To Barbara with love, Kate and Michael

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Chapter 3

Strife and hope in the lives of a scientist couple

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Years of 1939–1945

Michael: My professional career started with a considerable delay. I matriculated from high school in 1939 at the age of 18 with a summa cum laude degree, but was not accepted by any Hungarian University because I have been a Jew. The Numerus Clausus law restricted the admittance of Jews.

I became a mechanic student in an agricultural factory, with the aim to help my father repair the equipment in our farm. Shortly after I earned my diploma, I was called into the service and assigned to a mechanical army unit to build prefabricated bridge structures at an industrial center in Budapest, the capital of Hungary. As a Jew, I served the army in my civilian clothing and wore a yellow armband. Otherwise, I was well treated, as were other skilled workers, and had the privilege to leave the camp for the weekend.

By the end of 1943, the course of World War II took a visible turn: the Soviet troops recaptured their own country and were

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chasing the Germans out of Poland. The Allied troops landed in Sicily; soon Rome fell and Mussolini was out of power. Hungary, a trusted ally of Hitler, changed its mind by refusing to commit more troops to Germany; moreover, a secret Hungarian delegation traveled to Turkey to make contact with the Allies. Hitler could not tolerate this, and in March of 1944, Germany occupied Hungary. The situation for Jews changed immediately: Jews had to wear a big yellow star on the upper left side of their clothing and were collected into ghettoes. In Budapest, I met two Czech Jews who told me about the extermination of Poland's Jewish population in Auschwitz. There was no doubt in my mind that the same would happen with the Hungarian Jews. In order to save my parents, I traveled to our home in a small village, with false identification papers for a Christian civilian in my pocket. I begged my parents to go into hiding, but they refused, saying that God would save them. Within 3 months they were gassed in Auschwitz.

With German troops on Hungarian soil, we became the target of bombing by the Allied air forces. The army's industrial factories in Budapest were severely hit, and we were evacuated to a suburb. Our factory also employed civilian mechanics. One of them was living in the same suburb. Once he took me home, introduced me to his wife, and told me I could hide there should the need arise. In the middle of October 1944, Soviet troops entered Hungary; to counteract the military situation, an extreme rightwing political party seized power and announced the liquidation of all remaining Jews. Under darkness, I went to the home of my friends, who took me to their attic. After a week, he was very upset when he told me that their neighbor questioned his wife about her frequent visits to the attic. The neighbor asked point-blank whether they were hiding somebody in their house. Hiding Jews was punishable by execution. My friends were frightened and asked me to return to the factory. By the end of November, Budapest was surrounded by the Soviets from the East and

South and the German-Hungarian army retreated to the West. Our factory's new headquarters were scheduled to be in a small city, 60 miles west of Budapest.

Buchenwald

Michael: In the early morning of 4 December 1944, we were directed to a train station. Unexpectedly, we were handed over to a police unit that jammed about 90 of us into a cattle wagon where we were kept for 21 days. Before leaving for the train station, we were given a 2-day supply of food; otherwise, we received bread just twice in the entire 3 weeks. However, we never received water, and after a while we started to drink our own urine. Hunger, smell, and disease transformed the cattle wagon into hell; one person was dead and two young men could not walk when the train stopped in front of the Buchenwald concentration camp. On the top of the main entrance there was a sign 'Arbeit macht frei' (Work makes you free). All of our belongings were taken away. We each got an identification number, one striped uniform, and a pair of wooden shoes. Then, we were taken to a half-filled barrack and were told to find a place on any of the bunks. (Buchenwald had over 100 barracks, each for 500–600 persons, where people were laying side by side).

Our days were always the same. In the morning we got 'coffee' (black colored water) and 1/2 ounce of bread, and at noon we got a cup of sugar beet soup. In the afternoon there was Zähl-appell, head count by a German guard; it was usually dark when this ended, and we went to sleep. There was no activity in the barracks; we just stayed on the bunks to warm each other, because it was winter and the temperature was below 20°F. Some people came down from the bunks to search for those who were near death and took their bread away; this was the only way to get extra food. The number of dead persons ranged from 10 to 20 per barrack per day; the naked bodies were piled up in

front of the barracks and were delivered by wheelbarrow to the crematorium.

