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# ROUGH AND ROCKY ROAD TO THE RETINOID REVOLUTION

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**Key Words** stress, cyclic AMP, tryptophan oxygenase, steroid hormones, vitamin A

■ **Abstract** Some of us who were born in the middle of Europe between World Wars I and II had to face quite a few unusual challenges that we all met in different ways. I was born and raised in Prague, Czechoslovakia, a country at the time of my birth that was governed by a Western style of democracy, which was later destroyed by the occupation by Nazi Germany and subsequently by the takeover by the equally cruel Communists. Life required special means of adaptation to the changing living conditions and a great deal of luck to survive. After graduating from the School of Technology, I started working in the Department of Medicine at Charles University in Prague as a clinical chemist in endocrinology. This work was followed with training in basic biochemistry and the study of metabolic changes in stress. This rather diversified research, due to my changing of workplaces, led to the findings that diet can change enzymatic activity of liver tryptophan oxygenase. For a short time I worked on the metabolism of cyclic AMP in *E. coli*, and at the age of 41, I made a risky move and succeeded in escaping with my family from the "paradise of communism." The reasons for this decision will become clear. After settling in the United States, I worked on the mechanism of activation of liver tryptophan oxygenase by cyclic AMP and eventually moved to the Vanderbilt University School of Medicine. There I initially worked on the mechanism of action of steroid hormones and finally on the molecular mechanism of action of retinoids, retinol, and retinoic acid. Also in cooperation with neonatologists, I initiated studies on prematurely born human neonates which led to successful supplementation of these patients with vitamin A. The work from my laboratory and my coworkers eventually became recognized.

"Who does not remember the past, is condemned to repeat it."
*George Santayana (1863–1952)*

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## PROLOGUE

To be asked to write a prefatory chapter for the *Annual Review of Nutrition* represents for me a great honor. This task has motivated me to reminisce on the past. I am very lucky for I inherited mixed genes and had the tremendous support of my immediate family to be able to accomplish in nutrition research what has brought me this honor. Looking back it is not possible to separate my education, work, and eventual accomplishments from the political turmoil in Central Europe where I was born between World Wars I and II. Postwar events eventually caused me to escape from communism. Only by being forced to leave my home country and being so generously accepted by the people of the United States via Brandeis University did I accomplish my life's goals. America gave me shelter and the long-term financial support to be able to do the research of my interest.

Since 1974 the number of published papers on retinoids has grown exponentially (29). Bollag, the father of therapeutic use of retinoids, recently proudly called this unprecedented phenomenon in this field a "revolution" (2). Deciphering how vitamin A works on the molecular level has been my interest for close to 30 years. In the United States, I have fulfilled my scientific and personal dreams, and now I can look back on my rather turbulent past in peace.

## Origin

I was born in 1924 in Prague, in the country then called Czechoslovakia, now known as the Czech Republic. I am the youngest of three siblings. My brother, ten years older than I, eventually became a nephrologist at the Charles University School of Medicine in Prague. My sister is five years older than I. My father was very successful in banking; he was eventually the president of a Czech bank and rose to this position with the equivalent of a high school diploma. Strangely enough coming from a Czech family, my father was born in Plovdiv, a town that is now in Bulgaria. My grandfather received his Doctorate of Jurisprudence in Prague, but he and his family moved to Plovdiv for his work. My grandfather was there to introduce legislation in Eastern Rumelia. At the time, this part of Europe had been liberated from Turkey. In addition, while living in Plovdiv my grandfather was a war correspondent for two Vienna newspapers during the Serbian-Turkish conflict. To me he was a man of a rather adventurous character. My grandmother, his wife,

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was Jewish. Mixed marriages at that time were very rare and the fact that my grandmother was of Jewish blood later influenced our lives. My grandmother was a very brave lady, as she had to raise her three children alone, after my grandfather died in his thirties. My father had two siblings. His older sister, Marie, was also born in Bulgaria and became the wife of Alphonse Mucha, a well-known Czech painter of the Art Nouveau period. After the family returned to Bohemia, my father had a younger brother, who in family tradition became a doctor of jurisprudence and democratic politician. My mother was the daughter of a farm manager.

