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TIBOR FARKAS
1929–2003

A Biographical Memoir by

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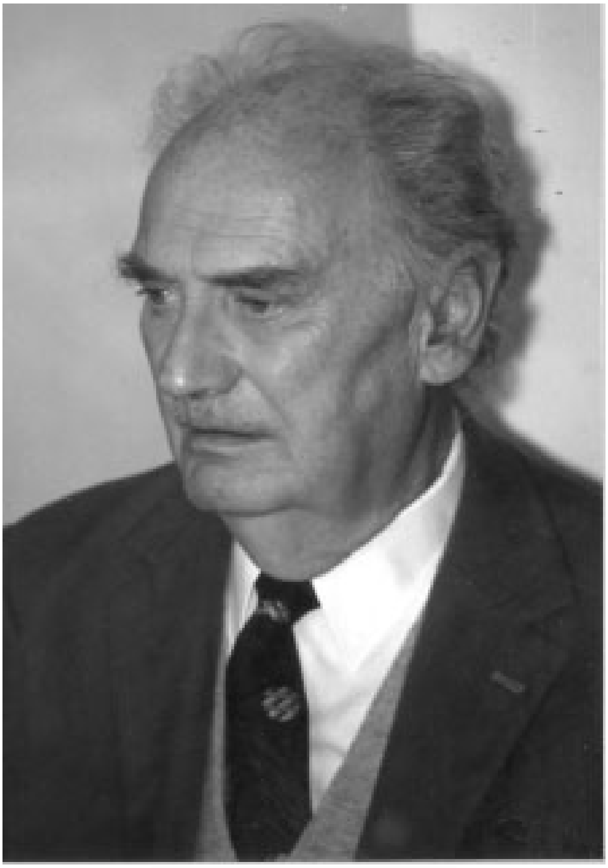
Biographical Memoirs



TIBOR FARKAS

June 8, 1929—November 15, 2003

BY ISTVAN CSENGERI AND JOHN E. HALVER

TIBOR FARKAS WAS a dedicated Hungarian scientist in lipid biochemistry and physiology who was able under political and economic constraints to maintain a productive life in science. He was born, lived and worked in eras and areas with many challenges, dangers and adventures. However, throughout his life, until his sudden death in November of 2003, he was a diligent, enthusiastic, inventive, patient, and consistent investigator able to overcome the difficulties bestowed by the social and political environment during his life of research work.

His life started at the dangerous southern borderline of Hungary in 1929. The Trianon Peace Agreement, dictated after World War 1, reduced Hungary to roughly one third of its former area, disconnecting families and relatives. This Agreement, and the subsequent political events in Hungary, caused many troubles and even tragedies in the lives of his parents, and in his own life as well. In spite of many difficulties and conflicts, Tibor finished secondary school and university studies with excellent marks.

He began his scientific career at the Institute of Biological Sciences of the Hungarian Academy of Sciences at Tihany (Hungary) in 1955. His first studies dealt with micro chemical analyses of lower aquatic crustaceans. His interest soon turned from the depot fats to fatty acid synthesis and regulation of fatty acid metabolism. Beginning in 1971, at the Biological Research Center of the Hungarian Academy of Sciences (at Szeged, Hungary) his studies focused on the involvement of fatty acids and various lipids (phosphatides, cholesterol, phospholipid molecular species, etc.) in the structure and functioning of biological membranes and in temperature adaptation of lower and higher animals (crustaceans, fish, rat, birds, etc.) and plants (algae, wheat, rye, etc.).