We were isolated from the outside world, although frequently new prisoners were brought in from evacuated concentration camps. From this, we concluded that Germany was not doing well in the war. In March of 1945, we heard the bombs falling on the neighboring towns, and by the end of that month the Allied airplanes were flying at low altitude over the camp. Until 6 April, food was distributed in the barracks, but on this day we were told to go into the center of the camp for the soup and bread. While on the way, I noticed that after obtaining the food, people were not allowed to return to their barracks but were moved outside of the camp. I decided to skip the meal. The next day, five of us escaped to an already empty barrack and went into hiding. Soon this became a common practice; to counter it the German guards searched the empty barracks and shot to death whomever they found. Our group dug into the earth below the bottom bunks, and we were holding our breath when the Germans came. We did not eat for 5 days, but had access to water.

On the morning of 11 April 1945, we heard gunfire that became stronger and stronger, and in the afternoon, the German guards left the watch towers surrounding the camp. In a short while, we saw the motorized American army moving toward the camp. Hot food was served that evening. Many people ate so much that they died during the meal. According to my information, at the beginning of April there were about 60 000 prisoners in Buchenwald, of which 35 000 were evacuated and 25 000 liberated. I weighed 92 pounds and was diagnosed with lung tuberculosis.

After a month of hospitalization, I felt strong enough to return to Hungary. The war was over and my wish was to become a physician. In September of 1945, I started my medical studies at the University of Budapest. The American Jewish Organization provided financial support for my education, since my parents did not survive the Holocaust. I was

living in a student dormitory and got three meals per day. I will always be most grateful to the American Jewish Community for its generous help.

Auschwitz

Kate: I was born in a small Hungarian town, which had good schools, theaters, a museum, and a concert hall. My father was a respected medical doctor, who combined patient care with successful research. In his lifetime, he had more than 150 publications, and in his retirement he wrote a book about Nobel Prize Winners in Medicine and Physiology [1]. My mother was an X-ray technician and worked with my father. My brother was 4 years older than I. We were well off, we had a nice house with a beautiful garden. I learned to play the piano and dreamed of becoming a concert pianist, but the war interfered.

I was 15 years old in the Spring of 1944, when the Germans occupied Hungary. My father and brother were called into the labor camp service for Jews. My mother and I went into hiding, but soon we were captured and deported to Auschwitz. When we got off the wagon, the announcement came that children under the age of 16, adults over 45, and pregnant women should go to the left; the others, capable of hard work, should go to the right. I wanted to go with my mother; after all, I was already 15, I was athletic and I could pass for 16. But my mother believed that children would have an easier task, so I went to the left. Then suddenly, I was overcome by fear and wanted to join my mother. By hiding and running, I got away from the group sent to the left. This was my first escape from the gas chamber. I joined my mother.

In Auschwitz, our heads were shaven, our clothes and even glasses were taken away. We obtained one piece of cloth, wooden shoes, and were sent to a large barrack. Our routine was the same each day: twice a day we participated in Zählappell, which was also our mealtime, 'coffee' in the mornings

and turnip soup in the evenings. After Zähl-appell we marched to the latrine, and then back to the barrack. In the barrack, we sat in rows on the floor. The space was very tight. The first woman in the row leaned to the wall and sat with her legs spread apart. The second one sat between the former's legs, also spreading her legs, until we were all sitting in rows. It was most embarrassing: some of us could not withhold urine for the whole day or night, so we sat in our urine.

The most fearsome event was the 'Selection': we got undressed, showered, and then marched in front of the notorious German officer, Dr. Mengele, who divided us into groups. In the Fall of 1944, he sent me to the left, the group of the weak ones to be gassed. Instinctively, I exclaimed to Mengele, in German, that I was young and strong, I wanted to work. He hit my behind with his cane and said 'go', and I ran to the right to the group of the strong ones. This was my second escape from the gas chamber. However, in the meantime, I lost sight of my mother and never saw her again.

