of cancer to whom I tried to " sell" this story.

SEE Ginsburg I, Theodor Dishon, Marianne Bloch and Gross J. Thermostable Cytotoxic Factor in Normal Human Serum Active Against Landschutz Ascites Tumor Cells. Exp Biol Med J 1961: 107:235-240

and : Isaac Ginsburg and T. Dishon. Separation of Anticomplementary Material and Plasminogen from a Cytotoxic Factor Active Against Ehrlich Ascites Cells in Cohn Fractions I-III by Fluorocarbon and n-Butanol. Exp Biol Med. 1967: 126, 564-568

Cleveland Ohio: the strange story on anti cancer therapy which never actually happened

One day, in 1968 during my work with Jack Gross and Dishon, things had " heated up " when Gross called me to tell that he made a contact with a person in Cleveland Ohio, USA named Rand, a rich inventor, who together with a cancer investigator, De Carvalho from Portugal, built a laboratory in which a new technique was developed to obtain specific antigens from cancer cell which upon injection to animals, could develop " specific" cytotoxic) (**horgay taiim**) antibodies against human cancer cells.

He asked me to spend a month in Cleveland learning the methods developed by them. Again, I left my family for a month and upon arrival in Cleveland we came to a huge house with

servants all in black suites. I also met a German Pathologist who had arrived for the same purpose. Rand, a "genius", invented the electrical razor and the washing machine among many additional inventions. He was very intelligent and charming but always drunk after supper and had to be carried away to his room by the servants. Next morning we travelled to the laboratory. What I had seen there during my visit was the strangest thing I have experienced. The so called laboratory was actually a hangar filled with centrifuges, homogenizers and freezers full of tissues taken from cancer patients at the Cleveland Clinic, a part of the famous Case Western University hospital. The main technique they developed was to put the cancer tissues in a huge **blender,** separate proteins from DNA and RNA by extraction with fluorocarbon (an agent used in refrigerators) and then, injected them to animals as done in regular immunizations. They waited several weeks, bled the animals, separated the plasma which hopefully contained tumor **specific** antibodies and mixed it with extracts from normal tissues. This removed all the antibodies to normal tissue **while leaving only cancer specific antibodies** for use in patients. Finally, the specific antibodies were injected to ***melanoma*** patients. I went to the hospital and was shown a near complete regression of the skin cancer only a few days after receiving injections of the **" specific antibodies".** This was too good to be true. However, the idea behind the method to produce antibodies against cancer cells was great but not totally a new one. In 1962, Dr. Bertil Bjorklund, a Swedish Immunologist, began a long term program of testing in healthy humans volunteers what might turn out to be an anti cancer antibody.

However, the preparations of the vaccine in Cleveland was under poor hygienic conditions which eventually led to the closing down of this laboratory by the medical authorities because some of the samples used to inject patients, were found to be contaminated with bacteria. However the question whether cancer cells possess **specific antigens** which can be used to genefate cancer specific antibodies, is still controverisal.

During my stay in Cleveland, I also learnt that all efforts by De Carvalho and Rand to present their results to oncologists and to immunologists at the hospital, had failed. Nobody was interested in a "crazy" non – conventional idea. This was when I decided to step in. I got in touch with an old friend, Melvin Kaplan, an eminent immunologist at Western Reserve University. I told him about the Rand - De Carvalho story and suggested that it might be reasonable to at least to listen to their talk and if found un scientific, also to strongly criticize their results. Upon return to Israel, I learnt that De Carvalho finally gave the lecture which was accepted very favorably and nobody doubted that this approach might one day be useful to destroy cancer cells. However, this issue further raised a hot debate whether cancer cells really possess "specific" antigens on their surfaces and that immune therapy might be the right future to combat cancer.

Upon returning from Cleveland, I wrote a long protocol indicating that although . Iwas not sure what the small bottles used for treatment contained. I definitely saw with my own eyes cancer regression

Jack Gross was excited and decided to start a project based on Rand's-De Carvalho's findings. Dishon and myself followed the protocol, produced the antigen mixtures from cancer cells, injected the material to

donkeys in the old zoo in North Jerusalem, bled the animals after several weeks, and got plasma and were prepared to start a Clinical trial. In those days it was realtively easy to obtain permission to conduct trials on humans before the strict regulations today on human experimentations (The Helsinki accord). Then, one morning Gross asked us to hand him over all our protocols and told us to forget all about the project, **End of story** !!!. Since Gross had since passed away we could not find out any explanation why he had decided to cancell the proje

Additional investigations:

Phospholipids inhibit cytotoxic antibodies and the toxin produced by bacteria which produce periodontal disease)

During our studies on the cytotoxic effect of Streptolysin S on cells, we also showed that purified phospholipids, a group of sybstances which form the cell membranes and also found in egg yolk when added to cancer cells before the addition of antibodies directed against the cells, very significantly inhibited their destruction. This was probably due to a competition with the membrane pohosphilipids .

I. Ginsburg . Action of phospholipids on the cytotoxic effect of rabbit anribodies against Ehrlich ascites tumor cells. Brit J. Exp Pathol 1960,41

Similarly, phopspholipids were also found to inhibit the killing of neutrophils (white blood cells) induced by a toxin produced by a microorganisms which is involved in the generation of periodontlal duseaeses.

Ginsburg I, Chi-Cheng T, SM Warren and Taichman N S.

Phsholipids inhibit cytitoxic effects of Actinobacillus actinomycemcomitans leukotoxin on human polymorphonuclear leukocytes . Inflammat ion 1982: 6,365

The study was performed inphiladelphia in one of my short visit there .

To sum up : These studies might be important since phospholipids may be used clinically as agents capable of protecting against cell killing by toxins and by cytotoxicc antibodies generated in infections and inflammation.

I Joined the Faculty of Dental Medicine

Work at the Department od Experimental Medicine and Cancer Research went on very smoothly. We had nice people around us such as Fany Dolzanski, Amirav Gordon, Gershon Zeitzik, a wonderul academic atmosphere and we managed to publish interesting papers (**see List**). Although life in the Academy looked promising, J Gross argued thathis laboratory was too small and able to support only one student, at a time, which might have seriously affected my future academic activities.

Then, out of the blue, something happened which had changed my life. One bright morning Gross calls me and said: Look, you are a gifted and very productive investigator but, I am sorry to tell you that I have only one room to give you which will definitely slow down your academic activities. I was just informed that the School of Dentistry will soon be separated from the Medical school and will become an independent Faculty under Ino Sciaki who will be the new dean of Dentistry. Why not apply for a job as head of Microbiology and immunology ?. I suggest that you immediately

prepare a research plan, budgets and manpower and send it over to Prof. Ino Sciaki. I happily did what he had suggested and after a short period I received a phone call from Sciaki asking me to come over. Upon arrival, I saw him smiling. He opened a drawer in his desk and pulled out **4 keys** to rooms on the third floor of the Dental School in Ein Kerem and handed them to me saying: " this is yours, start working !! " This is how I became an active member of the School of Dentistry, head of Oral Biology and later on, also the Dean of the Faculty.

To this day, I still occupy the same small room on floor III room 411 used as an office - laboratory and still use the same table and chair given to me in 1974 as a gift from my Vice Dean, Avraham Rappaport

At that time, I was fortunate to have received a very large grant ($ 50,000) from the NIH to work on streptococci. This helped to start very interesting years of work with many students working on their M.Sc and Ph.D thesis which yielded many publications mainly on streptococci some of which are presented below.

The following publications 1976 – 1982 published mainly with MN.Sela (my Ph.D student) several MSc students, scientists from abroad and colleagues from the Ministry of Health.These focused on the effect of cationic peptides on bacteria, on tissue lesions induced by microbial products, on bacteriolysis and on the release of LPS (endotoxin) from intestinal bacteria.

The reason why 16 publications on these subjects had been published in the Journal, Inflammation, was the support by Gerald Weissmann, the Editor in Chief of the Journal who greatly appreciated our "Synergism" concept of cell damage in

inflamamation and infection as a reasonable explanations for many human disordres. He also invited me to serve on the Editorial Board of the Journal. Weissmann is a highly intelligent investigator, has a wide horizon not only in Medicine and in pathology but also in philosophy, education and ethics (see his impressive list of 455 publications !!!). He is now the Editor in Chief of the prestigious FASEB Journal -The Journal of the Federations of American Soscieties of Eperimental Biology.

We also later on wrote two major contributions on the role of cationic charges in the activation of leukocytes and the generation of oxygen radicals

***In 1987*: Isaac Ginsburg Cationic polyelectrolytes: a new look at their possible roles as opsonins, as stimulators of respiratory burst in leukocytes, in bacteriolysis, and as modulators of immune-complex diseases (areviewhypothesis). Inflammation 1987;11(4):489-515.**

And in 1989: Isaac Ginsburg. Cationic polyelectrolytes: potent opsonic agents which activate the respiratory burst in leukocytes. Free radical research Communications 1989;8,11-26. And the review in Inflammation .

These reviews summarized the concept that cationic proteins released in inflammation may also serve as non – specific antibodiy – like agents capable like antibodies to opsonize microorganisms (cover themrem) by and also other particles for phagocytosis not only by "

prfessional phagocytic " cells such as neutrophils and mavtroages but ,also by other cell types . See also our paper

Ginsburg I, Sela MN, Morag A, Ravid Z, Duchan Z, Ferne M, Rabinowitz- Bergner S, Thomas PP, Davies P, Niccols J, Humes J Bonney R. Role of leukocyte factors and cationic polyelectrolyte in phagocytosis of group A streptococci and Candida albicans by neutrophils, macrophages, fibroblasts and epithelial cells: modulation by anionic polyelectrolytes in relation to pathogenesis of chronic inflammation. Inflammation. 1981: 5, 289-312..

also

I Ginsburg, SE Fligiel, RG Kunkel, BL Riser, J Varani Phagocytosis of Candida albicans enhances malignant behavior of murine tumor cells Science 1987: 238 ,1573-1575

The following is a list of publications from 1976 - 1982 part of which were performed by M.Sc students and by my Ph.D student, Michael Sela :

Ginsburg I, Sela MN. The role of leukocytes and their hydrolases in the persistence, degradation, and transport of bacterial constituents in tissues: relation to chronic inflammatory processes in staphylococcal, streptococcal, and mycobacterial infections and in chronic periodontal disease. CRC Crit Rev Microbiol. 1976: 4.249-322

Ginsburg I, Lahav M, Ne'eman N, Duchan Z, Chanes S, Sela MN. The interaction of leukocytes and their hydrolases with bacteria in vitro and in vivo: the modification of the bactericidal and bacteriolytic reactions by cationic and anionic macromolecular substances and by anti-inflammatory agents. Agents Actions. 1976; 6, 292-305.

Ehrlich J, Sela MN, Lahav M, Ginsburg I.
The bacteriolytic effect of human dentoalveolar purulent

exudates and leukocyte extracts. Refuat Hapeh Vehashinayim. 1977:26,39-44.

Sela MN, Lahav M, Ginsburg I.
Effect of leukocyte hydrolases on bacteria. IX. The release of lipoteichoic acid from group A streptococci and from Strep. mutans by leukocyte extracts and by lysozyme: relation to tissue damage in inflammatory sites.
Inflammation. 1977:2,151-64.

Ferne M, Duchan Z, Rabinowitz-Begner S, **Sela MN**, Ginsburg I. The effect of leukocyte hydrolases on bacteria. XII. The release of lipopolysaccharide (LPS) from Salmonella typhi by leukocyte extracts, lysozyme, inflammatory exudates and by serum and synovial fluid and the modulation by anionic and cationic polyelectrolytes of LPS release and the sensitization of erythrocytes. Inflammation. 1978: 3,59-80. No abstract available.

Bab IA, **Sela MN**, Ginsburg I, Dishon T. Inflammatory lesions and bone resorption induced in the rat periodontium by lipoteichoic acid of Streptococcus mutans. Inflammation. 1979:3,345-58.

Lahav M, Ne'eman N, **Sela MN**, Ginsburg I.
Effect of leukocyte hydrolases on bacteria. XIII. Role played by leukocyte extracts, lysolecithin, phospholipase a2, lysozyme, cationic proteins, and detergents in the solubilization of lipids from Staphylococcus aureus and group A streptococci: relation to bactericidal and bacteriolytic reactions in inflammatory sites. Inflammation. 1979: 3, 365-77.

Ne'eman N, **Sela MN**, Chanes S, Bierkenfeld L, Kutani D, Lahav M, **Ginsburg I** Effect of leukocyte hydrolases on bacteria. XIV. Bacteriolytic effects of human sera, synovial fluids, and purulent exudates on Staphylococcus aureus and Streptococcus faecalis: modulation by Cohn's fraction II and by polyelectrolytes. Inflammation. 1979 :3. 379-94.

Sela MN, Natan G, Lahav M, Ginsburg I, Dishon T.
Bacteriolytic activity of human gingival exudate.
Inflammation. 1980 :4,195-203.

Ginsburg I, Dishon T, Duchan Z, Garfunkel AA. Modulation humanlymphocyte transformation by bacterial products and leukocyte lysates. Inflammation. 1982 :6,31-38.

Work with Ithak Ofek (a Ph.D student) and Sonia Rabinowitz from the Ministry of Health

Ofek was my last Ph.D student and today is an emeritus Professor in Tel Aviv University working on cranberries polyphenols. The following is a list of publications of Ofek performed with our goup.

***Sela MN, Ofek I,* Lahav M, Ginsburg I.**
The effect of leukocyte hydrolases on bacteria. XI. Lysis by leukocyte extracts and by myeloperoxidase of a Staphylococcus aureus mutant which is deficient in teichoic acid, and the inhibition of bacteriolysis by lipoteichoic acid. Proc Soc Exp Biol Med. 1978: 159,126-30. No abstract available.

Ofek I, Bergner-Rabinowitz S, Ginsburg I. Oxygen-stable hemolysins of group A streptococci. VII. The relation of the leukotoxic factor to streptolysin S. J Infect Dis. 1970;122,517-22.

Glaser M, Nelken D, Ofek I, Bergner-Rabinowitz S, Ginsburg I
Alpha globulin decreases resistance of mice to infection with group A Streptococcus. J Infect Dis. 1972 Oct;126(419-56.

Ofek I, Bergner-Rabinowitz S, Ginsburg I.
Oxygen-stable hemolysins of group A streptococci. 8. Leukotoxic and antiphagocytic effects of streptolysins S and O. Infect Immun. 1972 6459-64.

Ofek I, Bergner-Rabinowitz S, Ginsburg I. Oxygen-stable

hemolysins of group A streptococci. 8. Leukotoxic and antiphagocytic effects of streptolysins S and O. Infect Immun. 1972;6.459-64..

Bergner-Rabinowitz S, Ofek I, Fleiderman S, Zohar M, Rabinowitz K, Ginsburg I. Evaluation of streptozyme and antistreptolysin O tests in streptococcal pyodermal nephritis. Appl Microbiol. 1973 ;26,56-8.

Glaser M, Nelken D, Ofek I, Bergner-Rabinowitz S, Ginsburg I Alpha globulin decreases resistance of mice to infection with group A Streptococcus. J Infect Dis. 1973;127,303-6..

Sela MN, Ofek I, Lahav M, Ginsburg I.
The effect of leukocyte hydrolases on bacteria. XI. Lysis by leukocyte extracts and by myeloperoxidase of a Staphylococcus aureus mutant which is deficient in teichoic acid, and the inhibitionof bacteriolysis by lipoteichoic acid. Proc Soc Exp Biol Med. 1978;159126-30..

Additional paper published with my colleagues

Gazit E, Ginsburg I, Harris TN. Dialyzable form of an extracellular streptococcal toxin causing histopathologic and biochemical changes in rabbits. Proc Soc Exp Biol Med. 1972 140,1025-9.