## Early Development (1924–1939)

Until 1938, my life was rather quiet and could be considered middle-class in a country governed by the democratic principles of a constitution established by the president of the country, T.G. Masaryk. He happened to be married to an American lady. Because of my family's mixed blood my father fostered a very simple principle. He regularly told us to always follow the "Golden Rule" and to never generalize. In the fall of 1938, my sister left to study in the United States as an exchange student at Smith College and then Mills College. At that time I had finished grade school and began attending the gymnasium, a public school that was equivalent to high school in the United States. After my sister departed, the government of France and England struck the Munich Agreement with Hitler. According to the agreement, Czechoslovakia had to give up the Sudetenland territory. This act was followed on March 15 of 1939 by the occupation of the western part of Czechoslovakia. This occupation included Prague, where my family resided. With this act Germany established "The Protectorate of Bohemia and Moravia."

## War Years: Life Under the Nazis (1939–1945)

Soon it became evident that the Nazis intended to enforce some of their laws in the occupied territories. Particular laws pertinent to my family had to do first with the definition of who is a Jew by the so-called "Nuremberg laws," and second with who is defined as a mixed breed. According to these laws my father was a first-grade mixed breed and I was a second-grade mixed breed. The first law was enforced soon after the war broke out in 1939, and we did not know whether and when the second law would be enforced. In fact, the second law for the first-grade mixed breed was not enforced until the beginning of 1945. At that time first-grade mixed-breed men were sent to labor camps in Silesia. Luckily for us, my father was not taken. Unfortunately, some of our friends were. My father was not taken because he was able to obtain a fraudulent birth certificate, which stated that his mother was actually christened. In spite of this my father was dismissed from all his banking functions. The atmosphere in my family was that of constant apprehension.

As for me, I enjoyed my student years at the gymnasium. There I started playing basketball by joining a club in Prague named AC Sparta. I played for several years in premier league competition. In retrospect I believe that the sport induced me to have a competitive spirit that was instrumental in preparing me for the very competitive

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environment of scientific research. After graduation from the gymnasium in 1943, I had to cope with another challenge. The Czech government offered to the Nazis every Czech citizen who was born in the year of 1924 to help with the "total" victory in the war. Most of my peers were sent to work in Germany, but I was allowed to stay at home. My assignment was to work in the chemical laboratory of a factory building airplanes. My duties were to run the quantification of nickel in steel. In the laboratory I found an unused electrochemical instrument called a polarograph. We were supposed to work six 12-hour days each week. I was not very busy and, consequently, very bored, so I attempted to use this instrument.

Polarography was invented by J. Heyrovsky, professor of physical chemistry at Charles University in Prague. Professor Heyrovsky's father had been professor of Roman law at Charles University and coincidentally was a colleague of my grandfather's brother Karel Chytil. My great uncle was professor of Art History at the same institution. Already prior to the war J. Heyrovsky was a well-known researcher. Even though the Czech universities were closed by the Nazis in November of 1939, Professor Heyrovsky—because of his accomplishments and mainly because he had students and coworkers from Germany and Japan—was allowed to keep a small laboratory in one of Charles University's buildings. Since I wanted to set up an assay for the determination of copper in human serum, I needed his help. I was motivated to determine copper levels by a desire to help my brother with his studies.

Professor Heyrovsky allowed me to visit him in his laboratory. Here was my first exposure to a great scientist. He counseled me on my approach to the problem during several sessions. I am indebted to this gentleman, for he introduced me not only to the application of polarography but also gave me the following advice: (*a*) Always make sure that your experimental results are reproducible. (*b*) Make sure that you are familiar with the history of the problem. (*c*) The interpretation of results is secondary to the reproducibility of your results, for interpretation may undergo changes as the field progresses. Lastly he told me that if he were younger he would study biochemistry. In 1959 J. Heyrovsky was awarded the Nobel prize in chemistry for his invention of polarography.

I immediately started reading biochemistry from a textbook inherited from my brother and began setting up calibration curves for copper determination. Soon after I began those studies, the United States Air Force to my great joy was successful in bombing the factory. The laboratory I was working in, including "my" polarograph and all my belongings, was completely destroyed.

## Postwar Years (1945–1948)

When the war ended, the emotional balance in our family was rather unsettled. In total we lost eight blood relatives, including a five-year-old boy. Only two came back from the camps. In addition, my father's brother—a Freemason who was taken prisoner at the end of 1944—died in the "Small Fortress" situated in Bohemia, close to the town of Theresienstadt, where he was infected by typhoid fever. On the

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other hand, the defeat of Germany in 1945 and the liberation of Czechoslovakia were followed by the reopening of the universities. At that time biochemistry courses were not offered, so on the advice of my father I applied to the School of Chemical Technology in Prague in order to become a chemical engineer. This is a less well-known school than Charles University but it has produced two Nobel prize-winning alumni: Leopold Ruzicka (1939) and Vladimir Prelog (1975), both of whom received the prize in chemistry.