After understanding the key role of the polyunsaturated fatty acids (PUFAs) and phosphatidyl glycerides in the structure and functioning of biological membranes, his research was extended to human health aspects, focusing upon prevention of cardiovascular diseases by dietary omega-3 or (n-3) PUFAs, and on brain development and pathology in relation to the lipids. His scientific work was an inquisitive adventure, from the question of why the melting point of depot fats of poikilothermic crustaceans is lower than the ambient temperature (1958) to questioning "Do We Need a New Paradigm?" (2003) alternative to the fluid mosaic membrane model by Singer-Nicholson. Besides solving questions, he was involved in the simplification, modification, and elaboration of several methods in lipidology. He had worldwide cooperation and teaching activities, creating the "BRC-school" of lipid scientists. With Chinese and Russian scientists, he was among the few (some ten) Foreign Associates active in the former socialist-communist block who were elected into the National Academy of Sciences of the USA. He was elected in 1989.

EARLY LIFE AND FAMILY

Tibor Farkas was born June 8, 1929 in Budapest. His father János Farkas and his mother, Katalin Kiss, went to

Budapest for his birth because they were alarmed by the unstable regional conditions near their hometown area in southern Hungary. Katalin's family, being Hungarian, had been deported from Vajdaság (Vojvodina) after the Trianon Peace Agreement turned that prior portion of Hungary over to Yugoslavia. Subsequently there was violence between the ethnic Serbs and ethnic Hungarians in that region. In order to avoid the violence, János and Katalin moved closer to Budapest. János became a teacher at Kelebia elementary school and Tibor began and completed his primary school studies at Kelebia (Hungary). During his early school years, Tibor systematically prepared himself for becoming a medical doctor. He copied and drew anatomical pictures, prepared skeletons and skulls of small animals, and focused upon common physical features of different animal forms. In 1940, he started his secondary school studies in Kiskunhalas at the Áron Szilády Reformed College (established in 1664). During World War II, from 1941 to 1944, Tibor Farkas continued his studies at Szabadka, at the Hungarian Royal Boys' College.

After the war his school studies were endangered so he returned to Kiskunhalas as a private pupil in Szilády College. After completing secondary school studies in 1948, he applied to university, planning to be a physician, veterinarian or a forester. However, his application to the university was rejected for political reasons. He was unacceptable by the communist government because his family had owned land and also because they were Christians. In those days people who personally owned land or a business over a certain limit were considered an enemy of the State and could be "domestically deported" (imprisioned). The Communist Party also wanted to eliminate all religious beliefs, therefore Christians were considered enemies of the State.

He tried to enter the navy but his application was also refused.

Two years later, owing to a good recommendation by a friend who was a Communist Party member, and to some relaxation in the education policy in Hungary, his application to the university was accepted. He began his studies in biology and chemistry at the Attila József University at Szeged (Hungary) in 1950. After 4 semesters of study, he was selected to be part of a special university group for training research scientists at the University Loránd Eötvös at Budapest. Therefore, Tibor left the companionship of his good friends, girlfriend, and professors at Szeged, and moved to Budapest. Over the years, usually on summer holidays, these early companions maintained their friendships and met regularly.

In Budapest, the education was not as good as at Szeged, and university years were hard in other respects too. Tibor's older brother was in military service, his younger brother was in college studies, and his parents were helping to support them both. Therefore they were not able to help Tibor financially. Food shortages throughout the country were an everyday problem for the young student. He received his MSc degree in animal physiology in 1955. At that time, the graduated students were not allowed to select their work location; the government assigned them. Tibor was very fortunate, and was sent to an academic research institute at Tihany, a little village on the shore of Lake Balaton.

Before going to Tihany, Tibor started his 3-month compulsory military service. As a soldier, he had met his university schoolmate, Erzsébet Soóky (daughter of Erzsébet Lipokatity and Gábor Soóky who had a bad 'screening sheet' in the communist system because they had previously owned a restaurant). The young couple were married after Tibor finished the military service. They went to Tihany together and their daughters Katy (Catherina) (1957) and Rita (1959) were born there. In Tihany, the family bought a small, rocky building plot, and built their own house (with the help of one master builder) over a period of ten years. The family returned regularly to this lovely home in Tihany after moving to Szeged in 1971. Later Tibor's daughters and grandchildren visited them there for the holidays.