Ginsburg I. Mechanisms of cell and tissue injury induced by group A streptococci: relation to poststreptococcal sequelae. J Infect Dis. 1972;126, 294-340. Review (MENTIONED ABOVE)

Ginsburg I, Trost R. Localization of group A streptococci and particles of titanium dioxide in arthritic lesions in the rabbit. J Infect Dis. 1971: 123,292-6.

Ginsburg I .The focus of infection theory: a new look at the possible relation to poststreptococcal sequelae. Hum Pathol. 1971;23,45

Red cell-sensitizing antigen of group A streptococci. II.

Immunological and immunopathological properties. Isr J Med Sci. 1972 8(11):1807-16.

Stein H, Yarom R, Levin S, Dishon T, Ginsburg I, Harris TN. Chronic self-perpetuating arthritis induced in rabbits by a cell-free extract of group A streptococci. Proc Soc Exp Biol Med. 1973 143:106-12.

Ginsburg I, Ne'eman N, Lahav M.
Effect of cationic and anionic polyelectrolytes and antibodies on the lysis of micrococci and streptococci by leukocyte lysates and lysozyme. Isr J Med Sci. 1973 :9, 663-8.

This ended a very fruitful research mostly on group A Streptococci and their toxins .

The Samuel M. Robbins of Cleveland Ohio, USA story

Our studies on streptococci and their role in tissue damage reached a point where we had aslo to study in more detail what happens when the bacterial cells explodes and releases its intra cellular and cell - wall components especiallly after treatment either with penicillin or with cationic peptides from leukocytes .

However, at this point, we nearly ran out of funds. We understood that since no money could come from the university, we should look for a donor from outside. This is when a real miracle happened **!!!.**

It was in the early 1980 when one bright morning my door burst open and a funny looking old American person showed himself in. He had a red tie, embroidered shirt and trousers which looked like a chess board. The first words he said : Don't you know Sam Robbins of Clevelland Ohio USA ?. I am a dentist and the oldest fund raiser for Hebrew University and one who gave a lot of money for stipends for students. I said to him that since I was not a dentist, I did not have

the honor of hearing about him. Than he said, I am also an histologist and I want to know what are you doing in the Faculty ?.

After explaining at length what we were doing and showing him the list of publcations, he asked: do you have a computer ?. I said not yet. You cannot go on working like this, he said: Get on the phone, I want to talk to a computer company. On the spot, he ordered a computer, wrote a checque addressed tHhebrew Uiversity at a sum for $5500 (this was the price in those days) and said mazal tov, you will soon receive and IBM computer. But before he left, he asked me to hand him **all** the reprints of our published work and said: I am not giving money away so quickly " **Nobody is fooling Sam** !!! . I am going to show your work to my friends in NY University and also in Cleveland to get their opinion on the importance and standards of the work you are doing. He said shalom and left. From that day on, and for years afterword, he used to come over for the whole month of August, sat at my laboratory, drank tea, talked and talked but in every visit also offered me more funds for research but always took with him additional reprints to show to his friends. He actually had a very sad life. His first wife whom he dearly loved, passed away and his second wife was a difficult women. He cut his relations with his 2 dentist sons but gave each 1 million $ to set their practices, now they are important professors of Dentistry. However, the only one he had relations with, was his grand daughter whom he supported through college. It seems that he looked upon me as a kind of a "son". This is a possible explanation why he had adopted me. Then, a year before he passed away (see below), I managed to organize a ceremony where my laboratory was dedicated to Sam Robbins of Cleveland Ohio in memory of his late wife. The ceremony took place outside my small

laboratory, the participants were the President of the University, the Dean of Dentistry, photographers and my colleagues. Sam was so happy and said that he had been waiting for years for this moment to see that he had made a reasonable investment in the Dental school. He was really a good hearted person who did not have friends from amongst the Dental staff. A year later, it was about 15 years ago, he arrived in the summer and I realized he was not well. He said: Isaac, this is probably my last visit with you. I am very sick, I have lymphoma. It was quite a shock to me. I was so accustomed to have him near by every summer and to hear his chats. We sadly said good bye and left. Then, several months later, I learnt that he had passed away at the age of more than 90. I then received a note from the University that he had left me a largw some of money which was invested, in my name, with the Friends of Hebrew University in NY where the money was invested by their financial experts. As a results, I have been receiving 4 annual payments as interest, while the fund remains untouched which is a wonderful support for the laboratory all these years. Then, in 2008 came the financial crisis. Maydof, took half of my fund (The university lost 65 million $) but as of 2009, the payments were slowly resumed so, things are now nearly back to normal. This ends Sam Robbins' amazing story and every publication of ours has a special foot note: **This study was supported by an endowment fund by the late Dr. S. M Robbins of Cleveland Ohio, USA "**. To this day, I believe we have published about 100 !!! papers bearing the Robbins name as a kind supporterof our research work. I am sure he is very happy where he is now, believing he did the right thing by helping the development of the academic status of the University. The big Robbins Plaque is on the left side of my door,

floor 3, room 411 .

All these years and with Sam's generous support, we moved into interesting new fields of research but with struggles for a recognition of new ideas (thinking outside the box).

I became the Dean of the Dental School

In **1974** work in the laboratory went on nicely except for one "sad fact ": I was pressurized by the Faculty to become the next Dean of the Dental School. One of the reasons for electing me was because in 1974 I was promoted to a full Professor !!, and at that time, was the only person with this degree in the school. They all thought that I would be a better representative of the Faculty in the important permanent council of Deans the President and the Academic Secretary**.** This is a weekly get together of all 6 Deans of the University Faculties,. It discussed various burning problems at the university which included: future academic developments, budgets academic degrees, and promotions, teaching, future research and developments of faculties etc. etc.

When I had accepted this difficult task, the school was moving only very slowly as an Academic Institution and the strength of private practices among dentists, mainly at home, did not really allow the development of any serious clinical research, the base line of any academic university. Also, many Dentists held only partial appontments which was not good for an Acadmic University but, reality was economically stronger than anything. However, in a meeting with the president of the university, I raised a suggestion how

you might solve the problem of young graduates in Dentistry by preventing them from opening private practices at home and this coud pehaps solve the Academic difficulties of the Faculty. My suggestion was to allow the young clinicians with a DDS degree, to have a kind of private practice in the Faculty and as such, they will also help to instruct 5th ad 6th year students. Although he liked my idea, it was torpedoed by young doctors with an MD degree in Hadasah who argured that according to law, only senior lecturers are allowed a practice within the Hospital. So, my " bright idea " did not hold water !!!.

One major difficulty running the Faculty, was my total ignorance of the politics involved and the complex interactions among 1) Hadassah Women the main supporterof the clinics 2) the University (teaching all basic sciences in the first 3 years of schooling, 3) the Ministry of Health and 4) Alpha Omega Fraternity of Jewish dentists which helped to build the Dental School and used to donate but only about $ 25,000 !!!! a year, which was a joke.

Being totally ignorant of what has been going on, I was fortunate to have on my side Avraham Rappaprt (the vice Dean and Shula Koenig) as a devoted and very efficient advisers . Avraham came from an administrational position at Hebrew University and new all the "politics and tricks" involved and how to look for more funds to run the school. He was a very devoted hard worker, I would say, a **workholic** who never left the office before 2100 every night. I could blindly trust all his knolewe and useful administrational decisions. He was really a good friend, always meant well, but used to call me late evening to solve "burning issues ".

A significant help to run the school also came from the late Mario Ulmansky, a former Dean (a dentist Oral Pathologist) as a wonderful advisor. The worst of all, was my inability to judge clinical criteria and faced the chronic lack of funds for research and having to be away from the laboratory.

As a dean, I was obliged to participate in fund raising and in the annual meeting of Alpha Omega Fraternity which took place in various places in the US and Canada, always in cold December, when prices in hotels were low. Today, the 3 years of my Deanship seem so far away and many events have been totally erased from my memory except for struggling with clinicians to start doing research. I also experienced 2 unpleasant incidences where with the advise and support of 2 former Deans, I had to **let go** 2 heads of Departments and as of today, they ignore me when we meet, manly in the dining room.

All in all, I believe that I had contributed something useful to the understanding that basic science is absolutely to advance the academic status of the dental profession. Today, we have a world appreciation of the very high levels of basic sciences and clinical research in the Faculty with more than 100 M.Sc and Ph.D students and the most important of all, students of Dentistry that in parallel to their clinical studies also work on their M.Sc Ph.D degrees. This will ensure that future teachers in Dentistry will also have scientific capabilities. Today, all the basic sciences in the faculty are under an Institute for Dental Sciences. The inclusion of basic sciences in clinical teaching, greatly contributed to the current excellent scientific status of the school.

The library committee of the Medical center

The late nineteen seventies and the beginning of the nineteen eightees were the years prior to the computarization of the Medical library. As a member of the Library committee we realized we had a very useful Medical and Science journals collections mostly in English, however the budget problem was always a main concern. I was asked by the members to take over of the committee where I served as chairman for more than 5 yeas. According to the affiliation agreement signed in 1925 !!! between Hadassah and the Hebrew University, all the budgets for books and journals in basic sciences and in clinical medicine about (50 : 50) should come exclusively from the University. With increasing prices of books and journals, we had to cut down the budget every year. The first thing we had to do was to agree to stop buying journals and books in German, French, Italian, Polish and Russian. So, the number of Journals went fown from 1400 to less than 1000. This was a serious blow to the strength of the library. However, today, upon the establishment of comuter sceinces, the problem was nealy solved and today we have access to all the medical information we need. The main sources are PuMed, the Library of Congress in the USA and of course, Google and Google Scholar.

The Bacteriolysis story.

Upon completing many studies on streptococcoi, we started an interesting new project to investigate not only how bacterial cells are killed by phagocytes but mainly, how various agents produced by leukocytes can also cause breakdown of the rigid bacterial cell walls and to induce lysis a process known as **bacteriolysis**.

The work to be presented was in collaboration with Milu Sdovnic, Meir Lahav and students. In 1922 Alexander Fleming has noted the occurrence in the tissues and secretions of humans and animals, and in some vegetable tissues of a bacteriolytic substance which he named : " lysozyme" which was active mostly on non – pathohenic microorganisns. He used a bacterial strain called **Micrococcus lysideikticus (a cousin of Staph aureus)** as a substrate for lysozyje which became a very useful tool to quantify this enzyme. However, later on, it became apparent that unlike this sensitive bacterial strain, the large majority of pathoghenic bacteria were highly **resistant** to the **direct** effect of lysozyme (see below). Lysozyme is also found in large amounts in saliva, tears and in the urines of patients suffering of myeloid leukemia. For some years, Dishon and myself gave a free service to Hadassah Hospital helping to **confirm** the diagnosis of myeloid leukemia based on finding large amounts of lysozyme in urine which came from the intracellular destruction of the cancer cells in the blood.

We decided to test the effect of lysozyme on Staphylococcal strains such as MRSA- **methicillin resistant staphylococcus aureus),** the nasty bacteria that are involved in skin wounds (**the same I had as a child**) and is a dangerous organism causing septic shock and infect mainly patients in hospitals. Therefore, it is highly suggestive that patients better leave the hospital as soon as possible.

Our understanding of the mechanisms of action ofbactericidal cationic peptides on bacteria came from the simple observation that cationic lysozyme **cannot** directly break the chemical bonds in the walls of the majority of bacteria since their walls contain a certain chemical structures - o-acetylation, which did not allow the enzyme to

interact with its target. Indeed, the removal of this cell wall structure by a simple laboratory technique, will now allow lysozyme to act directly as a bacteriolytic enzyme**.** Furthemore, although the enzymatic activity of lysozyme could be destroyed by heat treatment, it could still retain its bacteriolysis - inducing properties by activating a series of enzymes which regulate normal cell division indicating that the electrical charge on lysozyme but **not** its enzymatic activity, was involved.

Taken together, we argued that lysozyme, being a highly cationic protein (**having a positive charge**) might function **not** as an enzyme capable of breaking the bacterial cells from the **outside** but, similarly to penicillin, to affect the divsiton of the cells during normal growth and this, by activating a group of autolytic enzyme (capable of breaking the walls from **inside** the cells). This results in the release of cell wall fragments and also intracellular agents. Similarly to lysozyme, a large group of cationic peptides **(highly positively charged**) produced by leukocytes at sites of inflammation and which acted as defenders against bacteria, could also function like penicillin to break the cell walls from **within** the cells thus behaving as **Trojan horses**. Therefore, **bacteriolysis** might act as a duble edged sword as injuring tissues but also as activators of the immune resposes.

The extent of bacteriolytsis indced by cationic agents was also beautifully demonstrated by electron microscopy. We were fortunate to have with us **Milu Sadovnic** a devoted friend and colleague. He was a highly skilled worker who developed better techniques to prepare very thin sections of bacteria and tissues containing bacteria for use by electron microscope (EM) (magnifications of 20000 –

30000). He skillfully photographed the samples, which yielded very interesting results and also explanations how bacteria behaved inside phagocytes. Using EM allowed us to determine exactly where the intracellular enzymes destroyed the cell walls and how phagocytes which engulfed bacteria in inflammtory sites, where shown to slowly destroy **only the cytoplasm of bacteria** but did not touch the rigid cell wall which was responsible for the initiation of chronic inflammation probably due to the inability to get rid of the walls. We also think we know now why while when tested in vitro, cationic peptides induced the breakdwon of the cell walls, phagocytes failed to do so. We thought that his might probably due to the inactivation of the bacterial intracellular enzymes (muramidases which break the cell walls) by oxidants and proteinases generated inside the phagocytes. This resulted in the persistence and survival of whole cell walls in tissues causing chronic inflammation.This ideas was published in 1989

Ginsburg I. bacteriolys is isinhibited by by hydrogen peroxide and by proteinases. Agents Actions 1989;28,238–42.

The debate over bacteriolysis and the mode of its activation, lasted for years and to this day, the majority of investigators still state that cationic peptides kill bacteria by increasing the permability of the membrane causing cell death. On the other hand, we claim that the cationic peptides may **also** act by activating intracellular enzymes which break the wall from inside of the cells. However, I now believe that the 2 events might occur side by side, depending on how long you wait between the addition of the cationic peptides and looking for cell death and a later event of cell damage detected either by electron microsciopy or by the relase of radioactive cell wall fragments.

Taken together, over the years we managed to publish about **15** papers on the subject. This project was in collaboration with Milu Sadovnic and Meir Lahav and students but we also established a very fruitful collaboration with 2 German investigators, Peter Giesbrecht and Jorg Wecke from the Robert Koch Institute in Berlin (see list of publication on bacteriolysis). They had the most sophisticated electron microscopic equipment and a lot of knowledge on the chemistry of bacterial cell walls. Meir and myself also went to Germany several times to work with these investigators.

The following is a partial list of publications on bacteriolysis and additional subjects published during the years 1973 - 2008

Ginsburg I. Effect of Body Fluids and Macromolecular Substances on the Lysis of Group A Streptococci by Muramidases of Streptomyces albus. Isr J Med Sci. 1973: 9,663-668.

Ginsburg I, Ne'eman N, Lahav M. Effect of cationic and anionic polyelectrolytes and antibodies on the lysis of micrococci and streptococci by leukocyte lysates and lysozyme. J Infect Dis. 1973 1273:3-6.

Lahav M, Ne'eman N, Adler E, Ginsburg I

Effect of leukocyte hydrolases on bacteria. I. Degradation of 14C-labeled Streptococcus and Staphylococcus by leukocyte lysates in vitro. J Infect Dis. 1974 :129, 528-37

Neeman N, Lahav M, Ginsburg I. The effect of leukocyte hydrolases on bacteria. II. The synergistic action of lysozyme and extracts of PMN, macrophages, lymphocytes, and platelets in bacteriolysis. J Infect Dis. 1975 :131, 49-57.