During my studies, my sister came back from the United States with her husband. She was full of enthusiasm to help revive the country after the damage caused by the Nazi occupation. Her husband was given a position at the ministry of foreign commerce. Again we were living in a free country. I continued to play basketball and to travel with the team abroad. I was nominated to be a member of the 1948 Czechoslovak Olympic team, but I did not get to go.

## Communistic Putsch (1948)

In February of 1948, the Communist party of Czechoslovakia, with the help of the Soviets, successfully organized a putsch and virtually took power in every aspect of our lives. The democracy and free economy of Czechoslovakia was transformed into a dictatorship. This was the year Czechoslovakia lost its sovereignty. Luckily, I was very close to the end of my studies and was allowed to graduate with a degree in analytical metallurgy because the school was willing to give me credit for my employment in the laboratory during the war, which shortened my studies. Soon after the 1948 putsch, my sister's husband was fired from his government position and put on a leave of absence as an enemy of the "people" because he spent the war in the United States. A year after this verdict, my sister and her husband with a little son were able to cross the border illegally and immigrated back to the United States.

## Life Under Communists (1948–1965)

In the fall of 1949 I married my wife, Lucie, who was a much better basketball player than I, for she had more international experience and had represented Czechoslovakia many times before the putsch. Our wedding took place while her father, a former editor-in-chief of a daily Catholic newspaper in Prague, was serving time in prison. He was sentenced to 11 years of hard labor for writing a small pamphlet about the intolerance of religion by the Communist government. In similarity to the Nazi philosophy, the Czech Communists began to punish the members of families who were in jail for political reasons or had relatives living abroad. My father-in-law served eight years of his sentence, was released because of illness, and died very soon thereafter. My wife was dismissed from the university and was sent to work in a bakery. A year later the government confiscated my parents' house. We were evicted in a matter of two weeks and moved to a one-room apartment without a bathroom. Here our first child was born.

I was very lucky because after my graduation I learned that the Department of Internal Medicine at Charles University was looking for an analytical chemist

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to supervise and perform routine blood assays. The head of the department, Josef Charvat, M.D., hired me. He is credited with starting endocrinology in Middle Europe. He was an extremely nice person, a non-Communist who had been incarcerated by the Nazis during the war. He treated me extremely well, motivating me to study endocrinology. From him I learned that every experimental finding should be corroborated in the whole animal. I worked very hard on this principle.

At the time, Charvat was very interested in the phenomenon of "stress" as defined by Hans Selye. Already it was known that adrenals are involved in this phenomenon. From Charvat I learned quite a bit about glucocorticoids. Charvat was the one who triggered in me the curiosity to understand how steroids work. I was co-author with him on several clinical papers published in Czech. At the time we were not allowed to publish outside the Communist sphere. Professor Charvat was interested in exploring the role of sulfur containing amino acids involved in stress phenomenon. He asked me to determine methionine and cysteine levels in blood. At that time the only available method of quantification of these compounds was by microbiological assays using *Lactobacilli*. The purchase of biochemicals had to be planned a full year ahead of time of use because the Communists, immediately after the putsch in 1948, established a "completely planned society." I felt that I could not wait to accomplish my assignment, so I had to walk around begging people for small amounts of pure amino acids in order to be able to set up the media. As for the necessary *Lactobacilli*, I wrote Howarde Sauberlich who was then at the University of Wisconsin in Madison. He generously provided me with the microbes, and I was able to do the determinations. This was my first exposure to practical microbiology and my first contact with the free world after 1948. I was very happy working with Charvat, but unfortunately I was supposed to be inducted into the Communist army. Since individuals of my family background were then not allowed to bear arms, I faced the danger of ending up in a special military unit that served in coal mines. My wife's brother had served for three years in a coal-mining unit. Charvat understood my situation very well. He contacted Arnost Kleinzeller, M.D., Ph.D., and asked him if he could take me as a doctoral candidate. Such a position would allow me to get a deferment from the army.