In the Spring of 1945, the Soviet army was approaching Birnbaumel, my labor-camp. I was in the hospital with acute rheumatic fever, and when the Germans evacuated the camp they left one guard behind with the patients in the hospital. We knew that his instructions were to shoot and bury us. We were hysterical and we were screaming. Our screams were heard by a group of Soviet partisans, and they came to our rescue.

Unexpectedly, the Soviets took us into a camp of war prisoners and released us only in the late Summer of 1945. I returned to Hungary. I felt very much out of place. Among all of the Jewish girls in my age group, only three returned to my hometown. My two girl friends could not cope and committed suicide. I went to Budapest, finished high school, and subsequently studied physics and math at the university.

My father survived the war and remarried a girl slightly above my age. She could not tolerate my brother and me in their home, and we moved out. Then my brother got married

and I remained alone. I earned my scanty living by tutoring students in math. I did not have enough food, and had no heating in my room. I was saving money for a radio, but that took me 3 years.

The Szent-Györgyi Institute

Michael: The lectures of the Nobel Prize winner Albert Szent-Györgyi, Professor of Biochemistry, were the highlights of my medical education. The general interest in his lectures was so great that they were held in a theater, rather than in a regular auditorium, in order to accommodate virtually the entire medical community of Budapest. Szent-Györgyi transmitted his knowledge and enthusiasm to the audience so that everybody felt enriched in science. He convinced me that advances in human medicine will come from biochemical research. It became my dream to work in his laboratory, and I was overjoyed when he accepted me. There were two projects at the Szent-Györgyi Institute: the major one was on muscle proteins and the minor one was on blood coagulation. I was assigned to the blood group. Initially, I worked with Lászlo Loránd on factors involved in coagulation and later with Elemér Mihályi on the kinetics of the fibrinogen to fibrin transformation. I spent long hours at the Institute, and when I got home I pursued the medical school requirements.

The Szent-Györgyi Institute was an intellectual center in Budapest. Every scientist who visited Hungary also visited the Institute. In addition, leading artists, writers, and musicians were among our guests. The Institute provided full meals for everybody, and took care of basic needs such as shoe repair. The Institute was open day and night. The only excuse for not doing research was the daily volleyball game with the participation of Professor Albert Szent-Györgyi.

An interruption

Michael: The many sleepless nights affected my health. In the Spring of 1947, I started coughing, and during the evenings I noticed a fever. I went to a physician and got the worst news possible: the lung tuberculosis originally acquired at Buchenwald was reactivated. Immediately, I had to stop both my medical studies and my work at the Szent-Györgyi Institute. I was sent to a tuberculosis sanatorium up in the mountains, and underwent pneumothorax therapy. This involved compression of the lungs by introducing air between the pleurae, hence reducing the breathing activity and thereby promoting the healing process (no specific drugs were available at this time). In addition, all patients spent 5–6 h per day in the fresh air, laying on their backs to ensure proper ventilation of the lungs. I was eager to find some intellectual activity. The surgeon of the sanatorium allowed me to attend thoracic operations for patients whose lung cavernae did not disappear upon pneumothorax treatment. The rib cage in the back was cut with huge scissors so that the entire half lung collapsed. This was a major operation, lasting about 9 h, by the surgeon and his assistant, who was also in charge of the anesthesia. The patient was awake throughout the operation, because only local anesthesia was used. Surprisingly, the patients tolerated this drastic procedure well; even the wife of the surgeon underwent such an operation.