Lahav M, , James J, Ginsburg I. The effect of leukocyte

hydrolases on bacteria. III. Bacteriolysis induced by extracts of different leukocyte populations and the inhibition of lysis by macromolecular substances. Infect Immun. 1975 :11, 869-72.

Lahav M, e'eman N, Sela MN, Ginsburg I.
Effect of leukocyte hydrolases on bacteria. XIII. Role played by leukocyte extracts, lysolecithin, phospholipase a2, lysozyme, cationic proteins, and detergents in the solubilization of lipids from Staphylococcus aureus and group A streptococci: relation to bactericidal and bacteriolytic reactions in inflammatory sites. Inflammation. 1979;3,365-77.

Ginsburg I. Bactericidal cationic peptides can also function as bacteriolysis-inducing agents mimicking beta-lactam antibiotics?; it is enigmatic why this concept is consistently disregarded Medical hypotheses Medical Hypotyheses. 2004:62, 367-374,

Ginsburg I, Goultchin J, Stabholtz A, Neeman N, Lahav M, Landstrom L, Quie PG. Streptococcal and staphylococcal arthritis: can chronic arthritis in the human be caused by highly chemotactic degradation products generated from bacteria by leukocyte enzymes and by the deactivation of leukocytes by inflammatory exudates, polyelectrolytes, leukocyte hydrolases and by cell sensitizing agents derived from bacteria? Agents Actions Suppl. 1980;7,260-270.

This last paper was published as a result of our short stay in Minneapolis, Minnessota USA.

Two major **reviews** on cationic peptides were also published in 1987 – 1989:

Ginsburg I, Cationic polyelectrolytes : A new look at their possible role as opsonins,,as stimulators of the respiratory burst in leukocytes, on bacteriolysis and as modulators of immune complex disease. Inflammation

1987:11, 489

Ginsburg I (Invited review) Cationic polyelectrolytes : Potent opsonic agents which activate the respiratory burst in leukocytes .Free Radical Research 1989: 8, 11-26.

This paper dealt with the ability of cationic peptides to activate leukocyte to release oxygen radicals**.**

Finally, the following summarizing review from 2002 was a major effort to " sell " to the public the bacteriolysis concept and its importance in human and animal diseases.

Isaac Ginsburg. The role of bacteriolysis in the pathophysiology of inflammation, infection and post-infectious sequelae APMIS 2002 110: 753–70, 2002.
APMIS is for short: **Acta Pathologica Microbiologica, Immunologica Scandinavica**. As of today, this review had been cited **97** times.

To end the complicated story of bacteriolysis, and the asguments with other investigatirs, lets move to **2008**, when we were invited by the Journal **Experts Reviews in Antimicrobial Therapies,** to review a paper by Hancok (a famous investigator on cationic peptides who has about 30 Ph.D students !!! and is the one who never cited our papers). Reading his article, I realized that he **did** mention our theory but, **by chutzpa,** did not include a citation !!. I complained to the Journal about this unethical behavior and they responded by inviting me to present **our thoughts** on the subject. For this task I persuaded Erez Koren (my former Ph.D student) (**see studies on polyphenols below**), to become involved in writing the review paper. This was intended to cover **all,** the theories which argued the pros and cons of the killing and bacteriolysis induction by cationic peptides.

It is important to stress that because of antibiotics resistance, scientists are currently investigating the role of different new synthetic cationic peptides as replacements for antibiotics.

However, our review also dealt with the hazard involved in the production of new peptides designed to replace antibiotics. We warned that because of their positive charges, cationic peptides may also act as "double edged swords "causing un desirable bacteriolysis and the release of toxic bacterial product. The paper appeared in 2008 under the title :

Ginsburg and Koren E. Are cationic antimicrobial peptides also" double-edged" swords ?.Experts Reviews in Anti microbial Therapies, 2008:3, 437–451

The long struggle with investigators who were not willing to accept alternative ideas, stressed that persistence hard work and believing in your ideas, will eventualy pay off.

Our trip to Wahsington D.C – NIH (National Institues of Health)

Going back to 1969 we went on a sabbatical leave as a senior lecturer, I Joined Roger Cole at the NIH (Washigton D.C) who was working on microbial diseases and he happily invited me to spend a whole year in this wonderful place where hundreds of scientists were working in a huge campus. I travelled with Ruth and Michal, our youngest daughter (**There she learnt how to swim**) and managed to publish an interesting paper which is actually connected with the **Focal Infection Theory.** This argues that an injury in one part of the body joints, heart could attract bacteria from the oral cavity or fom the

intestines. Our study in Science from 1969 may supports this hypotheses .

Ginsburg I, Gallis HA, Cole RM Group A streptococci: localization in rabbits and guinea pigs following tissue injury. Science 1969; 166, 1161 1163.

We showed that that we can **track** how streptococci migrated to a remote inflammatory sites by using fluoresce antibody techniques.

Upon return, I was informed that I had been promoted to an Associated **Professor** good news !!

Studies on arthritis

A) Bacterial arthritis

Many bacterial species may cause arthritis in humans, a condition different from rheumatoid arthritis which is an outo- immune disease. Bacteria such as Staphylococci and streptococci causing skin wounds or infections in various other body sites, may be carried by the blood stream to from an infectious focus in joints injured by trauma or by non-specific inflammation. As already mentioned above, one example may be the migration of **oral streptococi** to injured joints (the famous discussion about the **focal Infection Theory**). However, without going into the endless discussions around this debatable theory, we neverteess performed several experiments which might support this theory. We spent some time studying the role of streptococcal cell wall components as agents capable of triggering arthritis in *laboratoiry animals . The following papers were published:*

Ginsburg I. The focus of infection theory: a new look at the possible relation to poststreptococcal sequelae. Hum Pathol. 1971: 3,345-347.

Isaac Ginsburg and Rama Trost. Localization of Group A Streptococci and Particles of Titanium Dioxide in Arthritic

Lesions in the Rabbit The Journal of Infectious Diseases 1971: 123,292-296

In these publications, we propsosed that the arrival and localization of bacteria in injured joints is carried out by leukocytes which act as "transporting buses".

In another paper we argued that small fragments of undegraded bacterial wall components which persisted for long periods within phagocytic cells, could induce an active release of enzymes which can cause tissue destruction. The failure to identify such wall components in diseased tissues may be due to the lack of adequate sensitive techniques to detect very small amounts of these wall components, shown to trigger chronic destructive arthritis in laboratory animals.

See–

Stein H, Yarom R, Levin S, Dishon T, Ginsburg I, Harris TN. Chronic self-perpetuating arthritis induced in rabbits by a cell-free extract of group A streptococci. Proc Soc Exp Biol Med. 1973 143:106-1012

Ginsburg I. Can chronic and self - perpetutating arthritis in humans be caused by arthrot ropic undegradd microbial cell wasll constituents: A working hypothesis . Rheumatology 1977:16,141-149

Isaac Ginsburg, Michael N. Sela. The role of leukocytes and their hydrolases in the persistence, degradation and transport of bacterial consttuents in tissues: Relation to chronic inflammatory processes in staphylococcal streptococcal and mycobacterial infections 1976 CRC

.

Summary :

Taken together, we believe that either live bacteria growing in one

part of the body or even dead bacterial cells, may be transferred (trans located) by leukocytes to remote tissues sites where they can establish a new inflammatory focus).

B) Rheumatoid arthritis

We then also spent some time studying a rat model of rheumatoid arthritis, an auto immune disease thought **not** to be caused by bacteria. Rheumatoid arthritis is today thought to be an auto immune disease and its cause is still **unknown.** The process involves an inflammatory response of the capsule around the joints secondary to swelling of synovial cells, excess synovial fluid, and the development of fibrous tissue in the synovium. The pathology of the disease process often leads to the destruction of articular cartilage.

Role of glod compounds: The application of gold compounds to medicine which is called "chrysotherapy" and "aurotherapy was first reported 1935 and found to reduce inflammation and to slow disease progression in patients with rheumatoid arthritis

In collaboration with Dr. Finkelstein from Albert Einstein Institite in Buenoa Aires in Argentina, Ruth Borinski (my student) and a few others, we published 2 papers dealing with the possible therapeutic effects of gold in adjuvant artritis models in rats.

Finkelstein AE, Ladizesky M, Borinsky R, Kohn E, Ginsburg I. Antiarthritic synergism of combined oral and parenteral chrysotherapy. I. Studies in adjuvant-induced arthritis in rats. Inflammation. 1988 12: 373-82.

Finkelstein AE, Ladizesky M, Borinsky R, Kohn E, Ginsburg I. Antiarthritic synergism of combined oral and parenteral chrysotherapy. II. Increased inhibition of activated leukocyte oxygen burst by combined gold action. Inflammation. 1988 12

:383-90.

We showed that combinations of oral gold and injected gold preparation (Auronafin), very markedluy inhibited the development of arthritis in rats and and that such treatment also inhibited the generation of oxygen radicals.

A short stay in Lund, Sweden

In 1982, I was invited to join a group of researchers in Lund, Sweden, a wonderful old and beautiful city, to work for several months with Poul Christensen and several of his colleagues. This yielded 2 interesting publications

Ginsburg I, Christensen P, Eliiasson I, Schalen C. Catinic polyelectrolytes, iquoid and leukocyte extracts modulate the binding of IgG to group A streptococcal Fc receptor. Acta Pathologica Microbiologica Scandinavica Series B: Microbiology 1982 161–168,

In another study we looked at an additional example of synergism which might occur when various agents act together to release toxic oxygen radicals from blood leukocytes

Ginsburg I. Borinski R, Lahav M, Matzner Y, Eliasson I, Christensen P, Malamud D. Poly-L-arginine and an N-formylated chemotactic peptide act synergistically with lectins and calcium ionophore to induce intense chemiluminescence and superoxide production in human blood leukocytes. Modulation by metabolic inhibitors, sugars, and polyelectrolytes. Inflammation. 1984 8:1-26.

However, we did not continue these studies and I have never heard from the group in Lund. It was very unfortunate that Jacom Matzner, the Dean of Medicine at Hadassah who participated in the project, was later on killed in a plane accident upon return from a meeting in Europe. This is the second time that colleagues of mine had been

killed in plane accident (**See Melvitzki** and my M.Sc thesis)

The lipoteichoic acid story (a cell – sensitizing agent)

Liipoteichic acid (**LTA**) known in the old literature as a cell - sensitizing agent, is a chemical structure which is attached to the cell membrane of streptococci and staphylococci but spreads out of the cells as thin threads. These threads help the bacterial cells to strongly bind to the membranes of host cells. LTA also acts inside the bacterial cells to regulate / control the enzymes which are responsible for the separation of the bacterial cells during normal growth. However, If the cells are interrupted either by antibiotics such as penicillin or by cationic peptides, it might lead to **bacteriolysis** (see above).

However, the early observations showed that **LTA** may also destroy red blood cells by binding to their membranes and after interaction with antibodies to streptococci, could induce cell aggregation (**agglutination**) and lysis. This phenomenon is called a " passive - immune kill process." This happens when antibodies are not directed at the cell membrane but, against substances bound to it from the outside. Our studies from 1972 shed more light on this process. In 1967 working with T. Dishon, we found that cholesterol and phospholipids in the red blood cell membranes, are some of the binding sites for this factor (**LTA)** and that purified phospholipids such as egg yolk lipids, could bind **LTA** before it can interact with red blood cells thus, preventing cell damage.

Dishon T, Finkel R, Marcus Z, Ginsburg I. Sensitizing Products of Streptococci. Immunology,1967:13,555.

Many years ago, studies by Harris from Philadelphia, my Post Ph.D

Mentor had shown that plasma of patients recovering from streptococcal infections, had higher levels of antibodies against this factor and that this could be used in the diagnosis of recent streptococcal infections and especially of rheumatic fever.

The cell – sensitizing agent was finally identified chemically in 1975 by Knox and Wicken as lipoteichoic acid – (**LTA)**. Here, I must tell an amazing story regarding Knox and Wicken realted to ethics. It appeared that these Australians worked in Cambridge University, the U.K, with the very eminent scientist, Sir John Baddily who was really the first to discover the chemical structure of LTA. During my correspondence with him (**see section on Ethics**), he mentioned to me that these Australians actually "stole " the chemical structure from him and that the only difference was **the new name !!! given to the agent**. This is how you become famous. !!!

Much later, when we were in Michigan (see below), we observed that human neutrophiles, the main phagocytic cells in blood, which had been treated by **LTA** followed by antibodies against LTA, became "activated" and released large amounts of the oxygen radical **superoxide** which immediately formed H2O2, a toxic agent for both bacteria and also for host cells.

Ginsburg I, Fligiel S G, varani J, Ward. Lipoteichoic acid – anti lipoteichoic acid complexes induce supeoxide generation by human neutrophiles Inflammation 1988: 12, 525

These findings led to an additional observation that neutrophiles pre - treated for a short time with compounds which bound to cell membranes also generate the oxygen radical supeoxide when treated by certain other membrane - active agents likely to be present in

inflammation and to increase cell damage .

Ginsburg I, Ward, PA , Varani J. Lysophosphatides enhance superoxide response in stimulated human neutrophils. Inflammation 1989:13,163-174.

This is an additional example of synergism which might occur in vivo.

The 2 publications described above may shed more light how LTA from bacteria can cause cell and tissue damage due to their ability to bind to the membranes of host cells and that antibodies developed against these microorganisms may not always be protective but can also act to destroy host cells . This is one of the paradoxes of biology.

Finally, our studies on **LTA** stimulated me in 2002, to review the world literature on **LTA** and its possible role in inflammation and infection. The paper appeared in the prestigious journal **The Lancet Infectious Diseases** under the title :

I.Ginsburg. Role of lipoteichoic acid in infection and inflammation. The Lancet Infect Diseases 2002:2, 171.

As of today, this review had been cited in the world literature **344** times !! Today, **LTA** is a big and cenral player in the understanding how leukocytes recognize bacteria and their products. Any one interested can find a lot of literature on Toll Like receptors a subject into which I cannot go into. This ended our studies on **LTA.**

The Padma -28 Project

Today there is a strong discussion / debate among scientists whether the use of herbals (**plants**) which contain a large mixture, sometime hundreds, of biochemicals and used in traditional medicines, is safe and also effective. The criticism why not to use mixtures of undefined

chemicals may be justified since no sufficient serious research had been done to assure their safety. However, we cannot ignore hundreds of years of the use of Traditional plants to treat cardiovascular, liver, skin disorders and many additional diseases. Many modern medications are today based on plant origins.

A large body of evidence is available today on the Tibetan Medicine herbal preparation, PADMA - 28, used for centuries to successfully treat diseases characterized by heat, meaning infections and inflammation.

In 1998 I was introduced to Tibetan plants research by Dr. Serra Sallon, a pediatrician in Hadassah and an expert on Medicinal plant therapy. She made contacts with PADMA Inc. a firm in Switzerland (Director Herbert Scwable) which for years, had been selling a preparation named **Padma -28** which according to Tibetan Medicine, is highly effective to treat cardiovascular disorders (diseases). I was excited by the idea suggested by the Tibetans Medicine and philosophy that only a mixture of agents, but not **single medicines** !!!, might be effective in vivo. This was also in line with our thoughts on " multidrug strategies" and the synergism concept we proposed later on in 1999 .

I Ginsburg. Multi drug strategies are necessary to inhibit the synergistic tissue damage and organ failure in post infectious sequelae mechanisms causing . Inflammopharmacology 1999,7: 207-217.

Isaac Ginsburg, Peter A Ward, James Varani. Can we learn from the pathogenetic strategies of group A hemolytic streptococci how tissues are injured and organs fail in post-infectious sequelae FEMS Immunology and Medical Microbiology 1999,25:325 - 338

Our first publication using Padma- 28 was in collaboration with Ann Arbor people .