I was again lucky when I learned that Kleinzeller was willing to take me. I owe Kleinzeller many thanks since I did not then have to go into the army. He was born in Czechoslovakia and earned his M.D. just before the beginning of the war. He went to England where he received his Ph.D. with Sir Hans Adolph Krebs (20). After the war he came back to Prague and was an ardent defender of Communist ideology. When I met him, he was head of the Department of Fermentation Chemistry in the School of Chemical Technology at my alma mater. Dr. Kleinzeller was known for his aggressive Communist ideology, but he accepted me, and I learned a lot from him. When dealing with him, I often went back to my father's "do not generalize" rule. Unlike many members of the Communist party he was an honest man. Quite a few members of the Czech intelligentsia for various reasons had become members of the Communist party. Many joined the party out of opportunism, some out of a desire for power, and some out of ignorance. When

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I asked him later why he behaved the way he was known for, he replied to me, "I believed in the philosophy." I began working in the mornings in the hospital with Charvat and in the afternoons in another part of the city with Kleinzeller, who was kind enough to sponsor my thesis to receive a Doctorate of Rerum Technicarum in 1952. I worked with Charvat for more than two years and collaborated on several papers that were published in Czech.

After I obtained my doctorate, Kleinzeller offered me a position called "aspirant" and my military service was again deferred. I was enlisted in a military program similar to the ROTC program in the United States. Kleinzeller gave me the task of finding a strain of actinomycetes in which one could produce vitamin $B_{12}$ for human use. I was lucky. With the help of a technician who did the microbiological assays for the vitamin, we found a strain which produced into the medium reasonable amounts of vitamin $B_{12}$. In addition, I found that the production of this vitamin could be augmented considerably by adding cobalt chloride to the growth medium. We were making arrangements to produce larger amounts of the vitamin when the project came to an abrupt end because Kleinzeller was dismissed from his position of department head and was moved to another institution. At the end of 1952, the Communist party put on trial a group of high-ranking members and quite a few were executed for treason. Historians researching this trial agree that the majority of these people were Jews and that the result of this trial was an act of anti-Semitism (15). Kleinzeller may have been dismissed because he was Jewish. In 1968 he moved to the United States and began working at the University of Pennsylvania Medical School. I am thankful to both Charvat and Kleinzeller for their help in postponing my military service and keeping me out of the coal mines.

When Kleinzeller was dismissed, I lost my job. Again I was lucky. The Institute of Nutrition was established under the auspices of the Ministry of Health, and the Department of Metabolism was formed. Otakar Poupa, M.D., who was originally interested in endocrinology, headed the department. Through my brother I received an offer to join Poupa's small group. Poupa at the time was not a member of the Communist party, and was a physiologist and a very good artistic painter. I had not expected to be hired because he knew of my political problems. He hired several other people. One of them, Hruza, M.D., was the head of the local Communist organization and was in charge of supervising the day-to-day progress in the research unit. I was primarily collaborating with him on the metabolic consequences of the adaptation of rats to trauma. We published several papers in this area (10). While working at the Institute of Human Nutrition I did not run into any problems because of my political views, for the local Communist organization was very small and tolerant. Another member of the laboratory, Lat, was studying the effect of nutrition on behavior in rats. Our work was quite productive and Poupa was able to fix it so that I was permitted to travel to the Western world twice. I was permitted to travel in spite of my coworker Hruza regularly mentioning that my sister lived abroad. My work went rather well, and I was able to gain independence by having my own project supported by the Institute of Nutrition.

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My own interests led me to pursue the original findings of Knox, who discovered that the activity of rat liver tryptophan oxygenase can be elevated by glucocorticoids and also by injection of tryptophan. I wondered whether or not switching rats from a control diet to a high-protein diet would induce the activity of this enzyme. The results showed that this was the case (5). When the results were about to be published, Sidney Chernick, who was at the National Institutes of Health (NIH), came to Prague to attend a symposium. I discussed my recent results with him and he suggested that I should pursue further training either with Gene Knox, Alfred Harper, or Nate Kaplan. At the time our laboratory was in the process of merging with the Institute of Physiology. This was done on the initiative of Poupa, who was attempting to become a member of the Communist party, and was successful with the merger. However, the local organization of the Communist party did not recommend him for membership. He eventually moved to Sweden.