The Straub Institute

Michael: After 6 months, I was released from the sanatorium under condition that ambulatory pneumothorax continue for the next 2–3 years. My first order of business was to pass my postponed medical school exams; it took me almost a year to catch up. Next, I wanted to return to the Szent-Györgyi Institute; however, things had changed quite a bit during the time I had been away. Szent-Györgyi had moved to America, and

Loránd, Mihályi, Laki, and A.G. Szent-Györgyi had also left Hungary. Straub, the discoverer of actin, was the new director. He came from Szeged, the second largest university of Hungary, and brought his entire staff with him. First, Straub was not willing to take me back. Fortunately, N.A. Biró, the only faculty member whom I knew from my previous stay at the Institute, asked Straub if I could work with him.

Straub had discovered that actin in its globular (G) form contained bound ATP, and that during polymerization of G-actin to fibrous (F) actin the G-actin-bound ATP was converted to F-actin-bound ADP while inorganic phosphate was liberated. Based on these findings, Straub developed a new concept of muscle mechanochemistry [2]. In Straub's word 'the change in the structure of the protein cannot be separated from the change in energy'. Biró and I were initially working on the accessibility of actin-bound nucleotide to enzymes involved in ATP metabolism. Soon, Straub asked us to study the G → F transformation of actin and the accompanying ATP → ADP transformation in live muscle. Straub observed that during winter hibernation the frog heart uses up all its free adenine nucleotide content and only the actin-bound nucleotide remains. This was the ideal system for our planned study, because in the absence of free nucleotides the bound ATP and ADP could be selectively released from the heart and quantified. Accordingly, Biró and I purchased several hundred frogs during autumn and placed them into the outdoor frog lake; when winter arrived, the surface of the lake froze and the frogs started to use up their excess ATP. Frogs were sacrificed in batches of six, and the hearts were dissected, cannulated, and transferred into the cold room to slow down the rate of spontaneous heart beat. Biró filmed the motions of the hearts while I froze the hearts in a dry ice/acetone slurry. The film revealed the functional state (e.g. systole or diastole) of the heart at the time of freezing. The acetone dried powder was extracted with trichloroacetic acid and used to quantify the ATP and ADP content of the heart. Since we did not have a

UV spectrophotometer, adenine nucleotides were determined by a viscometric method that was based on the difference in reactivity of actomyosin for ATP and ADP. The method was time consuming; a single analysis took about 1 h.

Biró's wife gave birth to a boy. He spent less time in the lab and I became the principal investigator. Straub was very anxious to know the results and visited me every evening. A friendly relationship developed between us. He learned my life story and I learned his. He was a true genius: at the age of 19, he isolated lactic acid dehydrogenase; at 24, he isolated diaphorase (the flavoprotein responsible for the oxidation of NADH); at 28, he discovered actin; at 31, he became institute director; at 33, he published a biochemistry textbook. He typed faster than the departmental secretary, learned Russian in less than a year, and was an excellent tennis player. He married a chemist and they had two girls. Once he expressed his opinion about contemporary biochemists, 'There are three types of biochemists: Type 1 sits at his desk and is thinking. He gets an idea, calls his technician, and asks her to prove his theory in the lab; Type 2 has no ideas, but reads a lot in the library. Eventually, he finds a new method that can be applied to an accepted research project; Type 3 has no ideas and no methods. He is working in the lab and eventually finds something new; then he goes to the library, reads what is necessary; then he goes back to the lab and continues the research. When enough data are collected, he develops a theory and writes a paper.' Straub classified himself as Type 3 biochemist.

In spite of all our efforts, we could not show that actin polymerization was correlated with ATP → ADP transformation in the beating frog heart. Biró and I informed Straub about the negative results. He asked a few questions, and then concluded: 'who can't lose can't win'. Biró and I were depressed, having spent 3 years on the project and ended up with nothing. We analyzed our data over and over, and came up with a few new ideas. However, Straub remained firm in his decision, the project could not be resumed. To revive our

enthusiasm, he outlined four criteria necessary for success in research: (1) one has to work hard; (2) one has to read a lot; (3) one has to evaluate the significance of the results; (4) one needs a little luck. In our experimentation, we fulfilled the first three, but we did not have luck. From this time on, we called luck the 'number 4 factor of Straub'.