Isaac Ginsburg, Milu Sadovnik, Sarah Sallon , Milo-Sadovnik, Goldzweig, Raphael Mechoulam, Aviva Breuer, Douglas Gibbs, James Varani, Stanley Roberts, Edward Cleator, Neirmal Singh. PADMA-28, a traditional Tibetan herbal preparation inhibits the respiratory burst in human neutrophils, the killing of epithelial cells by mixtures of oxidants and pro-inflammatory agonists and peroxidation of lipids of lipids Inflasmmpharmacology 1999 :7,47-62.

Here I must thank Kim Raisford the Editor – in - chieif of Inflammopharmacology for seeing the importance of this agent and additional papers and accepted for publication **6** of our contributions in his Journal Inflammopharmacology (see also below)

Our above mentioned study supported the suggested that Padma 28 may affect especially inflammation and also blood vessles. This publication was cited **51** times, and led to a series of additional papers using Padma 28 mainly as 1) anti oxidants, 2) inhibitor of growth factors, 3) in type 1 diabetic using NOD mice which naturally and spontaneously develop type 1 diabetes a disease similar to the human disease and 4) on wound healing in rats treated by cortisone which made them diabetic.

Healing effect of Padma 28 on skin wounds (collaboration with Jim varani (Michigan).

Padma -28 was found effective to heal skin wounds in rats by stimulating the migration of fibroblasts (**cells which form the**

connective tissue) and also to increase collagen which is an important compnent of the connective tissue formation due to the depression enzymes which destroyed collagen .

Aslam MN, Warner RL, Bhagavathula N, Ginsburg I, Varani J. Ginsburg, A multi-component herbal preparation (PADMA 28) improves structure/function of corticosteroid-treated skin, leading to improved wound healing of subsequently induced abrasion wounds in rats. J Investiative Dermatlogy, 2010;302,669-77.

An additional paper showed that Padma -28 could affect important growth factors related to many human disorders including cancer.

Navab R, Aingorn H, Fallavollita L, Sallon S, Mechoulam R, Ginsburg I, Vlodavsky I, Brodt P. PADMA-28, a traditional Tibetan herbal preparation, blocks cellular responses to bFGF and IGF-I. Inflammopharmacology. 2004;12, 373-89.

In the following publication we showed that Padma -28 also acted like **retionic acid,** used in **cosmetics** to correct facial wrinkles, but without causing any serious skin damage.

PADMA 28: a multi-component herbal preparation with retinoid-like dermal activity but without epidermal effects. J Invest Dermatol. 2005;124,524-529.

Warner RL, Bhagavathula N, Nerusu K, Hanosh A, McClintock SD, Naik MK, Johnson KJ, Ginsburg I, Varani J.MDI 301, a nonirritating retinoid, improves abrasion

***wound healing in damaged/atrophic skin. Wound Repair Regen. 2008:16,117-24*.**

In Collaboration with Hadassah workers, we have also reviewed the literature dealing with the effect of Padma 28 on inflammatory **cytokines**. Cytokines are small protein molecules (a **kind of hormones**) that are secreted by inflammatory cells and instruct and regulate the activities of many other cells telling them how to behave and where to go - intercellular communication

Barak V, Kalickman I, Halperin T, Birkenfeld S, Ginsburg I PADMA-28, a Tibetan herbal preparation is an inhibitor of inflammatory cytokine production. Eur Cytokine Netw. 2004 Jul-Sep;15(3):203-9.

Padma 28 may also affect beta amyloid deposition in brain cells in culture and how it might be related to Alzheimer's disease. In this disease an insoluble protein beta amyloid is deposited in brain cells which affect their electrical properties .

Isaac Ginsburg; Lea Rozenstein-Tsalkovich; Erez Koren; Hanna Rosenmann. The herbal preparation Padma® 28 protects against neurotoxicity in PC12 cells. Phytotherapy Research 2011:,25, 740 -743

Padma - 28 as an inhibitor of type 1 diabetes in NOD mice.

Type 1 diabetes is an **auto immune** disease affecting young children which results from the attack by T- lymphocytes on **insulin** - producing Langerhans cells in the pancreas. This results in an increase

in glucose in plasma and in the urine. Patients suffering of type 1 diabetes should receive insulin for life. We used a strain of female mice named NOD, which spontaneously develop diabetes at a very early stage of life. The disease in mice is nearly **identical** with the human disorder and studying these mice is easy to perform, but a bit expensive since every mouse may cost $70. Since this disease is also characterized by oxidative stress (over production of reactive oxygen compounds), it was reasonable to assume that **Padma 28**, a potent anti oxidant agent might be effective to suppress the disease.

For this project, we have recruited: Prof. Itamar Raz, the director of diabetes research at Hadassah, Dr. Lola Weiss an immunologist who then worked with Shimon Slavin (head of Tissue Tansplantation Department), Vivian Barak the head of Tumor Markers Laboratory, department of Oncology, Hadassh, Prof. Nurit Kaiser from the Department of Endocrinology (**hormones**). We started injecting extracts of either under the skin or intra peritoneally and also fed the mice with padma 28, by mouth, via a canulla (**a tube**) directly into the stomach. We then measured the amounts of glucose in urine every week and analyzed the pancreases of the dead animals by histology (looking at the tissues sections under the microscope) and also measured the amounts of insulin remaining in the pancreas. The results were clear. After 24 weeks, either all 8 mice in the control group (which received only injections of normal saline **(may me lach physio lo gim**), or fed Padma 28 by mouth, died, their pancreases did not show any traces of insulin and their Langerhans cells in the pancreases were heavily invaded by " killing T cell lymphocytes". On the other hand**,** all 8 mice which had either been injected with Padma -28 under the skin

or intra peritoneally survived**,** their urines did not contain any glucose and their pancreases contained normal amounts of insulin . However , most importantly, the T- lymphocytes which are responsible for destroying the insulin – producing cells, did not invade the insulin - forming cells.!!! . This was probably due to the ability of Padma 28 tio increase the levels of IL-10, a type 2 cytokine (see below) . Howevwer, the failure to prevent diabetes by feeding Padma 28 by mouth, might be explained on the basis (**see our studies on polyphenols below (2008 – 2014**) that even if animals ate huge amounts of plants and fruit rich in anti oxidant polyphenols, the amounts reaching blood is very very low which cannot serve as an anti diabetes agent. However, giving Padma 28 into the skin or intra peritoneally circumvented the digestive track to allow the poylphenols to reach plasma in higher concentrations and very fast. In our study on diabetes, we also found a marked change in the immunological responses and the most important finding was that the amounts of IL - 10 (an anti inflammatory hormone), in the animals which had survived, were very high, indicating that Padma - treatment induced a typical anti inflammatory response which suppressed the toxic activity of auto - immune T- lymphocytes. Our study is very interesting and it would be important to study if Padma 28 might also be beneficial to prevent type 1 diabetes in young children .

Lola Weiss, Vivian Barak, Itamar Raz, Nurit Kaiser4 Reuven Or, Shimon Slavin, Isaac Ginsburg, Herbal flavonoids inhibit the development of autoimmune diabetes in NOD mice: proposed mechanisms of action in the example of PADMA 28 Alternative Medicine Studies 2010; volume 1:1-6

Since I do not believe anybody will ever read this paper, I made it a habbirt to send this paper to key investifators hoping that if they see the paper on the computer they **may read it.**

An additional paper on Padma 28 was published in Inflammopharmacology. It analyzed several parametrs of infllammatory agents under the effe of the Tibetan mixture

See - ***Isaac Ginsburg; M Sadovnik; S Sallon; I Milo-Goldzweig; Urij Rampampam; Aviva Breuer; D Gibbs; James Varani; S Roberts; E Cleator; N Singh.PADMA-28, a traditional tibetan herbal preparation inhibits the respiratory burst in human neutrophils, the killing of epithelial cells by mixtures of oxidants and pro-inflammatory agonists and peroxidation of lipids. Inflammopharmacology 1999:7,47-62.***

The Interational Congress of Tibetan Medicine

In 2003, I went to Washigton D.C to participate in the second International Congress on Tibetan medicine meeting Tibetan monks (**neziirim**) specialist in Tibetan Medicine describing the use of herabal (**Tzi mchi**) mixtures to treat various clinical disorders. The story I am going to tell you is unvelievable. The hall in the hotel had small cubicles where a Tibetan Doctor sat in front of a small table. The only instument he had was a aglass and a glass rod. For this occasion, several pateints from the NIH -(National Institutes of Health) hospital were invited. The Tibetan Doctor had never seen the patients before and knew nothing about his clinical condition. The Doctors were asked to diagnose these patients. What I saw was not real. The patient sat

down and the Doctor tuched his pulse and started to count the beats**.** He said to us that a Tibetan specialists can differenat least 32 different pulses !! and make a diagnosis not only of heart conditions. After seveal tense minutes, he smiled, asked for the urine sample brought by the patient and started to rapidly mix it with the galss rod until a foam was formed. He looked at the foam and said: based on the heart beats and looking at the urine, I am sure the suffers of a severe liver disease!! The scilence arroud us was so great that I could here my own heart beats. The NIH Doctor who accompanied the patient was stunned and asked (via an interpreter): how is it possible ?, you have never seen this patient before and how do you do it ?. The Tibetan Doctor said to him: this is the result of years of practice, listening to hundreds of pulses and looking at hundres of urine samples. Natural Medicne had been arround for hudreds of years. Later on this morning, we learnt that nearly 90 % !! of the NIH patinets who participated in the meeting received a correct diagnosis of their disorder. This teaches us that Traditinal Medicine can still do wonders. This is part of the ongoing "battle" between conventional amd nonconventional medicine.

Effect of Padma 28 on neurtoxicity in PC 12 cells (A model for Alzheimer's disease)

The study investigated the protective effect of Padma 28 and of certain polyphenols on the neurotoxicity to PC12 cells induced by several neuro toxins including n the protein amyloid-beta (Aβ), known to be involved in, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD), respectively. The decrease in cell viability induced the toxins was significantly

attenuated by **Padma 28** treatment. Also, a decrease in the oxidative capacity of PC12 cells treated with Padma 28 was noted, indicating that the decrease in cell viability induced by the toxins might have been the result of an oxidative stress which could be attenuated by Padma 28 acting as a potent antioxidant. **Padma 28**, which is available in Europe and USA, seems to be a promising candidate for the treatment of CNS diseases.

Ginsburg I, Rozenstein-Tsalkovich L, Koren E, Rosenmann H. The herbal preparation Padma® 28 protects against neurotoxicity in PC12 cells. Phytother Res. 2011 May;25(5):740-3.

The Role of Indian plant mixtures as anti cirrhosis (Sha che met) agents

In an additional paper, we described the properties (**tchu not**) of another traditional preparation, the **Indian** herbal mixture **Trifala .**This is a mixture only of 3 traditional plants used in India for hundred of years to treat **liver** diseases. In this case, our study described the inhibition by Triphala of the development of cirrhosis in the liver. This is a serious disorder caused by hepatitis C virus and is very common in chronic alcoholics. Liver inflammation and changes in metabolism induced by alcohol is followed by the formation of "fibrosis (**fibrosis comes from the word fibers** – **si vim),** a acondition where normal liver cells are replaced by a fibrous tissue which is ieventually fatal or necessitates a liver transplant**.** This disease kills millions mainly in Russia. We undertook this project in collaboration of **Rifaat Safadi** from the Liver Department in Hadassah. In this model, we

injected a water- extract of Trifala to rats which had been pre - treated by a chemical substance (carbon tetrachloride) which induced **fibrosis**. We found that we could totally prevent this disorder by injecting the plant mixture under the skin and also offered the immunological mechanisms by which triphala acted to prevent fibrosis.

Ginsburg I, Koren E, Horani A, Mahamid M, Doron S, Muhanna N, Amer J, Safadi R. Amelioration of hepatic fibrosis via Padma Hepaten is associated with altered natural killer T lymphocytes. Clin Exp Immunol. 2009: 157,155-64

An additional recent study showed that Triphala extracts could also prevent asthma in a mouse model

Amjad Horani, David Shoseyov, Isaac Grinsburg, Rufayda Mruwat Sarit Doron and Rifaat Safadi
Triphala (PADMA) is associated with alleviation of bronchial hyper-reactivity in a murine model mimicking asthma Ther Adv Respir Dis. 2012 :6.199-210.

Travel to Portugal and Poland to meet Padma representatives (ne zi gim).

Padma company in zurich, Switzerland, is an old institution selling padma 28 product to many countries. This is how they make good money. They have representatives in various countries. According to the Tibetan formula which had been in use in Tibet for hundreds of, only mixtures of several agents might be efficient to treat many kinds of disorders. One of the ways to spread the sails Padma 28 is to invite experts to present scientific information how

and why to use Padma 28 in various diseases. I was "sent" by Padma Co to Portugal and later on to Poland to " push" sales. Lets first go to our trip to Portugal. Together with Ruth, we flew to Lisbon, the capital of Portugal, visited the beautiful city and listened in the town square to beautiful folk songs from Portugal and South America. Then, a representative of a company working with Padma, picked us at the hotel and took us to the lecture room. It was the yearly scientific meeting of internalists (I was supposed to explain how and why Padma may be good for you. The lecture (in English) was scheduled to begin at 1600. We arrived on time, met the representatives and also a translator. Time was advancing, and only very a few people showed up. We waited and waited and finally, around 1730 !!! the lecture room was full. When I later asked my guide why was it so late ? He smiled and said:" Don't you know" This is **Portuguese time** !!! We, need a long **siesta** (afternoon sleep). This is indeed a very sleepy country, no rush anywhere. The lecture was well accepted and there were many questions asked about the efficacy (of Padma in heart diseases, which I tried to answer to the best of my knowledge. I hope my lecture helped the sails of Padma 28 in Portugal. We were lucky in our trip since a year previously, Portugal hosted an international event and for this occasion, built new highways. However looking back, this might have been the beginning of the economic crisis we see today in this country. From Portugal we went north to **Sant Diego de Compostella in north Spain,** a sacared city for Christians where they believe Jesus's brother Jacob, was finally brought from Jerusalem and buried in Spain.