Little did I know that this merger would change my life and the life of my family forever. In order to work with Nate Kaplan at Brandeis University, even without my family, I needed the permission of the local organization of the Communist party at the Institute of Physiology of the Czechoslovak Academy of Sciences. The structure and administration of the Czechoslovak Academy was shaped after the Soviet Academy of Sciences and still exists in similar form even today, 14 years after the Communists were pushed out by the so-called velvet revolution. The infrastructure appears not to have changed from the Communist one. The Institute of Physiology, established within two years of the putsch, was directed by Dr. Servit, who was originally a practicing physician. But the actual business in the institute was supervised by the local organization of the Communist party. The local organization worked in secrecy and had final decision power in every aspect of the institution's activity. The number of Communist members in the Institute of Physiology was high in comparison to other institutes. Many were graduates from medical school but had never practiced medicine. Most of them were extremely active party members, and quite a few joined the party before or shortly after the 1948 putsch. From the outside this group of individuals behaved like a gang. The leaders of this gang were Bures and his wife, Buresova, both of whom were working on the mechanism of memory in the brain. Bures was a strong follower of the ultraleft-wing power prescribed by the party. In addition (as it was found later) he was an active agent of the secret service. Other members of the gang were Capek, Gutman, Hahn, Hruza, Hudlicka, Krecek, Kreckova, Jelinek, Martinek, Vrbova, Lodin, etc. Some of these people were involved in dismissing students from the universities soon after the Communist putsch in 1948. An outstanding exception was O. Koldovsky. He did not join the party, and we collaborated on some work on intestinal development (18). He eventually immigrated to the United States with the help of Norman Kretchmer.

I waited for the decision on whether or not I would be allowed to travel abroad to work with Nate Kaplan. After two long years I received a message from the chairman of the Communist party, Jan Bures, via his wife, that I would never get permission to travel abroad since my sister was in the United States without their

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consent, and because of my inability to travel, the institute could not continue to support my independent project. For these reasons I would be transferred to the group headed by my former coworker and comrade Hruza. Such a verdict would mean in reality that I would be demoted to a technician.

I managed to cope with this verdict only because my experience in playing basketball had accustomed me to winning sometimes and to losing sometimes. I had the future of my three children in front of me. In an act of desperation I decided to talk to Ivan Malek, M.D. He was the founder and first director of the Institute of Microbiology of the Academy. I explained my problem and asked whether he would support my transition to his institute. Such an action was very unusual under the Communists. He agreed to do it and added me to the staff of Margita Kohoutova, M.D. I knew her from my time with Kleinzeller. In contrast to Jan Bures's decision, here I was given the freedom to choose the research problem, but I had to work with bacteria and not rats. Even a young person was assigned to work with me as an "aspirant." What a difference that made in my life. But Nate Kaplan was very persistent and now bombarded Malek with requests to allow me to come and work with him. To my surprise I learned that I was given permission to leave the country, but for only six months and without my family. My wife and our three children had to remain in Prague as hostages.

I left for Brandeis in March of 1964 and worked on the question of beta-galactosidase isoforms in the small intestine (6). Nate and all the Brandeis people I met treated me extremely generously. I was fortunate enough to attend the International Biochemical Congress in New York that year. At the meeting Kleinzeller told me that the leadership of the Communists in the Institute of Physiology in Prague protested my being granted permission to visit Kaplan. I also saw my sister for the first time in 18 years. After my return to Prague I was exposed to several months of interrogation by the secret police. Approximately every two weeks, they would take me and interrogate me. In spite of such unpleasant distractions, I was able, with the help of my aspirant, to finish and publish a paper about the existence of a specific enzyme capable of splitting cyclic AMP in *E. coli*. This was the newest evidence that this bacterium is able to produce and split cyclic AMP (3).

## Escape from the Paradise of Communism (1965)

After the very traumatic experience of being interrogated by the secret police, my wife and I, having in our minds the future of our children, decided that we would try to escape from the country. We started to plan our escape with Jan Lukas, a close friend and an excellent Czech photographer, his wife Milena, and their two daughters. Because we were never allowed to travel as an entire family, even to the Soviet Union or other countries under Soviet domination, we decided to split the family into two parts. My wife gained permission to go for a short holiday with our two small daughters (9 and 7 years old) to Yugoslavia. Several months prior to that I received a document, not a passport, allowing me to participate at the Biochemical Congress of European Biochemists in Vienna. Ironically, I received

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the permission after the meeting had concluded. The document had an unused box into which I wrote the name of our eldest child, our 11-year-old son. We divided our family and my wife went by train to Yugoslavia with the girls, and I and my son left the same day by car to Vienna. A week later, with the help of our friends Jan and Milena Lukas, my wife and our two daughters crossed in the Lukas's car into Italy by illegally rushing through the barrier between Yugoslavia and Italy. We left everything which we owned behind, never recovering anything, but we knew we were finally free.