FATTY ACIDS IN PLANKTONIC CRUSTACEANS

In the Biological Research Institute at Tihany, Tibor Farkas started an organized collection of planktonic organisms in the winter of 1955. He studied the chemical methods available in the literature while on a short fellowship study at the Institute of Inland Fisheries, East-Berlin, Germany, with Werner Steffens and Marie-Luise Albrecht. Farkas adapted methods to determine gross nutrient and energy contents of planktonic crustaceans in order to have an insight into the mass balance of Lake Balaton, the largest lake in Central Europe. He was surprised by the high fat content of the collected species and observed some differences between the species. However, the species related factors were not sufficient to explain the differences. Therefore, he began to study the fatty acid composition. At that time, the gas chromatographic method for the analysis of fatty acids was just under development and was not available in Hungary. Composition data were available only when researchers used a complex set of methods from titrimetry and iodine number through catalytic hydrogenation, crystallization of bromides and salts of the fatty acids, paper and column chromatography and separation of urea complexes. Farkas adapted most of these methods with significant modifications and applied these to extracted lipids, and noted that his paper chromatograms of the summer and winter samples gave different patterns.

In 1958, a newly graduated scientist, Sándor Herodek (the director of the Tihany institute in 2004) joined the studies. With combined efforts they searched for an explanation of the observed differences in these fatty acid patterns. They found that the amount of the lead salt fraction solid at room temperature followed the same trend as the change in water temperature where the crustacean plankton organisms lived before sampling. The trends for the iodine numbers and for the contents of unsaturated fatty acids were opposite to the changes in the water temperature. Based on the observations, they assumed that the seasonal changes in the environmental temperature were responsible for the differences in fatty acid composition between marine and fresh-water animals. Then they started a systematic study on the seasonal effects in the fatty acid composition and collected crustacean samples from cold and warm seas. They analysed the iodine number of the fats of collected plankton and measured the environmental temperature from June 1959 to August 1961 and obtained data for two complete annual cycles of the relationship of the fatty acid contents and the environmental temperature. After discussing the results with James F. Mead, a respected lipid scientist at University of California, Los Angeles, they published the results in the Journal of Lipid Research (1964). They demonstrated that the highly unsaturated acids have a role in assuring the liquid state of the stored lipids. The melting point of the lipids of the planktonic copepods remained somewhat lower than the water temperature during the whole year. At the same time, the proportions of the C_{20}-C_{22} polyunsaturated acids in the planktonic crustaceans increased with decreasing temperature and in some species exceeded the values characteristic of marine animals.

For several years Farkas's main focus was on metabolic and hormonal control of lipid mobilization, but his interest frequently returned to temperature effects on lipid metabolism of crustaceans. In 1968, he was a visiting scientist in the former Soviet Union and collected fish and plankton in the White Sea near the North Polar Circle. (Here, he had been allowed on board by the local fishermen from Tsupa village after taking the "protecting drink" made of one cup of hot tea and a big portion of rancid butter.) Later he was invited by the Scripps Institution of Oceanography, University of California (San Diego, California) to take part in the SIO's Alpha Helix Program. He planned to take part in the Antarctic Expedition in 1971. However, his exit visa from the Hungarian government for travel to the United States was late and he arrived in Los Angeles in 1972. In 1972-73, he spent a year in the Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles, visiting regularly Andrew A. Benson's laboratory at the Scripps Institution of Oceanography (La Jolla) for collecting marine plankton with Judd C. Nevenzel. In the Laboratory of Nuclear Medicine and Radiation Biology, they did experiments labelling lipids in marine copepods with ^{32}P-orthophosphate and ^{14}C-acetate. Some of their results on the role of phospholipid fatty acids in temperature acclimation of *Procambarus clarckii* showed that the higher unsaturation of fatty acids, synthesized at 5 °C, constituted an effective response for modification of the composition of lipids to maintain adequate functioning of the membranes at the lower temperature. These data were presented first at the AOCS' meeting in Mexico City, in April – May, 1974. These findings were later published in *Lipids* (*1981*).