Padma 28, Lecture in Los Angles (LA) - late summer (2002)

Just before Ruth became sick and later on passed away, (December 1, 2004), we went together on a long trip to the west Coast of USA. After covering hundred of miles in the north part of the West Coast, we finally arrived in Los Angeles and settled down in a nice hotel on 14,000 Wilshire Boulevard only 25 Km long !!. The purpose of our visit was to deliver a lecture on **Padma - 28** and on the Hebrew University Hadassah Medical Center, to a group of Hadassah women in LA and to try to raise some funds. Before leaving, I was informed that the Hadassah women chapter was particularly rich and very pro Israeli and a supporter of Hadassah. On the eve of the lecture, I spoke by phone with a girl who offered to pick us up from the hotel. She sounded not a real American but it was not important until later on that evening. At 1700 a nice tall girl with a brown complexion and a peculiar English accent came with a huge 4x4 car, greeted us and we could hear that she had not been born in the USA. After a short drive into Beverly Hills, probably the richest suburb in America (unbelievably beautiful but "filthy trich" - not nice to say), we arrived at a real " castle " you often see only in movies. We were introduced to the women of the house who apologized that she still had a lot to do in the kitchen. We went into a huge room covered with beautiful modern and classical pictures, very rich carpets, beautiful lamps and a huge oval table filled with every type of fruits and cakes you could think about. During our stay in the room, many women carrying pots and pans crossed the room on their way the kitchen. In the mean time, several

young man brought plastic chairs (more then 100) and filled a wing of the room, preparing for the lecture. We still had no idea who these people were but listening to voices from the kitchen, we picked up a foreign language. Still, a mystery, the young girl who brought us in, introduced herself as a member of Hadassah women (**Neshot Hadsaah)** representing an **Iranian** chapter of old and newcomers from Iran. This explained that at least my host, managed to get out of Iran with all their possessions. Today, LA has hundreds of thousands Iranians many of whom are well to do. This chapter is comprised mainly of University academicians, doctors, dentists, lawyers, merchants, etc. People started coming in and the big hall was packed. I was very trierd fafter the long car drive (probably 300 miles that day) and hoped I would not fall a sleep. Finally, all were siting down and I introduced myself as a former Dean of Dentistry and a Professor of Microbiology at Hebrew University and in very simple words, explained what I was doing with Tibetan plants and why performing research on mixtures of traditional plants, may explain why using only a single medicine at a time, might not be fully effective and that, therefore, you probably need **combination therapies**. The second half of my lecture was devoted to Hadasah and what was going on in the hospital, and also spoke about the Dental School. Finally I thanked them for helping Hadassah. I was then greeted by a young man who introduced himself as a surgeon in one of the big hospitals in LA and very proudly stated that his chapter donates an annual sum of **$ 25,000** !!!! to Hadassah . At that moment, I thought I wanted to burry myself deep in the ground to realize that such a rich but

stingy community is so proud of themselves. After the lecture, I was surrounded by several young people who asked a lot of questions about Padma- 28 and took my phone umber and address and promised to be in touch (**nobody ever called me**). The next event that evening, took place outside in the huge garden all lighted by beautiful lamps and the food was fantastic. After several hours, mixing with the people and listening how they managed to get away from Iran, about Israel, Hadassah and the University, it was time to say thanks and good bye and went back to our hotel in the same huge car. This ended our last trip together and later on, Ruth fell ill, from which she had never recovered.

ANN ARBOR MICHIGAN 1986 - 1996

The synergism concept as a basis to explain how cells and tissues are destroyed in infectious and inflammatory conditions.

In previous sections, we discussed the strategies by which streptococci which produce large numbers of enzymes and toxins, migrate in tissues and probably kill target cells by a collaboration (synergism) among several of the their secreted mtabolites. As mentioned above in 1994, we proposed this concept in a "theoretical " paper which raised many questions regarding other sytems which supports the old saying: " Two are also better then one .T his is the hebrew biblical saying

משלי "בַּעֲמָלָם, טוֹב שָׂכָר לָהֶם-יֵשׁ אֲשֶׁר: הָאֶחָד-מִן, הַשְׁנַיִם טוֹבִים"

Isaac Ginsburg. Can hemolytic streptococci be considered "forefathers" of modern phagocytes? Both cell

types freely migrate in tissues and destroy host cells by a "synergistic cross-talk" among their secreted agonists. Comparative biochemistry and physiology. Part C, Pharmacology, toxicology & endocrinology 1994;109,147-158.

Ginsburg Kohen R. Cell damage in inflammatory and infectious sites might involve a coordinated "cross-talk" among oxidants, microbial hemolysins and ampiphiles, cationic proteins, phospholipases, fatty acids, proteinases and cytokines invited review in Free Radial Research. Free Radic Res. 1995:22,489-517.

Isaac Ginsburg, Milu Sadovnic / Gamma globulin, Evan's blue, aprotinin A PLA2 inhibitor, tetracycline and antioxidants protect epithelial cells against damage induced by synergism among streptococcal hemolysins, oxidants and proteinases: relation to the prevention of post-streptococcal sequelae and septic shock. FEMS Immunology and Medical Microbiology 22 (1998) 247-256

The years **1986 – 1996** left a greate mark on our research work and understanding of inflammation. In **1986**, a sabbatical leave approached and I had to find a good place where a whole year with no administration and committees, would be dedicated to work and to learn new things. Since my work was mainly on inflammation and infection, I remembered talking during a scientific symposium with a certain professor of pathology named Peter Ward, the head of the Pathology Department, University of Michigan. I wrote to him and Looking at my C.V, he was happy to invite me as a visiting Professor starting September 1986.

Ann Arbor a real academic paradise - is a small town 75,000 inhabitants + 42,000 students !!! all around the University campus. It has a wonderful Medical School, a school of Music and 2 concet halls.

The town is all green and the Huron River passes at the edge of the city and is always full of gesse. These gave up migration because they are alwats fed corn by animal lovers and this, against the local law.

During our stay in Ann Arbor I developed a special close friendship with Jim varani with whom I worked side by side on many subjects (see llist of publications). He is an outstanding scientist from whom I learnt a lot, a wonderful human being with whom I also had many non – scientific fruitful discusions, mainly about the Middle East and the Palestinian issues. His generous hospitality all these years made our stay very pleasent. Today, I am in contact with him by phone and we still exchange publications, views and ideas on inflammation and wound healing.

During the years 1986 – 1996 !! Ruth and myself had visited Michigan again and again for a total of 4.5 years, where interesting studies had been performed also with Douglas Gibbs and with the blessings and a generous financial support by Peter Ward, the head of the Departmert, an a world expert on inflammation, with whom I am also still in touch by phone.

In Ann Arbor, we enjoyed going to the School of Music several times a week to listen to students presntations and to guest musicians. However, Ruth, did not enjoy organ recitals performed by the most famous world players. She always said that organ music irritated her and was not pleasent. Ruth had a wonderful time, shopping, reading a lot and we used to either swim every evening or also walk - 2 -3 miles along the Huron river.

Upon arrival in the end of summer of 1986, one morning, a young man, Douglas Gibbs, came in and introduced himself as a biology

teacher who wanted to dip his fingers in research during the summer holidays. Peter Ward, suggested that I take Douglas under my wings. Duglas was a very bright young man, very enthusiastic and a wizard in computers and in general technology, and could fix anything !!. By the end of summer, Douglas accepted my advise and instead of returning to school as a Biololgy teacher, agreed to start a Ph.D path under the leadership of Peter Ward. Douglas was a very good listener and I helped him with tissue cultures, inflammation , leukocytes and oxygen radicals.

I am happy that during these long periods we had manged to publish the following papers:

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J. S. Warren, P. A. Ward, K. J. Johnson, and I. Ginsburg. Modulation of acute immune complex-mediated tissue injury by thepresence of polyionic substances. Am J Pathol. 1987 :128, 67–77.

Ginsburg I, Fligiel S E G , Kunkel Riser B L* Varani J*. Phagocytosis of Candida albicans enhances malignant bBehavior of murine tumor Cells Science 1987,238,1537

J. Varani, I. Ginsburg, L. Schuger, D. F. Gibbs, J. Bromberg, K. J. Johnson, U. S. Ryan, and P. A. Ward Endothelial Cell Killing by Neutrophils : Synergistic Interaction of Oxygen Products and Proteases. American Journal of Pathology, 1987:135, 435-438

J. S. Warren, P. A. Ward, K. J. Johnson, and I. Ginsburg Modulation of acute immune complex-mediated tissue injury by the presence of polyionic substaces. Am J Pathol. 1987 : 128, 67–77.

Shapiro DN, Varani J, Ginsburg I D. Activation of a murine T-cell hybridoma by cationized bacteria Immunology 1989: 67, 478-483

Ginsburg I, Gibbs DF, Varani J. Interaction of mammalian cells with polymorphonuclear leukocytes: relative sensitivity to monolayer disruption and killing. 1989 Inflammation.1989;13(5):529-42.

Varani J, Ginsburg I, Schuger L, Gibbs DF, Bromberg J, Johnson KJ, Ryan US, Ward PA. Ginsburg I, Endothelial cell killing by neutrophils. Synergistic interaction of oxygen products and proteases. Am J Pathol. 1989;135,435-438.

Schuger L, Johnson KJ, Ryan US, Ward PA, Varani J Vascular endothelial cell killing by combinations of membrane-active agents and hydrogen peroxide.. Free Radic Biol Med. 1989:7,369-76.

Ginsburg I, Gibbs DF, Schuger L, Johnson KJ, Ryan US, Ward PA, Varani J. Vascular endothelial cell killing by combinations of membrane-active agents and hydrogen peroxide. Free Radic Biol Med. 1989; 369-76.

Varani J, Ginsburg I, Gibbs DF, Mukhopadhyay PS, Sulavik C, Johnson KJ, Weinberg JM, Ryan US, Ward PA. Hydrogen peroxide-induced cell and tissue injury: protective effects of Mn2+.Inflammation. 1991 15.291-301.

J. Varani, J. Jones, M. Dame, C. Sulavik, D. F. Gibbs, and K. J. Johnson3, James Varani, Isaac Ginsburg, Lucia Schuger. Effects of all-trans retinoic acid on neutrophil-mediated endothelial cell injury in vitro and immune complex injury in rats. Am J Pathol. 1991 139: 901–909.

Ginsburg I, Misgav R, Pinson A, Varani J, Ward PA, Kohen R. Synergism among oxidants, proteinases, phospholipases, microbial hemolysins, cationic proteins, and cytokines. Inflammation. 1992;16(5):519-38.

Ginsburg I, Mitra RS, Gibbs DF, Varani J, Kohen R. Killing of endothelial cells and release of arachidonic acid. Synergistic effects among hydrogen peroxide, membrane-damaging agents, cationic substances, and proteinases and their

modulation by inhibitors. Inflammation.1993:17, 295 -319.

Ginsburg I, Misgav R, Gibbs DF, Varani J, Kohen R. Chemiluminescence in activated human neutrophils: role of buffers and scavengers. Inflammation. 1993;17,227-243.

Ginsburg I, Mitra RS, Gibbs DF, Varani J, Kohen R Killing of endothelial cells and release of arachidonic acid. Synergistic effects among hydrogen peroxide, membrane-damaging agents, cationic substances, and proteinases and their modulation by inhibitors .Inflammation. 1993 17295-319

Ginsburg I, Varani I . Interaction of viable group A stretococci and hysdrogen peoxide in killing of vasculare endothelial cells Free Radical Biology & Medicine,1993:14, 495-500

Ginsburg, I Ward P A, Varani. J. Can we learn from the pathogenetic strategies of group A hemolytic streptococci how tissues are injured and organs fail in post-infectious and inflammatory sequelae ? FEMS Immunology and Medical Microbiology 25 (1999) 325-33.

Can Phagocytosis of Candida albicans by cancer cells change their invasive (pol sha nut) properties ?

Working in Michigan, I one day asked myself: can microorganisms found in large amounts in the oral cavity and in the large intestines also interact with cancer cells and if so, can their phagocytosis change their spreading behavior in vivo ?. My idea was to use our older finding showing that various particles including microorganisms which had been coated by cationic peptides, could be phagocytosed

much faster by cells and also activated their production of oxidants.

The following 2 papers on the subject of cationic peptides and oxidants are shown :

Ginsburg I, Cationic polyelectrolytes : Anew look at their possible role as opsonins,,as stimulators of the respiratory burst in leukocytes, on bacteriolysis and as modulators of immune complex disease. Inflammation 1987: 11, 489

Ginsburg I (Invited review). Cationic polyelectrolytes: Potent opsonic agents which activate the respiratory burst in leukocytes. Free Radical Research 1989: 8, 11-26.

We decided to explore this " crazy " idea by chosing a **sarcoma** grown in tissue cultures**..** We prepared suspensions of E.coli. Streptococci, and Candida albicans - a yeast causing serious oral infections, skin and vaginal disords . its appearance in the oral cavity in large numbers is common in cancer patients and in people in stress. We mixed the highly cationic Histone the protein which covers the negatively charged DNA in the nucleus with the bacteria and added them to the sarcoma cells. The next morning we found that very large numbers of Candida cells were seen **inside** the sarcoma cells indicating that they had been pahgocytosed. We then collected the sarcoma cells, and injected them into the hand pad of mice. We wanted to see whether presence of Candida inside the cancer cells might affect their growth in vivo. Also, we looked for the formation of metastasis in the lungs. It usually takes about 1 month for the cancer cells to grow in the hand pad to a size of 1 cm and by that time, the cancer has already migrated to the lungs. We were very surprised to find out that while sarcoma cells which had eaten streptococci did not grow at all, animals injected with cancer cells which ate E. coli grew similarly to controls, animals injected with cancer cells which ate Candida, grew

much faster and sent metastasis to the lung much earlier. The results suggested that certain microorganisms may perhaps be able to change the kinetics of growth of cancer cells in vivo . However, looking at the hand pad, where cancer cells were packed with Candida, we were surprised to see millions of neutophiles surrounding the cancer cells. It is quite possible that these inflammatory cells helped the cancer cells to go through the blood veseles by secreting enzymes which destroyed the connective tissue and thus allowed the cancer cells to migrate and to localize in the lung, killing the animals much faster. The paper we wrote was published in the very prestigious Science Journal

Isaac Ginsburg; S E Fligiel; R G Kunkel; B L Riser; James Varani. Phagocytosis of Candida albicans enhances malignant behavior of murine tumor cells. Science 1987:238,1573-5.

However, it seems that this paper was read only by a very few people and I regret that we have not continued this project. However, the use of positive electrical charges on cells also raised a very interesting question: can bacteria coated by Histone a strong cationic protein which binds to to DNA in the cell nucleus also influence the behavior of immune cells ? This led to an interesting publication showing that cationized streptococci may affect T- cells .

Activation of T – cells by cationized streptococci.

In Michigan, I got together with the immunologhist, David Shapiro, to examine the possibility that streptococci coated by nuclear histone, a highly positively charged protein, might activate T- lymphocytes, which are involved in cellular immunity and in the destruction of insulin - producing cells in the pancreas, and auto immune diabetes model

see below our work on Padma 28 and Type I diabetes.

We found that the cationic streptococci strongly bound to the surfaces of the T – cells and stimulated them to release pro inflammatory cytokines those hormone – like agents released in inflammation which can change the inflammatory reaction. Indeed, this little trick acted successfully and it is possible that such events may also happen in vivo when dying neutrophiles release large amounts of cationic peptides and histone is released fom nulcei of dead cells. This research was published in 1989 under the title :

Shapiro DN, Varani J, Ginsburg I. Activation of a murine T-cell hybridoma by cationized bacteria. Immunology. 1989;67, 478-83

It is important to note that a recent paper claimed that histones released from injured clls are the main cause of mortality from septic shock

After I had left Ann Arbor in 1996, Douglas continued working with other membres of the Department, got his Ph.D degree, but finally decided to leave biology to devote his time to his real interest, computer sciences. He is now a senior worker in the very large computer section of the Deparment of Pathology which also includes serving clinical pathology responsible for diagnosis of disorders and also serving a large part of South Michigan.

All in all the fruitful years, 1986 – 1996 yielded no less than **16** publications which focused mainly to explain the role of synergy among a multiplicity of pro - inflammatory agents (oxidants, proteolytic enzymes, phopspholipases, **(enzymes which destroy the membranes**) cationic peptides, bacterial toxins, cytokines, etc)

as possible main causes of tissue damage in inflammation and infection. Therefore, I later on suggested that it is reasonable that only " cocktails" of inhibitors may be able to better prevent the toxic effects induced in infection and inflammation caused by a cross talk among a multiplicity of pro- inflammatory agents. The work in Michigan was actually directly related to my work with Ron Kohen in Jerusalem.

My close collaboration with Jim varani even after I had left Ann Arbor ,resulted in several key publications

In 1993, still in Ann Arbor and in collaboration with Jim Varani and with Ron Kohen we investigated which main agents are involved in the generation of oxidants by activated neutrophils. Using the luminol – dependent chemiluminescence technique we identified the need for a neutrophile enztme (NADPH oxidase), the enzyme myelo peroxidase hydrogen peroxide generated folloing phagocytosis and traces of divalent metals fresent in the buffers used. This led to a publication in 1993 which for the first time had identified several of the players invoved in the generation of light by activated neutrophiles .