## First Years in the USA (1966–1969)

Nate Kaplan, through Lawrence Levine, an outstanding immunochemist in the Department of Biochemistry, arranged a working permit for me at Brandeis. My sister's family arranged for us to get a green card, and we began our American lives in the Boston area. Kaplan let me work in his department and in a relatively short time I received an offer to work at the Southwest Foundation for Research and Education in San Antonio, Texas. There my work was funded by a grant from the Morrison Fund and later by NIH. I explored how cyclic AMP works in the activation process of tryptophan oxygenase. I was able to show that the activation is due to the presence of xanthine oxidase in liver extracts and purines formed from cyclic AMP worked as substrates for xanthine oxidase. Thus although the original observations are reproducible, cyclic AMP is not directly involved but is a source of purines. Our original interpretation had to be corrected (7, 17). During my second year in Texas, A.R. (Tony) Means, now at Duke, came back from Australia, where he had been a postdoctoral fellow. In 1968, at the International Meeting of Endocrinology in Mexico City, he introduced me to Bert O'Malley, who at the time was at NIH and was in the process of negotiating a move to Vanderbilt Medical School.

## The First Vanderbilt Years (1969–1973)

Soon after the meeting Grant Liddle, M.D., chairman of the Department of Medicine at Vanderbilt, offered me a junior faculty position in his department. I accepted, for I always dreamed of working in a medical school again, as I had with Charvat. Dr. Liddle was a well-known endocrinologist and was a friend of Charvat in Prague. In 1969 Tony Means and I moved to Nashville, where Bert O'Malley was very successful in building a group of researchers interested in the molecular mechanism of steroid hormone action. Among the researchers were David Toft, who discovered with Jack Gorski the estrogen receptor; Thomas Spelsberg, who came from Hnilica's laboratory and investigated nuclear proteins; and Chuck Strott and Bob Northcutt, who worked as both physicians and endocrinologists. Later Jeffrey Rosen and Bill Schroeder came as postdoctoral fellows. This was a very productive group that worked together well as a unit (23) or individually (11). Although all the members were much younger than I was, I learned a great deal from them. I am especially thankful to Bert O'Malley who taught me, among other things, how to improve my grant writing, a task at which he has always been a

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master. He also helped tremendously with my verbal presentation. At the time my English was very rudimentary. My research contribution was using microcomplement fixation as an immunochemical method. We used this method for probing the structure of chromatin and the effect of estrogen on its structure, tissue specificity of nuclear proteins (11) and their immunohistochemical localization in the cell nucleus. The members of the group were very productive and became visible in endocrinology. We presented our work at the first session on hormone action at the endocrinology meeting in St. Louis in 1970 (22).

## Later Years at Vanderbilt (1974–Present)

I believe it was in 1973 when Bert O'Malley received an offer to go to Texas. At this time the situation with all members of his group at Vanderbilt was somewhat unstable. Bert accepted a chairmanship at the Department of Cell Biology at Baylor Medical School in Houston and invited most of the members of the group to join him. Quite a few moved with him, with the exception of Spelsberg, Toft, and Northcutt, who went to the Mayo Clinic. Strott went to NIH, and I was the only one who stayed behind at Vanderbilt. I transferred from Medicine to the Department of Biochemistry, where I remained until my retirement. The faculty in this department consisted of very nice and competent people who worked with a small number of students and associates on independent problems. For example, Stanley Cohen discovered and worked on epidermal growth factor (EGF). Harry Broquist worked on lysine metabolism, and John Coniglio, on lipids. Conrad Wagner still works on folic acid, and David Puett, now at the University of Georgia, on protein structure. Bob Neal studied toxicity and later Fred Guengerich, who was Broquist's student, studied cytochrome P450.

Just before I made the decision not to go to Baylor—my family and I were not prepared to make another major move—I was introduced by my first graduate student (George Catignani) to Mark Bashor, who helped me change my research interest. My new research interest has stayed with me until the present. My focus became the mechanism of action of the vitamin A retinol and its natural derivative, retinoic acid. We wondered how retinoids are involved in performing their essential function in tissues other than the eye.