The systematically collected analysis data on the fatty acids on the planktonic crustaceans were summarized and published in 1979 in *Comparative Biochemistry and Physiology* (Vol. 64B:71-76). In this paper, on the basis of polyenoic fatty acid patterns, the studied species fell into three ecological groups of the possible four: (A) active only in the summer and forming resting eggs – all of the Cladocerans; (B) active only in the summer and spending winter in resting stage copepodit form – some of the Copepods; (C) active only in the winter and spending summer in the form of resting stage copepodits – some other Copepods. In these A, B, C groups the polyenoic fatty acid levels differed strikingly, for instance the level of the docosahexaenoic acid ranged 1.46±1.36; 5.23±1.49 and 16.78±4.80, respectively.

Farkas continued research on the planktonic organisms later in Hungary with Crustaceans (*Daphnia magna*) and Copepodes (*Cyclops strenuus*, *Cyclops vicinus*), and designed similar experiments to those in the Los Angeles studies. In these studies, the incorporation of the ^{14}C-label of the acetate into the individual fatty acids was measured by preparative radio-gas-chromatography, developed in the Institute of Biochemistry, BRC (Szeged, Hungary). Results of these experiments demonstrated clear involvement of the polyunsaturated fatty acid in the regulation of the composition of membrane lipids in relation to the temperature adaptation of lower poikilothermic animals (published in *Lipids* in 1981 and 1984). Later, the relationships were confirmed by the results of studies with Calanoid Copepods collected in India and Norway (*Farkas et al., 1988*). In a further study Farkas and his coworkers investigated the composition of lipid classes, as well as the molecular species composition of subclasses of choline and ethanolamine phosphoglycerides in a marine amphipod crustacean (*Gammarus* spp.), collected in the Baltic Sea in relation to environmental temperature. In these studies, the environmental temperature had little effect on fatty acid composition. The phospholipid vesicles of crustacean collected at 8°C were more disordered than expected compared to those obtained from animals collected at 15°C. They concluded that, in addition to variations in the levels of sn-1 monoenic and sn-2 polyenic phospholipid molecular species with temperature, ethanolamine plasmalogens may play a role in controlling membrane biophysical properties in marine amphipod crustaceans (*Lahdes et al., 2000. Lipids 35:1093-1098*).

Although, the term "homeoviscous adaptation" originated with Michael Sinensky (*Sinensky, 1974. PNAS, 71:522-525*), Tibor Farkas's pioneering studies on the fatty acid composition of crustacean lipids starting in 1958[1], contributed to the understanding of the role of lipids in the mechanism of temperature adaptation. His last research idea (elucidating the involvement of adaptive lipid composition changes in the speciation processes of a Caspian Sea crustacean transported to the Finnish Bay) was stopped by his death at the stage of preliminary sample collections.

REGULATION OF FATTY ACID MOBILIZATION, MEMBRANE RECEPTORS

During the Tihany years, Rodolfo Paoletti (Institute of Pharmacology, University of Milan, Italy) invited the two lipid scientists, Tibor Farkas and Sándor Heródek into his laboratory for one year. In 1963 Tibor Farkas started the studies on regulation of fatty acid mobilization, concentrating his efforts on the effect of catecholamines on fatty acid release from adipose tissue of lower aquatic vertebrate. He observed that epihnephrine and other cathecolamines did not increase the rate of fatty acid production in the adipose tissue of fishes (carp, pike-perch, bream, etc.) and amphibians. On the basis of these experiments, a more generalized theory was developed relating fatty acid type to lipoproteins specific structure for animals living in different temperature environments. One set of experiments demonstrated that the common carp fed more unsaturated fatty acids in the diet had better overwinter survival in fish farm outdoor ponds than those fed on wheat or corn.