Ginsburg I, Misgav R, Gibbs DF, Varani J, Kohen R. Chemiluminescence in activated human neutrophils: role of buffers and scavengers Inflammation. 1993;17,227-243.

Anti oxidants suppress the toxic effects of alcohol on stomach

In 1995, and in between our travels to Ann Arbor, I got together with

Moshe Ligumsky, a gastro enterologist in Hadassah (unfortunately passed away several years ago), to invesigate how can you attenuate the injury induced by putting alcohol into the stomach of rats ? Suspecting that alcohol might also function to generate oxidants we have chosen a cocktail comprised of manganese, glycine (an amino acid) and beta caroten - the pink color of carrots as a possible inhibitors of alcohol toxicity to the tomach. We also tested this cocktail using cells in culture. The cocktail proved an excellent inhibitor of cell damage.

Ligumsky M, Sestieri M, Okon E , Ginsburg I. Antioxiodants inhibit ethanol, - induced gastgric injury in trhge rat . Scand J gastroenterology. 1995:30,854-860

To sum up, our research seems to have opened key questions regarding the concept of synergism. If cell killing in inflammation is a result of a "cross talk " among leukocytes and their pro - inflammatory agents, then, it is so reasonable to assume that multi drug strategies, might be needed do a better job at least to slow down organ failure . Only time will say whether this approach can be maetrialized (le hi yot me **yu se met) .** However, in the mean time

I approched this concept at least theoredtically 1999, by writing a "provocative " paper to summarize the idea of synergism as a major phenomenon in cell damage during infections and inflammation. It was poublished under the title **:**

I **Ginsburg. Multi drug strategies are necessary to inhibit the synergistic tissue damage and organ failure in post infectious sequelae mechanisms causing causing tissue damage and organ failure in post infectious sequelae.**
Inflammopharmacology 1999,7: 207-217

This paper is a challenge for investigators since all 150 clinical trials of

septic shock**,** resulting from severe microbial infections which had been conducted with an expense (of billions of dollars, failed to save lives since they had all used only **a single angtagonist , at a time, but not cocktails.** This is of course my **own idea** and I am fully aware of the difficulties of conducting clinical experiments using cocktails since the FDA - Food and Drug Administraion, puts strict regulations over combination therapies.

After leaving Ann Arbor in 1996, I continued my contacts with Jim Varani and after Ruth had passed away in 2004, I had retuned to Ann Arbor for 1 month every year until 2011, **Role of lipoteichoic acid in infection and inflammation.** to continue working with Jim Varani mostly on **Padma 28** effect on wound healing in diabetic rats. We also studied the effect retinoic acid (used in cosmetics of facial (wrinkles**)** and on on leukocyte toxicity to cells.

Aslam, Muhammad Nadeem; Fligiel, Helene; Lateef, Humaira; Fisher, Gary J.; Ginsburg, Isaac; Varani, James. PADMA 28: A Multi-Component Herbal Preparation with Retinoid-Like Dermal Activity but Without Epidermal Effects. The Journal of Investigative Dermatology 124:2005. 524-529

Muhammad Nadeem Aslam; Roscoe L Warner; PP 93-100Narasimharao Bhagavathula; Isaac Ginsburg; James Varani. A multi-component herbal preparation (PADMA 28) improves structure/function of corticosteroid-treated skin, leading to improved wound healing of subsequently induced abrasion wounds in rats. Archives of of Dermatological Rresearch. 2010;302, 669-677

The Polyphenols project (2008 – 2013) PP 93-100

An interesting new turn in our research had started in 2008 when I got together with Ron Kohen from the School of Pharmacy (see the **20** papers we published together) to instruct Erez Koren, a pharnacist, who had just started his Ph.D thesis. Erez was an M.Sc student with Kohen working on oxidants,. Our planned study was to examine the role of polyphenols, a group biochemicals found in large amounts in colored vegetables fruits and beverages (red wine, coffee, tea, Cacao, pomegranate, cranberry juices and colored vegetables such as tomatoes apples and carrots). These chemicals are considered very potent anti - oxidants and also bactericidal agents. Eating very day large amounts of colored fruits and vegetables and drinking red wine regularly, is the main part of the Mediterranean diet (shown to protect against the development of atherosclerosis. **The French Paradox** tells you that you should always consume fatty foods together with plyphenols such as present inlarge amounts in red wines. Something in wine prevented the fatty acids from reaching the blood where they could oxidize LDL, the good cholesterol. This field of research is toady very hot and slowly but surely, people have started to better understand the importance of eating less fatty meat and fatty cheeses, the main cause of the high mortality due to myocardial infarction which is also affects general health and the economy. This research involved analyzing either the effect of purified polyphenols (which we buy from companies) or simply prepare water and solvent extracts from plants.

Erez showed wonderful technical skills and diligence was an excellent judge of results and their meaning, a good writer and had a lot imagination and creativity, for we were blessed. We developed

a wonderful teacher student friendships, and are happy that our research came up with exciting new discoveries. All in all, we published **about 15** papers, in reputed journals, many of them as part of Erez's Ph.D thesis. One large review on polyphenols from 2011 was invited by the Editor of Archives of Biochemistry and Biopohysics.

Erez koren my last Ph.D student and a close collaborated in many recent poblicatios

Erez was also a partner in our review on cationic peptides (see above).

When we moved to work on polyphenols as anti oxidants, we new that oxidants generated in vivo due to metabolism have dual

functions. While small amounts are absolutely necessary for normal metabolic functions, higher amounts, are also highly toxic to cells and tissue. It is believed today that hundreds of disorders in humans are caused by oxidative stresses which affect many metabolic pathways**.** Therefore, understanding how anti oxidant polyphenols function might help to better control human diseases. However, it is also well known that people who eat fatty foods but drink red wine, have very low levels of heart diseases and this is known as the French Paradox. You see this phenomenon in Greece, South Italy and in Spain, all living along the shores of the Mediterranean sea. This may be the key to understand how a correct diet, rich in vegetable and fruits may protect our hearts. One of the problems with polyphenols is that although you can daily consume huge amounts of fruits and vegetables rich in polyphenols still, the amounts of polyphenols which reach blood which may be effective to protect against oxidation of the bad cholesterol **(LDL)**, are extremely low which cannot explain how plants rich in polyphenols are able protect our hearts. We shall deal with this problem also later on where we **proposed** that the protective effects of polyphenols are not only in blood but actually starts in saliva and as shown by **Gorelik et al 2008** but later on mainly in the stomach.

Erez first showed that the total amounts of anti oxidants such as vitamin C, and E, both known anti oxidants, which can be absorbed by cells in culture are strongly regulated. Thus, if you use large amounts of one vitamin, the amounts of another anti oxidants in the cell, goes down but high concentrations of anti oxidants such as

vitamin E increased all cause mortfality. Our study on the subject using tissue cultures was published in 2008 .

Koren E, Zverev I, Ginsburg I, Kohen R Supplementation with antioxidants fails to increase the total antioxidant capacity of several cell lines in culture. Biomed Pharmacother. 2008;62,179-88

To measure anti oxidants polyphebnols and vitamins, we used a new assay developed in 2004.

Ginsburg I ,Sadovnic M, Oron M Kohen R Novel chemiluminescence-inducing cocktails, part II: Measurement of the anti-oxidant capacity of vitamins, thiols, body fluids, alcoholic beverages *and edible oils Inflammopharmacology. 2004:12, 305–320*

In this simple to perform test, we use a **Luminometer** which measures visible light generated by a special cocktail of chemicals which yields H2O2 and hydroxyl radical. In this method, many anti oxidants, including polyphenols from foods, can lower or even totally depress the light wave created, pointing to their oxidant scavenging abilities. This simple method is very sensitive and can measure extremely low concentrations of vitamins C and E, anti oxidants in plasma, in whole blood, bacteria, saliva and in food products. This enabled us to measure anti oxidant levels not only of single agent but also by combinations among 4 - 6 materials.

We also made an additional step and developed a novel method to quantify anti oxidants.

Erez Koren, Ron Kohen, Ginsburg I. A Cobalt-Based Tetrazolium Salts Reduction Test To Assay Polyphenols. J Agric Food Chem. 2009:57,7644 - 7650.

Erez also helped a pediatrician from Bikur Cholim Hospital to finalize an interesting paper on anti oxidant levels in glycogen storage disease.

Koren E, Lipkin J, Klar A, Hershkovitz E, Ginsburg I, Kohen R. Total oxidant-scavenging capacities of plasma from glycogen storage disease type Ia patients as measured by cyclic voltammetry, FRAP and luminescence techniques. J Inherit Metab Dis. 2009 Oct;32(,651-659.

How anti oxidant polyphenols are thought to act in vivo ?

Now, it is time to ask the most important question: how do polyphenols from foods actually work to prevent **atherosclerosis**?? and how work by Shlomit Gorelik, Joseph Kanner, and Ron Kohen had advanced our understanding of this field of research.

In 2008, Ron Kohen's group made an important breaking observation by suggesting a new hypotheses that polyphenols **do not** actually function in blood but mainly in the stomach which explains why drinking red wine rich in polyphenols togheter with fatty foods could prevent later on the oxidation of LDL in plasma. They argued that soon after eating fatty meat, one can find in the stomach oxidized fatty acids which, which if not neutralized, will eventually reach plasma to oxidize LDL (the good cholesterol). However, drinking red wine rich in anti oxidants polyphenols while eating red meat or probably also fat cheeses, can destroy the oxidized lipids and thus prevent them from reaching blood. The study to prove this point involved 30 students. 10 ate 200 gr of fat meat, 10 had meat mixed with red wine and 10 had meat cooked with red wine. The results were clear cut.

While those students who ate meat, had high levels oxidized fatty acids in blood, thoes who ate meat and red wine (mixed or cooked with), had practically no oxidized fatty acids in their plasma. These findings suggested that polyphenols from wine acted in the stomach and not in blood **!!!.** This actually revolutionized our understanding of the French Paradox and why wine can prevent heart diseases if consumed together with red meat. The same phenomenon was recently found by a student working also on coffee polyphenols.

Shlomit Gorelik, Moshe Ligumsky, Ron Kohen and Joseph Kanner. A novel function of red wine polyphenols in humans: prevention of absorption of cytotoxic lipid peroxidation products The FASEB Journal. 2008:22,41-46.

A little later, we came up with a series of experiments which added new observations and understanding of the role of saliva and its proteins as major solubilizers of polyphenols making them more available as anti oxidants . **Therefore, protection against atherosclerosis may starts already in the oral cavity (see below)**

Taken together, it is highly recommended that every consumption of food rich in fats should be accompanied by drinking either tea, coffee or additional drnks rich in polyphenols .

Polyphenols bind to cells.

As work continued, we also found that when food extracts and beverages such as red wine, pomegranate, tea, cocoa etc, all rich in anti polyphenols came in contact either with bacteria from the oral cavity or from the intestines and also with blood cells. These are

always found in the mouth due to tooth brushing, use of tooth picks, during orthodontic treatments, in trauma, or in inflammation, the anti oxidants polypohenols can strongly bind to their surfaces which might better protect them against damage caused by oxidants generated especially in inflammatory conditions (**see below studies on saliva**) These results were published in 2009 and 2010 .

Erez KOREN, RON KOHEN, HAIM OVADIA, AND ISAAC GINSBURG Bacteria Coated by Polyphenols Acquire Potent Oxidant-Scavenging Capacities. Experimental Biology and Medicine 2009: 234 940–951.

Erez Koren, Ron Kohen and Isaac Ginsburgץ. Polyphenols enhance total oxidant-scavenging capacities of human blood by binding to red blood cells Experimental Biology and Medicine. 2010: 235, 689–699

Can you measure oxidant scavenging abilities – OSA in whole blood ?

Work with polyphenos went on very nicely. We knew already that these compounds can strongly bind to red blood cells and to bacteria and to increase their OSA mainly due to the enzyme catalase, which rapidly breaks down H2O2 to water and oxygen. We also found that very small numbers of red cells can protect other cell types against killing by H2O2. Then, one bright morning we had an "illumination". which we wondered why it had not been observed before, either by us or also by others. We questioned : what is the real OSA in blood as routinely measured in various clinical disorders ?. Is it due to anti oxidants in plasma, in red blood cells, platelets, lymphocytes or to combinations among all agents. Screening the world literature had shown that **practically all t**he measurements of anti oxidants levels

in various diseases reported, were exclusivelyin **plasma**, known to be rich in the anti oxidants, uric acid**,** vitamins C and E and Bilirubin**.** We wondered: **why are we throwing out the red blood cells and use only plasma ?.** Using our sensitive luminescence tests adapted to be used for whole blood (see above), **we found that red blood cells and platelets are actually responsible for 90 % of the OSA in whole blood !!!.** It seems, therefore, that practically **all** the publications in the literature which had measured anti oxidants in plasma alone, might be **worthless.** This observation was a real **revolution** and we thought it might definitely cause a great shock among investigators who had based all their obsevarions using only plasma. We were also afraid that no Journal would accept such a **provocative** paper which would definitely hurt many investigators. Finally, it was a **" bright**" idea to send a short Letter to the Editor describing our observations to the most prestigious medical Journal, the **New England Journal of Medicine**. We waited 2 weeks and then the fantastic news came that the Journal accepted our Letter and only corrected the style to match the Journal. It appeared as:

Isaac Ginsburg , Kohen R and Koren E. Quantifying Oxidant-Scavenging Ability of Blood. New England Journal of Medicine 2011 364: March 3 .

Our observations are important and I am sure that from now on, people who will read our Letter, will use our luminescence test and change their evaluations of the anti oxidant abilities by **using whole blood.**

In 2010 and with 3 papers with novel observations and a letter to the Editor, I went to Duesseldorf in Germany to deliver a lecture at the university. My host, Helmut Sies, probably one of the most important

scientists in the field of polyphenols, liked my lecture and as I was ready to leave, Sies suggested that, acting as Editor of the Journal, Archives of Biochemistry and Bioiophysics, he invites us to write a review article on polyphenols also describing our contributions to this field of research. Writing a critical review is quite a difficult task and it took several months to complete the manuscript. Finally it was published under the title.

Isaac Ginsburg Ron Kohen′ Erez Koren. Microbial and host cells acquire enhanced oxidant-scavenging abilities by binding polyphenols. Archives of Biochemistry and Biophysics 2011: 506, 12-22

This paper was written during phone consultations with Erez who was in Boston as a post – doctoral fellow. It involved tens of hours of phone discussion until we finally completed the task. We hope somebody will read this review. It takes some time for people to cite papers after they appear in journals .

The results which had shown that bacteria coated by polyohenols acquired a much higher anti oxidative properties, led to an interesting project testing how tea polyphenols could prevent inflammation in the urinary bladder. This was performed with Dr.Shilo Rosenberg , a urologist.

The role of tea polyphenols as inhibitors of inflammation in the bladder

Infections of the bladder (UTI – Urinary tract infections) which is very often caused by E.Coli strains ,is a very common disease which affects large numbers of people, especilly women. The bacteria get into the bladder from the outside, adhere to the walls of the bladder

and induce a very irritating inflammation. Treatment of bladder infections by antibiotics is often unsuccessful causing a lot of discomfort. About 2 years ago I was approached by Shilo who worked with Prof. Dov Pode the head of the Urology Department in Hadassah. Shilo suggested to perfom his Doctoate thesis in my laboratory. The idea was to inject live cultures of E.coli directly into the bladder of female rats using a canula and if inflammation is induced, to try whether water extracts of green tea known as potent anti oxidnants and also bactericidal, might prevent the infection. Shilo is a very bright and diligent learnt all about polyphenols and how to quantify them and also solved the techniques necessary to perform the experiments with rats. He has recently summarized his results in a paper in which he suggested that the polyphenols in green tea acted not by killing E.coli but by preventing their adherence to the surface epithelium of the bladder. This is the first time that tea was used as a " medicine" in the bladder and hopefully this simple inexpensive technique may be used paricialrly in women who suffer more than man of bladder infections

Rosenberg S1, Horowitz R, Coppenhagen-Glazer S, Pizov G, Elia A, Gofrit ON, Ginsburg I, Pode D.
Intravesical administration of green tea extract attenuates the inflammatory response of bacterial cystitis - a rat model. BJU Int. 2013 Oct 31.:

Erez had left me for Bston in 2011 and I searched for new ideas how to continue working with polyophenols. The idea that metabolism of foods actually starts in the in the oral cavity was obvious but no studies had been published describing what really happens when polyphenols from foods interact with saliva. This issue will be analyzed in the following section.