The so-called nonvisual functions of vitamin A were very much neglected. It had been known since 1924 that when animals, including humans, are fed with a diet lacking retinol, severe histological changes in various organs occur reproducibly. Many organs, including the lungs, testes, etc., undergo histological changes. For instance, the epithelium of the trachea and of lungs undergoes keratinization. The pulmonary epithelium stops producing cells making mucin and surfactant and becomes full of keratin. This process is reproducible and reversible. When animals are re-fed with retinol, the morphology reverts to that of normal cells. In addition, the action of vitamin A is tissue specific, because some of the tissues in vitamin A–deficient animals undergo changes other than keratinization. The dominant theory as to how vitamin A works already established in textbooks

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on nutrition and biochemistry was that retinol in the form of retinyl phosphate works as a coenzyme for enzymes involved in glycosylation of proteins (12). This proposition did not make sense to us, because the crucial compound retinyl phosphate had never been seen, but more importantly it seemed to us that this mechanism could not be completely responsible for the action of reversibility of the vitamin. Even more so, the action of retinoic acid, the most potent form of the retinoids, could not be explained by the proposed mechanism (8). The histological changes seen in vitamin A deficiency and recovery are very much reminiscent of those observed during the estrous cycle. So our working hypothesis was that vitamin A works similarly to steroid hormones (1).

Consequently, we embarked to answer this question. Mark Bashor had joined my laboratory as a graduate student, and we asked whether a molecule exists, similar to steroid receptors, which could be detected by a labeled ligand using sucrose gradient centrifugation. The answer was yes. Mark showed that the binding component is a protein of a molecular weight of approximately 15 Kd. This protein bound retinol with high affinity and appeared not to bind retinoic acid. In addition, we demonstrated that this retinol-binding protein, cellular retinol binding protein (CRBP), is regulated by glucocorticoids (32). CRBP is now well characterized. The amino acid sequence is known, X-ray crystallography has been done, and specific antibodies are available. While Mark Bashor was characterizing the binding properties of this protein, David Ong joined my laboratory as a postdoctoral fellow. I gladly accepted him, and since 1974 we have worked together and later collaborated on this project for many years. David Ong came to the laboratory with a deep interest in protein purification. While attempting to isolate CRBP, he observed another protein eluting from the column. It was also characterized by specific binding to retinoic acid, and we called it cellular retinoic acid binding protein (CRABP) (25). This protein is now also well characterized. We know now a lot about these proteins, but their functions have not been completely delineated. It appears that they function in the esterification of retinol and intracellular formation of retinoic acid (27). Our results led to conclusions that these proteins are not receptors for retinol or retinoic acid, so we attempted to identify a receptor in nuclear extracts by labeling them with radioactive retinol and retinoic acid. However, we failed. As we suspected, we know now that chemical detection of true receptors requires much higher specific activity of radioactivity than compounds available to us at the time. However, in 1981 the receptors for retinoic acid (but not for retinol) were discovered and described in parallel publications. Interestingly enough, two Canadian postdoctoral fellows—Petkovich, who had previous experience working with retinoic acid and worked in Pierre Chambon's laboratory (28) and another post-doc working with Ron Evans (13)—discovered the receptor for retinoic acid by attempting to characterize genes for estrogen or glucocorticoid receptors, respectively. Thus these authors demonstrated that retinoic acid indeed works like steroid hormones by a mechanism involving nuclear receptors.

Another question that we wanted to answer was how expression of many genes is influenced by the action of retinol and/or retinoic acid in the whole animal.

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Labeling of testicular proteins and subsequent two-dimensional analysis of the proteins showed that the expression of many genes is induced or inhibited by retinol or retinoic acid (14). Thus it is now accepted that retinoic acid works by a mechanism similar to the action of steroid hormones and involves the cell nucleus where some of the genes are activated and at the same time repressed (24). Since I began my interest in the mechanism of action of vitamin A–like compounds, many people have joined this field and the progress in this area has become unprecedentedly great.

Some time ago we measured changes in the levels of the proteins CRBP and CRABP in developing rat lungs. We found striking differences in the levels of CRABP in the lungs postnatally. This is the time when alveolization of lungs develops (26). Moreover, it has been reported that the macroscopic picture of vitamin A–deficient lungs is much affected. I developed an interest in human lung development after I became a member of the Neonatal Pulmonary Center at Vanderbilt (director M.T. Stahlman, M.D.) soon after I joined the faculty. Originally we were measuring levels of the glucocorticoid receptor in lungs of neonates who died after being born prematurely. When we looked macroscopically at these lungs, we found a striking similarity to the lungs of rats fed a vitamin A–deficient diet. They are both hemorrhagic. When one looks at the histology of these animals, the trachea and the bronchopulmonary tree show a rather intensive keratinizing metaplasia. To us this suggested consequences of vitamin A deficiency. In collaboration with Dr. Stahlman and her coworkers, I determined that retinol blood levels from neonates who died prematurely at the Vanderbilt nursery showed lower values than control patients. This was found in 100% of the premature babies (30). The low levels of vitamin A suggested a vitamin A deficiency (9). I was able to convince the leadership of the center to reproduce these findings and subsequently to explore the vitamin A status of their prematurely born children using criteria such as measuring the level of retinol in various organs. We then started a double-blind study, giving retinol to patients and measuring their pulmonary functions and comparing the value from children maintained on control treatment. After publication of the evidence that this intervention had beneficiary effects (31), an independent, multicenter study supported by the NIH corroborated results obtained by us at Vanderbilt (33).