FISH OILS AND HEALTH

Farkas suspected the highly unsaturated fatty acids in marine fish oil would have a beneficial effect on human health as well. John Halver had available molecularly distilled hering oil for his feeding experiments, and Tibor requested 20 liters for diet testing. Together they proposed adding 20 gms daily to the current diet used in an institution of hypercholestermic patients. After six weeks of fish oil in their diet, the total blood cholesterol dropped significantly and the attending physician recommended the inclusion of EPA and DHA sources for hypercholestermic patients. These experiments generated media coverage on the value of polyunsaturated fatty acids in the diet, especially of the elderly, and were only one of the many demonstrations of the value of marine fish oils in the diet of humans.

Another interesting experiment with fish oils in brain function was a study on senile rats at the Biological Research Center in Szeged. Old rats in a colony which were tested for memory ability in a series of mazes, had lost rapid response to the challenges, and were about to be destroyed in favour of a new young set of experimental animals. Young rats navigated the maze for their rewards in one or two minutes. Mature rats in two or three minutes, and old rats took 4 or 5 minutes. Farkas took 70 senile rats, divided the group in two parts of 35 rats each, fed one group 10% fish oil in the diet, and the other group on the standard ration. After 3 weeks on the test diet, the rats were tested in the maze. The group on the standard diet took 4 to 5 minutes in the maze, while the fish oil fed group were able to solve the maze in about 3 minutes. This experiment stimulated the latest Farkas efforts to understand the possible role of DHA, the major lipid in the brain, on brain cell structures and memory zone impulse transmission.

Tibor was always interested in the role of long chain polyunsaturated fatty acids in cellular membrane structures. He pursued several studies to elucidate how these polyunsaturated C20 and C22 fatty acids were incorporated into the lipoprotein structure of cell membranes. He subsequently showed that these EPA and DHA acids had a coiling configuration, which allowed membrane flexibility which was dependent upon the type of fatty acid in the lipid portion of the cell membrane lipoprotein structure. The more unsaturated fatty acids present, the more flexible the membrane became and the lipids in cold environment species had to have more DHA present in order to survive.

Tibor even developed a system to hydrogenate unsaturated fatty acids in living tissues and then dramatically show that these membranes lost their flexibility and vitality. These studies led to investigation of brain lipid structures and brain functions.

In other studies he found that an adrenergic system consisting of adenyl cyclase-phosphorylase exists in the frog adipose tissue. He related the “antilipolytic” effect of the catecholamines to their hyperglycaemic effect and guessed that in fishes and in other lower vertebrates (in contrast to mammals) catecholamines have no role in mobilizing the fats in free fatty acid form. They have, however, the same mechanism as mammalian adipose tissue for stimulating lipolysis and glycogenolysis. The subject is still not elucidated and his related publications, although published mainly in Hungarian periodicals, are cited in recent articles too. All of these studies were summarized in his PhD dissertation: “Studies on the regulation of mobilization of triglycerides in lower vertebrates” finished in 1969.

After obtaining his PhD, he returned frequently to the subject of fatty acid mobilization either while investigating other subjects (*e.g.* phosphatidylcholine breakdown in rat liver plasma membrane: *Nemecz and Farkas, 1980. Biochem. Pharmacol. 29:2521-2623*; protein kinase C translocation in human amnion cells: *Premecz et al., 1987. FEBS Letters, 226(1):13-16*; opioid receptors in frog brain membranes: *Benyhe et al., 1989; Neurochem. Res. 14(3):205-210*; *etc.*), or with the development of methods (use of cation-exchange thin layers in adenyl cyclase assay: *Tomasz and Farkas, 1975. J. Chromatog. 107:396-401*).