The saliva story.

It is ususally accepted that metabolism starts in the stomach soon after food intake. However, it is also so obvious that metabolism may actually start already in the oral cavity and that saliva (**rok**) plays an important role in chewing and softening (of food and in the breakdown of carbohydrates (**pach may mot -by the enzyme beta-amylase**).

Whole saliva is a very dilute fluid, composed of more than 99% water containing complex mix of fluids from major and minor salivary glands and from gingival crevicular fluid, It also contains oral bacteria, food debris and aslo blood cells. The average daily flow of whole saliva varies in health between 1.0 and 1.5 L. Saliva is composed of a variety of salts, over one thousand different proteins, immuno- globulins,

hormones, nucleic acids, digestive enzymes such as alpha amylase and the nitrogenous products, urea and ammonia. Saliva is particularly involved in lubrication, buffering action, maintenance of tooth integrity, physico- chemical defense, antimicrobial defense and wound healing, taste and early digestion. It is also important in biofilm formation on tooth surfaces, in bacterial adhesion may also assist as an important source for genetic and forensic profiles and to maintain mucosal integrity of the oral and upper gastro intestinal mucosal surfaces. Among the huge numbers of proteins in saliva, mucin at approximately 350 ug/mL, albumin at approximately 150 ug/mL and additional proteins play important roles in food softening

It is important to note that polyphenols present in large amounts in colored vegetables and fruits are agents usually not fully soluble in water, a fact which lowers their anti oxidant activities. However, we discovered that they can be quickly solubilized either by small amounts of alcohol (found in wines) or, mainly by mucin, a protein which is responsible for the viscosity of saliva (see paper below). This assumption was supported by our recent findings published and was an interesting **new** observation which sheds light on how polyphenols might be beneficial.

However, in the same publication, we also described a very important phenomenon which may have important implications to instruct people how to handle food and how fast you should swallow your food. Since we have already mentioned that both bacteria and red blood cells can strongly bind polyphenols to their surfaces which significantly increased their anti oxidant abilities, we asked a simple question: can polyphenols from food **also** bind to cells in the oral cavity such as on tongue, roof of the mouth, gingiva, teeth **.** If so, can bound

polyphenols act as depots which are only slowly released thus serving as anti oxidants for a long time capable of destroying excessive amounts of oxidants generated by bacteria and during inflammation ?. This idea was explored by performing a simple experiment. You simply hold in the mouth for 10 seconds small amouts of wine, coffee, tea, cacao, cinnamon etc. all rich in polyphenols, then you swallow them. Then, every 15 minutes you take small amount of saliva and measure its anti oxidant abilities using several methods. We found that high levels of polyphenols remained in the mouth for hours after drinking these beverges indicating that the polyphenols in these drinks were strongly bound to oral surfaces and could not easily be removed by flowing saliva .

This idea was also **original** and I am happy that it was recognized by a good journal.**:**

Ginsburg I, Kohen R, Koren E. Saliva
A Solubilizer of Lipophilic Polyphenols . Oral Diseases 2013 on line

We continued publishing on saliva :

Saliva increases the availability of lipophilic polyphenols as antioxidants and enhances their retention in the oral cavity

Isaac Ginsburg[a] Erez Koren Miri Shalish [b], Joseph Kanner [c], Ron Kohen [d] Archives of oral biology 57 (2012) 1327–1334

After publishing this paper, we decided to further spread our ideas on saliva and polypphenols and submitted a paper to the Editor to Oral Diseases, a key Journal. It was accepted immediately and I am happy that now, more people will learn about saliva and its role in human health and disease. Hopefully, somebody will read our papers !!! ???

In January 2013, I have not given up saliva research which now moved to the clinical side. We have recently established a clinical trial performed in the Orthodontic Department. A young Greek dentist joined the Department to engage in an International program i in which after 3 years he will become a specialist in orthodontic medicine. His M.Sc thesis will be performed partly in my laboratory. He will analyze the levels of anti oxidant levels in saliva of children undergoing orthodontic procedures known to be under mechanical and psychological stresses and in addition, they also always have small amounts of blood in their saliva due to trauma. Saliva was now taken from young children and frozen for future analysis,

Additional projects with colleagues :

A) The Effect of visible light on cytokines generation

Osnat Feuerstein; Isaac Ginsburg, Erez Koren, Ervin I Weiss, Rawi Assad, Yael Houri-Haddad. **Visible light promotes interleukin-10 secretion by sublethal .E. Garfield, "Bibliographic negligence: A serious transgression," The Scientist, 5[23]:14, Nov. 25, 1991.** ***ƒ fluences. Photomedicine and laser surgery 2011;29(9):627-33.***

In this study, we found that exposure to blue light at fluences of 27-108 J/cm caused a decrease in splenocyte viability lower fluences increased the secretion of cytokine IL-10, an a nti inflammatory agent, which was abolished by ROS (reactive oxygen species) scavengers. Exposure to light had no effect on the secretion of the pro inflammatory cytokines TNFα and IFNγ. Following exposure to light, more ROS were detected and the temperature measured did not

exceed 30.7°C. we concluded that blue light had a stimulatory effect on cell secretion of IL-10, mediated by ROS. Therefore, an increase in IL-10 might be a potential method for modulating the inflammatory processes of local disorders, such as periodontitis and arthritis.

B) Malaria and polyphenolls

Malaria is a serious disease prevalent in Africa and Asia. It is caused by a parasite which is transmitted by a female mosquito. Upon a sting, the parasite present in the saliva of the mosquito, immediately invades red blood cells where they multiply and finally the cell explodes releasing the parasite and so, it goes on and on. This causes the typical fever seen in Malaria. Certain strains can also cause a serious damage to the brain by clogging blood vessels. It is called Cerebral Malaria. The mortality from malaria is still very high and every year about 2 million people die of this disease. It might be important to find substances which can block the penetration of the parasite into red blood cells. In a search for such substances, we got together with Dr. Ariel Shabtai from Machon Vulcani, Neve Yaar, in the north part of the country, where he tries to increase the anti oxidant ability of milk from cows by feeding them with (pomegranate) known to contain high amounts of anti oxidant polyphenols. We are now in the middle of a M.SC thesis by Narkiss, using extracts from leaves of trees like **Elat ha mastic**, **Alon** all very rich in polyphenols. To affect the penetration of the malarial parasite to RBC . Her additional instructor is Ron Dizkovzky, a very bright investigator and an expert on the malarial parasite and Ariel Shabtay from Neve Yaar in the Galil. Preliminary results hinted that coating RBC by polyphenols from these leaves, may lower the penetration (of the parasite. This work is now going on .

C) Studies on Mycoplasma in collaboration with Shlomo Rotem (School of Medicine)

Yehonatan is a Ph.D student with Shlomo Rotem at the Medical School. Shlomo devoted most of his research to Mycolpasma a microorganisms without a cell wall which can cause severe lung diseases (pneumonia). This microorganism contains anti oxidants which can be measured by our luminescence method. The project led to paper sent for publication **(2013)**

The oxidant scavenging capacity of the oral Mycoplasma salivarium.
Kornspan JD, Ginsburg I, Rottem S.
Arch Oral Biol. 2013 Oct;58(10):1378-84

The reducing antioxidant capacity of Mycoplasma fermentans.
Yavlovich A, Kohen R, Ginsburg I, Rottem S.
FEMS Microbiol Lett. 2006 Jun;259(2):195-200.
PMID:

Intracellular location and survival of Mycoplasma penetrans within HeLa cells.
Tarshis M, Yavlovich A, Katzenell A, Ginsburg I, Rottem S.
Curr Microbiol. 2004 Aug;49(2):136-40.
PMID:

D) Studies on Listeria In collaboration with Dr. Ran Nir Paz Hadassah Hospital

Since word was spread around that we have a very good luminescence test to measure anti oxidants, several groups at the Medical Center contacted me asking for help. So I found myself also working on Listeria monocytgenes, a nasty microorganisms which infects foods, causes a lot of trouble in the intestines and is difficult to get rid off.

Every now and then you read in the newspapers that certain food products had been removed from the shelves because they were contaminated by Listeria. My contact was with Efrat an M.D who performed her Doctoral thesis on the subject with Dr. Ran Nir Paz a microbiologist in Hadssha Hospital. It dealt with the quantifications of anti oxidants in virulent and non – irulent strains. We got good results and hopefully they will be soon published.

E) Cationization of enzymes (collaboration with Ron Kohen)

Bacteria coated by positively charged peptides were eaten up much faster not only by phagocytic cells but also by regular cells. This suggested that if we change the charge on enzymes they might stick to target cells and function more efficiently as protectors against oxidants . Indeed it happened and we and Ron Kohen, published a apapers on this subject.

Gibbs D, Varani J, Ginsburg I
Formation and use of poly-L-histidine-catalase complexes: protection of cells from hydrogen peroxide-mediated injury. Inflammation. 1989 Aug;13(4):465-74.

F) A novel mechanism of cell damage, with Shaul Yedgar .

Many mechanisms have been proposed to explain how cells and tissues are injured either by bacterial agents or during inflammation. These include: oxidants, toxins which injure cell membranes, agents which induce **apoptosis,** a suicidal proces in which intracellular enzymes attack the nucleus etc. etc. In collaboration with Shaul Yedgar from the Medical School a new idea came up. We asked: is it

possible that oxidants such as H2O2 which are generated in inflammation may peal off substances from the cell surface to expose lipids in the membrane which then lead to damage and cell death. This again is a synergistic mechanism in which each factor alone is not toxic but if combined has potent cell - damaging effects A . This lead to 2 publications

Yedgar S, Dan P, Dagan A, Ginsburg I, Lossos IS, Breuer R. Control of inflammatory processes by cell-impermeable inhibitors of phospholipase A2. Agents Actions Suppl. 1995;46:77-84.

Dan P, Nitzan DW, Dagan A, Ginsburg I, Yedgar S. H2O2 renders cells accessible to lysis by exogenous phospholipase A2: a novel mechanism for cell damage in inflammatory processes. FEBS Lett. 1996: 25;38 375-378

EDUCATIONAL TRAVELS ABROAD

A Travel to Ethiopia

During my work I was exposed to sad realities of poverty and struggle for existence. I was part of teams of teachers sent by our University on 2 missions **1)**: to help teach Medical students in Ethiopoia and **2**) to start a new Medical School in the east part of Turkey (the city of Diarbakier - all Kurdish population).

The Ethiopia project was very interesting because Ethiopian students also spent some time in Jerusalem. Our mission was to deliver a course in Medical Microbiology including teaching of laboratory methods to isolate and characterize diseases caused by bacteria. We spent a whole month teaching in English with good results. However, on the way down to Adis Abebba, the Capital, we flew together with a young women, deputy of the Minister of Health of Ethipoia **.** She came to Jerusalem to visit her family living in Rechov Ha Chabashim. The family ran away from Ethiopia during the Italian occupation. With us,

was Dr.T Dishon, who in the past was in close touch with the Ministery of Health managed to get an agreement by the Minister to send as a gift, 30 million units of vaccination against 2 important diseases, Dyptheria which killed many young children in Ethiopian and tetatus which is very common in agricultural areas. A few days later she came back with a fallen face informing us that Haile Salassie, the Emperor of Ethiopia, could not accept the generous gift because **if the young people will not die of the diseases, they might die of hunger !!!.** This was a terrible and cruel reality that I shall never forget. Upon completing our teaching we went home with mixed feeling facing tragedies.

Travel To East Turkey

Some time in the seventies, Israel was asked by the WHO -Word Health Organization in Geneva to help the Turkish government to start a Medical School in Diarbakier, a large city in the East part of Turkey near the Syrian border, Population 700,000 kurds with only a very few Medical Doctors. Unfortunately, it was reported that child mortality in East Turkey approached 30 % which is a terrible number considering Turkey as a developed country. 4 of us flew to Ankara and the first visit was with the Dean of the Medical Faculty, a graduate (of an American University. He told us all about the plan to start a Medical School and that teachers from Ankara will fly to Diarbekier for short periods and that our task will be to select 30 students to start the projet. Before leaving his office I managed to look at his collection of Medical books. I was shocked to learn that all the books were dated back to 1950 !!!. It seems that a there was no money available to buy new books. I was supposed to give a lecture on Rheumatic Fever and streptococcal infections to more than one hundred students and

doctors. A young doctor was asigned to bean interpreter a who will stop me every now and then, to explain in Turkish what I had said. You can imagine that the lecture lasted more than 2 hours. I have no idea what he had told the audience. The tragic thing was that in Turkey in the 70ties thousands cases of rheumatic fever were diagnosed every year. This was because they did not have enough penicillin, the best and only effective drug to prevent rheumatic fever. This was really a terrible reality. I am sure that now things had changed for the better.

Diarbekier a city surrounded by a heavy granit wall (look at Google) was very poor (mostly Kurdish people) and the sewage was flowing along the streets. However, the bazar was very exciting and you could buy very nice silver ornaments which I brought for Ruth as presents. We started to interview young boys and girls after high school. I simply donot remember anything what we did after working hours. Unfortunately, I cannot get more information about our stay in Diarbakir since 2 of the participants, Benyamin Shapiro and Rami Rachamimov had passed away and Arnon Gunders had emigrated to South Africa.

I was so pleased to learn that today, there is a hospital in the city with 198 beds and many doctors. So, a good end to an interesting story. On the way back we stopped in capadocia to see the wonderful geological formation in Urgip (**look at google).a**

Travel to San Antonio, Texas

It was in one of the years we were in the USA (maybe it was in 1996) we decided to spend some time in the University of San Antonio,Texas. The Battle of the Alamo in San Antonio was a pivotal

event in the Texas Revolution. Following a 13-day siege, Mexican troops under President General Antonio López de Santa Anna launched an assault on the Alamo Mission near San Antonio de Béxar. The Alamo, originally known as Mission San Antonio de Valero, is a former Roman Catholic mission and fortress compound and was the site of the Battle of the Alamo in 1836.

I do not remember why we did choose San Antonio but, 3- 4 months during the winter were wnderul because of the nice wheather=**.** We were very happy that 2 Israelies, Ruth Borinski (my former PhD student) and Aaron Weinberg, a graduate of our Dental School, were working with Stan Holt on bacteria causing periodontal disease**.** San Antonio downtown is wonderful and the university Campus was very pleasant so was ourn stay. Ruth helped Ruth Borinski to care for her first born duaghter and I had good time in the laboratory working on my " old ideas " :How to inhibit a toxin made by the main agent involved in periodontal disease.This toxin causes hemolysis and also injures other cells. As expected the presence of certain lipids which builds cell membranes very strongly inhinited cell death caused by the toxin. We never published this work and only a few days ago, I found a whole manuscript including all the graphs which I forgot about, in one of the boxes above my desk (Ruth Borinski is now the Dierector of Kupat Cholim Laboratories in Jerusalem).

LAST SABBATICAL LEAVE IN AUSTRALIA AND NEW ZEALND

Towards the end of 1996, we planned our last sabbatical leave before retirement and decided to go far to Australia and new Zealnd for 4-5 months.