Within a few months, Straub had to face another problem with the actin-bound nucleotide: Szörényi, a Hungarian born but Soviet trained biochemist, reported that crude actin preparations contain enzymes, such as creatine kinase, myokinase, hexokinase, phosphorylase, and adenylate deaminase, involved in ATP turnover; he then postulated that ATP hydrolysis during actin polymerization was due to the action of these enzymes rather than to the actin protein itself. (There was also a political background behind the Szörényi and Straub dispute: to please the Soviet Union, the Hungarian government recruited Szörényi and built for him a Biochemistry Institute that was better equipped than the Straub Institute. The question was: who is the leader of the Hungarian biochemists, the Communist Szörényi or the Western-oriented Straub?) Straub asked me to try to purify actin free of the aforementioned enzymes. I had a very hard year. All classical purification methods (salt fractionation, precipitation with organic solvents, absorption techniques) failed, i.e. a denatured actin was isolated that had lost its ability to polymerize. I realized that only a specific precipitation method could lead to results (column chromatography was not an option at that time). Straub, when he discovered actin, had noted that bivalent cations in the millimolar concentration range precipitate actin [3]. Based on this observation, I was able to isolate by Mg^{2+} precipitation an actin that was free of all enzyme contaminants, while maintaining its capacity to polymerize with concomitant hydrolysis of the actin-bound ATP. We wrote a paper and sent it to Szörényi for review; surprisingly, he rejected the paper, stating that the results were in disagreement with those of his laboratory. Straub sent me to Szörényi's

lab with the purified actin so that they could test it themselves. A 2-day long excitement led to a complete surrender of the Szörényi group. The paper was published in *Acta Physiologica Hungaricae* [4]. Subsequently, I became interested in the interaction between actin and magnesium ions; thus, I measured the number of Mg^{2+}-binding sites of actin, the pH and salt dependence of the binding, and the effect of binding on the gel structure of actin [5,6]. The interaction between actin and Mg^{2+} is unique: in the 10^{-3} M range Mg^{2+} polymerizes G-actin; in the 10^{-2} M range Mg^{2+} precipitates either G- or F-actin; and in the 10^{-1} M range Mg^{2+} depolymerizes F-actin.

Marriage

Michael: Already in 1949 the Communist Party had a strong voice in the Hungarian Government. To get the vote of the intellectuals, they launched a program supporting science. The salaries of university personnel were raised; moreover, all research assistants received a salary. Thus, I suddenly had an income and as such lost my eligibility to stay in the free student dormitory. I was looking for an apartment.

Kate: I was living alone and yearned for a life companion. I was always hungry and gladly accepted leftover food. It happened once that I was slicing a hard piece of bread and cut my finger. The bleeding did not stop, and to get help I went to the nearby dormitory. A handsome medical student took care of me, we spoke for a long time and recognized that we had a similar background, similar interests: we resonated with each other. Shortly after our first chance meeting, we were in the same group of university students who were rewarded by a 2-week long summer vacation by the American Jewish Organization. There, Michael and I spent most of our days together, and within 4 days we were engaged.

Kate and Michael: We found an apartment (a single room in an apartment that was split into three parts), and purchased coal and potatoes for the winter. Our wedding was 10 weeks after

our engagement. Although this was a quick decision it was a good one. Now, we are anticipating our 49th anniversary.

Electron Microscope Laboratory

Kate: In its efforts to advance science in Hungary after the war, the government established the Electron Microscope Laboratory. The name originated from the first piece of equipment that was purchased from the West; subsequently, a Tiselius electrophoresis, an analytical ultracentrifuge, and a diffusion apparatus were obtained. The Electron Microscope Laboratory was a unit of the Hungarian Academy of Sciences and it was located in the basement of the Straub Institute.

In 1950, I was a third-year student in physics. I applied for one of the several openings, and was hired. I was delighted, as it meant that I could work in the immediate vicinity of Michael and in the field of his interest. Actually, our scientific collaboration began at this time.