Farkas stimulated other teams to investigate the regulation of metabolic processes at higher organization levels, that is, at the level of central and peripherial nervous system of higher vertebrates, including human beings, investigating the synaptic and brain membrane structures and receptor sites. Findings from incubation of rocker-cultured neonatal rat heart cells suggested that the phospholipase A_2-15-lipoxygenase pathway plays a role in the induction of beta-adrenergic supersensitivity in the cultured cardiomyocytes and points to a new physiological role of the lipoxygenase product 15-S-hydroxyeicosatetraenoic acid (*Wallukat et al., 1991. Mol. Cell. Biochem., 102:35-47*). Blood-free microvessels from brain cortex from weanling Wistar rats maintained on semisynthetic diets containing marine fish oil and sunflower seed oil had significantly less PGF-1-alpha, PGF-2-alpha and 12-hydroxyheptadecatrienoic acid in the capillaries of fish oil treated animals. The ratios of vasoconstrictor and vasodilator metabolites of arachidonate cascade were not modified by the diets. These results suggested that diet with fish oil reduces the arachidonate cascade in cerebral microvessels, an effect that may explain the efficiency of n-3 fatty acids in preventing some vascular diseases (*Kálmán et al., 1992. Neurochem. Res., 17:167-172*).

Farkas initiated another series of studies on the structural role and modulation effects of lipids on the signal transduction from membrane receptors to regulatory proteins localized to various cellular compartments (lipid polymorphism in G protein-membrane interactions: *Escribá et al., 1997. Proc. Natl. Acad. Sci. U.S.A. 94:11375-11380*; topological organization of the MHC class I and II antigens and signal transduction: *Jenei et al., 1997. Proc. Natl. Acad. Sci. U.S.A., 94:7269-7274*; supramolecular receptor structures in the plasma membrane of lymphocytes: *Damjanovich et al., 1998. Cytometry 33:225-23*; role of polyunsaturated fatty acids in the resistance against induced excitotoxic degeneration of cholinergic neurones: *Högyes et al., 2003. Neuroscience, 119:999-1012*).

FISH FATTY ACIDS, THERMAL ADAPTATION

Studying the fatty acid composition of crustaceans Tibor Farkas formulated questions regarding the origin (food or synthesis), fate (transformation and deposition), species specific differences and food chain relation of the polyunsaturated fatty acids (*Farkas, 1958. Annal. Biol. Tihany 25:197-207*). Parallel to their zooplankton studies, Farkas and Herodek sampled lipids from fishes, too. The results of the comparative study on algae, freshwater crustaceans and fishes, showed that the fatty acid composition of fish was similar to the food and C_{22} faty acids were more abundant in fats of predatory fishes than in the carp (*Farkas and Herodek, 1962. Annal. Biol. Tihany 29:79-83*). The combined fractionation and paper chromatographic studies on the incoporation of ^{14}C-labelled acetate into the liver fatty acids of bullhead, provided proof that the basic pathyway of fat metabolism in fish is identical to that of mammals, and that the bulk of C_{20} and C_{22} fatty acids in fishes originates from their food (*Farkas et al., 1961. Acta Biol. Hung. 12:83-86*).

The accumulation of dietary fatty acids was proved by feeding guppies on summer and winter zooplankton showing that winter zooplankton caused a higher proportion of C_{22} fatty acids (*Farkas and Herodek, 1964. J Lipid Res. 5:369-373*).

Ten years after the introduction of gas chromatography for the analysis of fatty acid methyl esters, Farkas and Herodek compared the fatty acid composition of fishes collected from locations differing in water temperature. From the results they concluded that the fatty acid composition of fish fats was determined by two processes. Namely, (1) synthesis of polyunsaturated fatty acids at the lower level of the food chain, and (2) dilution of exogenous polyenoic acids with endogenous ones. When they arranged the fishes according to the increasing C_{22} fatty acid content, the sequence corresponded to the arrangement of fishes according to decreasing environmental temperature (*Farkas and Herodek, 1967. Annal. Biol. Tihany 34:139-146*). Later, these processes of formation and transformation of polyunsaturated fatty acid in the food-chain were confirmed.