Adelaide, Australia 1996

We first went to Adelaide in Australia to spend one month in the Childrens and Womn Hospital with (**kill me if I remeberhis the name of my host !!!**) . I am also ashamed to admit that I do not remember what I had done there exept for living in a hostel and often visiting dowan town. What I do remember is a statue commemorating the Australian soldiers who died in Palestine during the first world war against the Turks. I shall do my best to remember the name of my host.

Christchurch New Zealand 1996

From Adelaide, we flew over to Christchurch in the southern island, a vrey quiet flat and clene city. I joined Christine Winerbourne, an old aquaintant from International Symposia we both attended in the past. She is a wold expert on oxidants of white blood cells and this was an ideal place to spend seweral months. We were housed in a Univeristy apartmement with TV where we could get the BBC in London to learn abot what is happenmg in the real world. N. Z is really at the end of the world and you feal unsafe realizing that the next station is the south pole !!!. It always rains (7 meters a year) especially in the morning and then suddenly strong winds disperse the clouds and the bright sun appears which dryes the rain in no time. I had to take a local RED bus to go the the University, a 15 minride . Everyone was very polite and friendly and I immediately felt at home. The Department was small and I enjoyed participating in their seminars. Since I had come only for a short time there was no reason to start a new project so, I listened to the problems posed by the Department members. I was surprised to see how they function. Money for research was very scarce so every experiment they planned had to

conside rthe smallest details. I do no think I learnt anything new during the 3 months we spent there but havig no obligations and since I paid for the trip and for our expenses from my Shabaton, we could travel around the country to see its wonderful scenerios (There are more sheep in NZ than people and we learnt a lot about farming, the wool industries and the about **zimmers** which at that time were not very developed in Israel and the way they treated guests**.** We had also the chance to meet the chairmnan of the Jewish community (only 10 families) an enginneer who ran a firm of inventors of all kind of new technologies which is so typical for a small but not a rich country. We can learn a lot how they handle their economy. We travelled a lot, saw the gazers in the north island and went south to see wonderful sea lions lying on the beaches and vsited wonderful rock formations. I am still in touch with Christine by e- mail and was sad to learn that her laboratory was badly hit by the severe eartquake several years ago. Taken together, our stay in NZ was not really professional but an opportunity to see this beautiful and friendly country. I still read with interest the very good work on leukoytes comming out of her Department.

2013 – 2015 new project on chlorhexidine and fermented papaya

A) **Effect of Microbicides (an enzyme which destroyes H2O2 on bacetria**)

Since the polypohenols project was nearly over, I went back to " classical" Medical Microbiology and became interested in the way pathogenic microorganisms protect themselves against the host.

One important enzyme produced by bacteria and by the host is **catalase,** which very rapidly decomposes H2O2, a known disinfectant

used by all of us to sterilize skin wounds. It is unfortunate that, since the development of new antibiotics is a slow and a very expensive project, many clinicians treat oral diseases induced by microorganisms and by fungi such as Candida albicans by the old microbicide chlorhexidine. Our current work was initiated due to an observation that catalase - rich Candida albicans has the potential to decompose H2O2 and to inhibit oxygen radicals, generated by a luminol - depended luminiscens assay Catalase was even coined a "virulence factor" becasuse of its ability to destroy microbicidal oxygen species and thus able to defend other cells. We have recently observed a paradoxical phenomenon whereby washed suspensions of Candida albicans which failed to significantly consume H2O2 could nevertheless do so if the cationic chlorhexidine (CHX) at 100 uM was added to the reaction mixtures.This phenomenon may be due to the ability of CHX a cationic agent to increase the permeability of Candida cell walls to allow penetration of H2O2 and its subsequent decomposition by intracellular catalase . However, the ability of Candida and CHX to act in this manner depended on the medium on which candida had been grown were grown. Thus, while Candida grown on surfaces of agar plates containing brain heart infusion, tryptic soy, Muller Hinton or blood readily allowed CHX to initiate the consumption of peroxide, cells grown on 7 additional media used to cultivate fungal cells, failed to do so and were therefore coined " resistant". It is important to note that non of candida cells grown on these 7 media possessed any active catalase activities. Howevwer, when Candida grown on these 7 media were transferred back to BHI medium, CHX regained its enhancing effects suggesting that we deal here with a phenptypic phenomenon. Chlorhexidine markedly potentiates the oxidants

scavenging abilities of Candida albicans

I. Ginsburg1 • E. Koren1 • O. Feuerstein2 • I. P. Zogakis3 • M. Shalish3 • S. Gorelik1 Chlorhexidine markedly potentiates the oxidants scavenging abilities of Candida albicans

Inflammophartmacology 2015 ;23;221-281

The abiliy of effect of CHX on catalase – rich Candida albicans may be important since by consuming peroxide both catalase negative anaerobes and facultative anerobes such as PG Fuso and carigenic streptococci , Current experiments are now in progress to further study this important phenomenon .

PAPAYA AND ANTI OXIDANTS

Phytother Res. 2015 May 31.
The Antioxidant Effect of Fermented Papaya Preparation in the Oral Cavity.
Fibach E, Ginsburg I.

Oxidative stress has been recognized to play important roles in various diseases, including of the oral cavity. However, nutritional supplementation of antioxidants to ameliorate the consequences of oxidative stress is debatable. One caveat is that oxidative status is often measured under non-physiological conditions. Here, we investigated the antioxidant potential of fermented papaya preparation (FPP), a product of yeast fermentation of Carica papaya Linn, under conditions that prevail in the oral cavity. Employing highly sensitive luminol-dependent chemiluminescence assays, we show that its antioxidant capacity was augmented by saliva (up to 20-fold, $p < 0.0001$, at 10 mg) and its components (mucin, albumin) as well as by red blood cells (RBC) and microorganisms present in the normal and pathological environment of the oral cavity. Polyphenols are major plant antioxidants. Using the Folin-Ciocalteu's assay, a very low amount of

phenols was measured in FPP suspended in a salt solution. However, its suspension in saliva, albumin, mucin or RBC produced up to sixfold increase, $p < 0.001$, compared with the sum of polyphenols assayed separately. The results suggested that these enhancing effects were due to the solubilization of antioxidant polyphenols in FPP by saliva proteins and the binding to RBC and microorganisms, thus increasing their availability and activity.

The synergism concept of cell amage in inflammation infection and in post infectious dsordes such as sepsis and septic shock.

Histone sepsis re discovery of the wheel and ethics

See pages pp 119-125

As discussed above, sepsis is a a condition which developed following the pnetration of microorganisms into the blood stream. This starts a very complicate series of reactios among microorganims blood vessels endothelial cells which cover the blood vessels, leukocytes addhiring to the blood vessels and the immune response of the host. If not treated early enough by antbiotics, 40 % of patents will die within 28 due to organ failure. In 2009, 2 publications had suggested that the main cause of death in sepsis is due to the release from live and dead white blood cells of a mixture of DNA and histone the highly based protein which forms complexesx with negatively charged DNA. Histone causes severe activation of the hosts immune system . However the 2 authors forgot to tell the reader that toxicity of histone to human endothelial lcells had already been shown by us in 1952 and later on, during the years 1987-1996 (see pages 8,32-33,85-87). However,none of these pioneering publications had been cited, which

is an un ethical act .

See Isaac Ginsburg, The disregard syndrome : is it a menace to the future of scicene (The Scientist 2001 January) .

Since the early 1980, I became involved in a" WAR" against dishonesty in science. I am not sure thereis anything you can do about this unethical stand of researhers. However, in my article in the Scientist I ha suggested to nominate in Journals " old professors" as reviweessince they will remember the old publications on the subject.

Two "breakthrough" articles in Nature Medicine from 2009 by Xu *et al* [2] and Chaput *et al* had argued to be the first to suggest that the main cause of death in sepsis maybe the release from neutrophil extracellular traps (Netosis) of highly cationic histones possessing high toxicity to endothelial cells. This commences immunological (cytokines storms) and coagulation cascades culminating in septic shock and death. In their study, Xu *et al* [2] showed that activated protein C (APC) a protease, cleaved histones and reduced lethality. However, blockade of APC activation exacerbated sub-lethal LPS challenge into lethality, which was reversed by antibody to histone. Chaput *et al* assessed the protective effects of recombinant thrombomodulin (rTM), which was approved in Japan for the treatment of disseminated intravascular coagulation (DIC) and is currently undergoing a phase III clinical trial in the United States. Both groups of investigators had concluded and advised that extracellular histones may probably be the potential molecular targets for therapeutics for sepsis and additional post infectious inflammatory and traumatic manifestations. If substantiated, the possibility that histone neutralization by heparins but especially by the newly reported, non-anticoagulant heparin [], may be a blessed future hope for critical care patients combating post-infectious and inflammatory sequelae.

SUBMITTED FOR PUBLICATION 2016

It may interest the readers that already during the years 1951-1965, Katchalski's group at the Weizmann Institute of Science in Rehovot, Israel, had described for the first time that the histone mimics, poly L-lysine and poly L-arginine, injured blood vessels of rats, activated blood coagulation but inhibited of fibrinolysis. Also, during the years 1986-1996, investigators at the Department of Pathology, the University of Michigan,Ann Arbor, USA, at the Institute for Drug Research, School of Pharmacy and at the Institute for Dental Sciences at the Hebrew University of Jerusalem, Israel, had already described, the killing of human umbilical cord endothelial cells by hitone and by additional poly cations especially if combined synergistically with oxidants .

PMNs recruited in sepsis which are attracted to and adhere to endothelial cells, undergo activation and release not only of highly cationic histones LL37 and elastae but also a plethora of pro inflammatory agonist such as oxidants, additional PLA2 as well as of many acid hydrolases which may reasonably act synergistically to permeabilze and destroy ECs .

Therefore, the concept that histones may exclusively act as a unique virulence factors should be doubted and re considered since toxic histones may probably **never** act on their own but always in in synergy with many of the pro inflammatory agonists released by activated neutrophils.

Since 2009, a large number of publications had also claimed a possible major involvement of nuclear Histones, citrunillated histone and histone ideacytilase in the pathogenesis of cardio vascular, hepatic , pulmonary, renal disorders and also in post traumatic episodes.

If toxic histones levels in plasma are significantly elevated in so many

diverse clinical disorders , can this cationic peptide be considered a novel alarmin which if effectively neutralizes either by heparin by actvated prtein C (a proteinase) or by antibodies to histone , might be the unique miracle virulence molecule inhibitors.

Since sepsis, septic shock, disseminated intra vascular coagulopathy(DIC), adult respiratory distress syndrome ARDS AND ALI - acute lung injury (ALI) and additional post infectious and traumatic sequelae are al regarded as distinct multi factorial episodes, it clearly explains the futile attempts over so many years, to prevent mortality by the **exclusive** administration of only a single antagonist at a time . This definitely calls for the urgent development and testing of appropriate cocktails of inhibitors to replaced the ineffective single antagonists. After all, today the treatment of AIDS and tuberculosis is successfully controlled by cocktails of drugs .

The following are several view points by experts on sepsis regarding the possible pathogenetic role of circulating histones in sepsis.

A - One possible answer to this controversial issue was recently offered to by an Editor of a Critical Care journal who had suggested that: " The recent focus on sepsis, both in terms of redefining the disease and specialities (Histones?) and from the perspective of the Joint Commission and publically reported Hospital JAMA 2016, had recently been shifted". " However, no concrete suggestions were offered where this shift should go?. Will this finally justify an urgent necessity to search for and certify cocktails of antagonists to replace the faulty single antagonists and also to look for novel early biomarkers to facilitate diagnosis

B-. While the arguments about histones role in sepsis are cogent, this is not going to engage enough of the readership to make publication priority for our journal.. The histone problem is an interesting one, but not one that is close enough to clinical decision making to gain the attention of our primary readership.

C - Reinforced by the recent Sepsis-3 papers in JAMA 2016 we need to move away from trying to find an all encompassing definition for sepsis (and by implication an all encompassing treatment) and instead, adopt the principles of personalized medicine and try and find more homogenous subsets of patients, probably based on the causative organism, to develop more targeted treatments. However, like your arguments, I find that this idea does not land on fertile ground at present.

.D - I am not surprised that there are prior papers about histones and cytotoxicity to endothelial cells; It's effects as an anti microbial molecule have also long been known . The jury is still out on the role of histones in sepsis although Dr Esmon argued in his lectures and publication from 2009 that he has clinical data on histones major role in death in sepsis. Let's hope for the sake of all the kids that die that it will work.

Reading these arguments one may wonder whether preventing readers from reaching conclusions about histones possible role in sepsis, this information should be ignored and thus buried until further notice

Taken together, it is still an enigma and also un reasonable why the commissions reports on sepsis and sepsis prevention from 2016 JAMA 2016 and the major authority in sepsis treatment, had totally **ignored** any of the dozens of publications since 2009 which had related elevated levels of circulating histones to the pathogenesis of sepsis and in additional post infectious and traumatIc sequelae .

Why not include a small section which brings the histone story to the awareness of the readers of journals dealing with critical care,

infections, sepsis and post infectious sequelae and let the readers judge for themselves about possible futures treatment schedules.

Furthermore, it is also surprising why not a single word was said in the JAMA reports from 2016 about the possible efficacy of the anionic non- anti - coagulant heparin which can still neutralize cationic histone. This maybe a blessed new development in the struggle against the adverse effects of post-infectious and coagulation sequelae.
However, although non – anticoagulant heparins might perhaps prove promising "magic bullets", their efficacy may unfortunately still be quite limited since treatment of sepsis patients may start long after the "horses had already left the stable" and this since a positive clinical diagnosis of sepsis and septic shock may last several hours - days after admission to ICU . At that time points, heparins action might be ineffective since the deleterious effects of the immune (cytokines storm) and coagulation cascades may have already been in full swing /gear and out of control. Therefore, a search for novel very early biomarkers might greatly improve early diagnosis and proper treatment may be by combinations between antibiotics and heparin.

Another aspect missing from the reports in JAMA 2016 is a consideration and advise why not to routinely use certain highly bacteriolytic beta lactam antibiotics in sepsis treatment which might be hazardous . This is because similarly to anti microbial cationic peptides, add **CAMPS** , and histiones, bacteriolytic antibiotics, can also activate nascent endogenous microbial autolytic wall enzymes (muramidases) which can release from microorganisms the highly phlogistic components capsular polysaccharides, endotoxin (LPS), Lipoteichoic acid (LTA) and peptidoglycan.

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Sepsis: "If We Cannot beat them Alone Join Them?" Research Article 1 Int J Microbiol Infect Dis. 2018; 2(3): 1-5 Iniernational Journal of Microbiology and Omgectiouys Diseases

Conclusion

It is reasonable to postulate that since the pathogenicity of sepsis, septic shock, ARDS, DIC and ALI are all distinct multi - factorial synergistic episodes, cocktails of appropriate antagonists yet to be defined which If combined on time with antibiotics and heparin, might be future effective agents to replace the failing single antagonist which had been tried unsuccessfully for so many years.

Some of the concepts involvomg synergitc aspect of post infectoious sequelae and bacteriolysis was recently published

Synergistic Aspects and Treatment of Sepsis and Septic shock - An Opinion Erez Koren and Isaac Ginsburg
J. Infectious Diseases and therapy 2016

Ginsburg Isaac1, Erez Koren1 and Osnat Feuerstein2ss

Is Bacteriolysis In vivo a Friend or a Foe? Relation to

Sepsis, Chronic Granulomatous Inflammation and to

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16 papers on the Effecti of lleukcyte hydrolases on bacteria

Bacteriolysis is inhibited by hydrogen peroxide and by proteases.
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Effect of **leukocyte hydrolases** on **bacteria** XVI. Activation by**leukocyte** factors and cationic substances of autolytic enzymes in Staphylococcus aureus: modulation by anionic polyelectrolytes in relation to survival of **bacteria** in inflammatory exudates.
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