The supplementation of vitamin A in prematurely born babies is not common in the neonatal intensive care nurseries in the United States. This may be due to the fact that knowledge of the nonvisual functions of vitamin A has not been shared with neonatologists.

Beneficial treatment of xerophthalmia by retinol has been known for many decades, but until recently only skin diseases were treated by retinoids, and newly synthesized compounds structurally similar to and having some retinoid activity have been introduced and tested. Apparently the "revolution in the action of retinoids" on the therapeutic level has not taken place yet. One of the explanations may be taken from a review by a pioneer in the treatment of xerophthalmia, which is still in endemic proportions in the underdeveloped countries and which is caused by lack of retinol in the diet. The author recently surveyed medical textbooks to

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determine the extent of mentions of symptoms of the disease and found that very little, if any, knowledge about xerophthalmia is presented to future physicians (21). Ignorance, in my experience, about the nonvisual function of the natural retinoids among physicians is even more striking.

## EPILOGUE

"Truth even unto its innermost parts"
*(From the emblem of Brandeis University)*

Looking back on my life, I believe that I should be thankful that despite many strange circumstances I was able to accomplish something of importance. Perhaps this is why I was asked to write this chapter. I retired from Vanderbilt in the year 2000, thus serving longer than I had ever expected. I can only hope that my memory is still serving me well and that the content of this chapter is accurate. The chapter has been written in a very personal way so that young people especially can be informed about how extreme political systems can influence peoples' lives. I also hope that I still do not suffer from the selective loss of memory which is a common ailment, I believe, of those (including scientists) who joined political systems which gave them power but none of the ethical restrictions dictated by Western-type systems.

Ironically but truthfully, one of the main reasons my family and I enjoy our present freedom is due to the actions of certain members of the Communist party at the Institute of Physiology of the former Czechoslovak Academy of Sciences in Prague. Interestingly, many of those members immigrated to the West in 1968, after the country was occupied by the Soviets (16), yet they left behind a country in disarray caused by the devious and cruel Communist ideology which many of them vocally and factually supported. Their leader, Jan Bures, M.D., however, did not choose to emigrate from Czechoslovakia, and it is interesting to note that in spite of his experimental work on the mechanism of memory, his own memory does not permit him to recall the harm he did as an implicator of Communist ideology (4, 19). Perhaps my family and I should in some strange way be thankful to him. Nevertheless, I can only conclude that I have been very fortunate that I can now peacefully reminisce about the past.

Still there are many people living and dead to whom I am indebted for helping me to be able to enjoy my retirement in a free country. My immediate family deserves my thanks. My wife, Lucie, worked for me for 22 years and helped me thereby to maintain the continuity of the everyday work in the laboratory. My interaction with my students, postdoctorate fellows, and coworkers in the United States was most enjoyable. ~~In addition to those already mentioned, other people contributed to the productivity of my laboratory~~. In addition to those already mentioned, other people contributed to the productivity of my laboratory. These were graduate students G.L. Catignani, M.M. Bashor, L.M. Canfield, H. Kylberg, E. Wilson, J.Y. Orrahood, B.W. McGuire, G. Liau; D.R. Appling, K. Knutson, S.R. Porter, J.A. Crow, D.R. Sherman, D.G. Stump, and E. Munson; postdoctoral fellows D.E. Ong, S. Takase,

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T.C. Tsai, M. Omori, R. Coquoz, and R.U. Haq; and of course our collaborators M.T. Stahlman, J.P. Shenai, R.D. Zachman, M.G. Rush, and G. Fex. I hope I did not leave out anybody.

I deeply appreciate the care given to me by Herman J. Kaplan, M.D., to maintain my health during my Vanderbilt years. I wish to thank my daughter, A. Chytil; my secretary for two decades, S. Heaver; and especially my research assistant for two decades, M. Hunt, for their invaluable assistance with this manuscript.

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