In 1974, the Fish Culture Research Institute (Szarvas, Hungary) and the Biological Research Center of the Hungarian Academy of Sciences started a cooperative research program on the fatty acid metabolism of fishes mainly for determination of the essential fatty acid requirements of the fishes and elucidating dietary effects in the fatty acid metabolism of common carp. This program addressed practical questions of the fish culture industry, however, it gave an opportunity for Farkas to return to the question of the involvement of fatty acids in the temperature adaptation of poikilothermic animals. At the beginning of the project, Farkas succeded with elaboration of a preparative gas chromatographic technique for analysing the incorporation of the radioactivity from ^{14}C-labelled acetate into individual fatty acids (*Farkas and Csengeri*, 1976. *Lipids* 11:401-407).

FISH AND FOWL BRAIN FATTY ACIDS

Art Hasler and Warren Wisby showed migratory salmon made their final orientation to stream branches by olfactory means. They built a tank and "trained" Rainbow trout to orient for food intake at various sites by adding phenol before feeding. They then diluted the odor to ultra micro quantities. Halver and Oshima made encephelographs of Steelhead, the sea-going Rainbow, and the electrodes in the brain responded positively to micro quantities of reagents including even sodium chloride. When Farkas heard of this work, and that the olfactory lobe was about one-third of the salmonid brain, he asked for this tissue to analyse for fatty acid content in the structure. In 1980 Halver collected Chinook salmon brains during the spawning procedures of these large salmon (10-20 kg) which had travelled thousands of miles in the north Pacific to the mouth of the Columbia River and then smelled their way home to the tiny water outlet at the Spring Creek National Fish Hatchery. These brain tissues were hand carried to Szarvas and Szeged, Hungary, where Tibor analysed them and found very high content of DHA in the olfactory structures. At about the same time Tibor had obtained Arctic temperature fish tissues and Halver collected tropical temperature fish tissues to compare with the temperate water fish of central Europe. Farkas and his team analysed the tissue fatty acids and found a direct correlation between fatty acid unsaturation and water temperature in which the fish were acclimated. Tropical fish had lower unsaturated fatty acid content in the cellular structures. Temperate water fish had more unsaturated fatty acids present, and arctic coldwater fish had high levels of polyunsaturated fatty acids in their cell structures. Farkas proposed and confirmed that the fatty acid type determined cell membrane flexibility with more unsaturated fatty acid, which had a much lower melting point, necessary for life and functions in frigid water temperatures. When Farkas realized that certain avian species had a very large optic lobe in the brain, he asked for Eagle brains but Halver convinced him to use Canada geese brains instead. He then collected Canada geese brains from a hunting club in central Washington State. These frozen brains were hand carried to Szarvas and Szeged where they were assayed. Again, high levels of DHA were found present in the brains. Farkas then asked for grizzly bear brains of animals feasting in Alaska on migrating salmon, which had high DHA content and to compare levels in the bears before and after hibernation in order to measure retention times for these essential lipids. Three bears feasting on salmon became available, but the samples of emerging bears from hibernation were never obtained. Then Farkas died.

Tibor Farkas was a fine gentleman, and intellectually active until the day of his death. He will be missed by many friends and co-workers in fatty acid metabolism studies, and in the newly emerging scientific research programs on the role of specific fatty acids in brain structure and functions.

NOTE

1"We feel being justified in assuming that the increase in the fraction solid at room temperature means at the same time a higher melting point of the fat. On this basis it can be established that in the course of the year the changes in the fatty acid eomposition demonstrated by paper chromatography are parallel with changes in the melting point of fat which in turn correspond to the changes in the temperature of the environmental water. This correlation is more marked with the Crustacean plankton than with the *Dicerogammarus*, as the former sample showed a more marked variability of the fatty acid pattern, too. We intend to continue investigations, involving new methods as well, in order to obtain more data on this question." (Farkas and Herodek 1959. Acta Biol. Acad. Sci. Hung. 10:85-90)

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