A young chemist, Lászlo Hegedüs, and I were in charge of the analytical ultracentrifuge and the diffusion apparatus. We had to bring these instruments into operation without any manufacturer's help, as the Communist Hungarian government did not allow foreigners from the West to enter the country.

I devised a cell for the diffusion apparatus which greatly facilitated the measurement of diffusion for macromolecules. In the late stages of pregnancy, I was diligently writing up the work [7]. Finally the paper was ready for submission, with the exception that the figures were scheduled to be picked up on the morning of 19 February 1955. The previous night, my labor started, but I went to the laboratory to put the figures into the manuscript. I finished by 10 a.m. and our first son, George, was born by noon.

Partition of the Straub Institute

Michael: I got my MD degree in 1951, by which time I was already a well-established member of the Straub Institute. Everybody expected that I would be appointed to the core faculty, but the Communist Party representative to the Institute had found out that my father was a farmer, in Communist terms a *kulak.* They did not take into account that my father had died in Auschwitz. The fact that I was born as the son of a *kulak* eliminated me from being a teacher of students who would later serve as physicians to the people of a Communist nation.

The Institute was teaching both biochemistry and chemistry, and this was a tremendous burden on Straub. He arranged that the Institute should be split into two parts, Biochemistry and Chemistry, each responsible for its own teaching. Straub became the director of Chemistry, since there were not enough well-trained chemists in the medical school. I ended up in Biochemistry along with the other MDs. Our boss, Vilma Sz. Hermann, a former collaborator of Szent-Györgyi, kept a strong grip on everything, including the local Communist Party. Eventually she arranged for my appointment as an Assistant Professor, with the responsibility of preparing the labs for the students.

Partition of the Straub Institute separated me from Biró and, thus, I became an independent investigator. There were two medical students and one chemistry student associated with me. Notable was our study on the actin–actin bond that I had submitted to *Biochimica Biophysica Acta* as a preliminary note in English [8]. I suggested that hydrogen bonds between actin–SH and actin–NH_2 groups play a primary role in the combination of actin monomers to form the actin polymer. Westenbrinck, the Editor of BBA, liked my note so much that he wanted to publish it as a full paper. However, permission was denied by the Communist authorities in Hungary and, thus, the full paper appeared in the *Acta Physiol. Acad.*

Sci. Hung. [9,10]. In collaboration with Kate and her boss Ferenc Guba (the co-discoverer of the Guba–Straub solution for myosin extraction from skeletal muscle), we observed that actin can be prepared without previous extraction of myosin. This work was published as a Letter in *Nature* [11] and as a full paper in the *Acta* [12].

Escape from Hungary

Michael and Kate: Going home from work on a cold winter night in 1953, we met a friend who whispered the news 'Stalin suffered a stroke'. We eagerly listened to the radio all night and learned that Stalin died. Everybody was afraid to show their true feelings, and by the time we attended the compulsory meeting at the university, organized by the Communist Party, we were crying. We were told that Stalin was our 'father' and without him we would have to work much harder to build communism.

The fight for power in the Soviet Union had beneficial repercussions in Hungary, as the country turned from international communism to national communism. The most important event of this evolution was the posthumous rehabilitation of Lászlo Rajk, a national Hungarian Communist, who had been executed by his Communist rivals. Actually, Rajk's grave was opened and his remains were reburied in the national cemetery. The hate against the existing communist system started to be expressed loudly. The prisons were filled with innocent people, freedom of speech was suppressed, borders were closed and mined, and there was little food, clothing, or heating coal. On 20 October 1956, at 1 p.m., a few hundred university students, ourselves included, started a march through Budapest. Along the way, we were joined by thousands of others and reached the Hungarian radio station by darkness. The leaders of the march requested that their demands to the government be broadcast. In response, the Communist leaders ordered firing into the crowd. The revolution began.

In about 2 days, the entire country joined the revolution. The Hungarian Communists asked the Soviet army units stationed in Hungary to intervene, but even this appeal failed because the Soviet soldiers were sympathetic to the revolution. During its short life time, the new Hungarian government, led by Imre Nagy, restored democracy and announced its intention to secede from the Soviet block. In their bargaining with the Hungarian government, the Soviets used the master of arguments, force. New Soviet troops were brought to Hungary and the weak Hungarian army was defeated within days.

In order to get help from the West, the border mines were removed during the revolution. Now the way was open to escape from Hungary. Kate was 5 months pregnant with our second child. After two unsuccessful attempts to get to Austria, we traveled toward the Yugoslavian border. Smuggling people to Yugoslavia was a very expensive business. At night, after giving all of our savings to a smuggler, we were shown the way to the border. It was a walk that we thought would never end. There was about one foot of snow on the ground and gunshots could be heard. Michael carried our 2-year-old son, while Kate carried a handbag containing our valuables and some food. We were walking for hours and hours until eventually we reached a very deep trench, the actual border that we had to cross. We were exhausted, and we put down our little son and he too had to walk. We stopped frequently. It was dawn when the Yugoslavian border guards noticed us. We were taken to a refugee camp, along with about 400 people. Food was scarce and sanitation was poor. Fortunately, Jews could emigrate immediately to Israel. Within 2 weeks we were on our way to Naples, where we boarded an Israeli ship sailing to Haifa. It was the middle of February, the Mediterranean Sea was beautiful, and we could relax.

The Weizmann Institute

Michael: We went to Hayogev, a moshav (agricultural community with independent farmers) near Haifa. A cousin took us into his home. I started job hunting, while Kate was waiting for the delivery of the baby. Aharon Katchalsky, head of the Polymer Department of the Weizmann Institute, was the only muscle researcher whom I knew in Israel. (In May 1972, Aharon was tragically killed by terrorists at the airport, upon returning home from a scientific meeting in Göttingen.) He was the creator of the artificial muscle engine, made of polyacrylic acid, that relaxed when the side chain groups became ionized at high pH and contracted when the groups were protonated at low pH [13]. I was very much impressed when Aharon demonstrated the magnitude of the contraction: a polyacrylic thread weighing 6 mg could lift a weight of 360 mg. Aharon greeted me warmly and invited me to his home. After supper with his family, I narrated the fate of the Hungarian Jews; it was early morning before we went to sleep. Since Aharon had no lab facilities for me, he introduced me to his brother Ephraim Katchalski, the head of the Biophysics Department, who squeezed me into a half-bench space to be shared with one of the PhD students. The Katchalskys arranged a moderate fellowship for me, and Abraham Oplatka generously shared with me his apartment in the Weizmann housing complex.

At the Weizmann Institute, I became acquainted with state-of-the-art physical and biological sciences. In Hungary, behind the iron curtain, we had no UV spectrophotometer, pH meter, radioactivity counter, high speed centrifuge, magnetic stirrer, ion exchange resin, or even perchloric acid. In Hungary, in the 1950s, we had no access to Western journals or books. In contrast, the library of the Weizmann Institute was excellent. My education was also facilitated by the visitors of the Institute, e.g. I could talk with Chris Anfinsen or Arthur Kornberg. While enjoying my new environment, my cousin called me

from Hayogev to let me know about the arrival of our second son, Francis (named after Straub). From the Weizmann Institute, at Rehovoth, it was a half-day bus trip to Hayogev and I was eager to see Kate and our sons. However, Kate was crying when we met, because the wife of my cousin, who was infertile, wanted to adopt our new baby. Upon returning to Rehovoth, I learned that Abraham Patchornik from Ephraim's department was just leaving with his family for a longer stay in Bernhard Witkop's laboratory in the US, and could rent his furnished house to us.

Ephraim's laboratory pioneered in preparing polymers from all physiological amino acids; these were considered the simplest model proteins with well-defined composition and structure [14]. My arrival coincided with the initial effort to develop biological applications of polyamino acids. I tested the effects of polyamino acids on actomyosin and myosin, and found that polylysine inhibited the Mg^{2+}-activated actomyosin ATPase under conditions when Ca^{2+}-activated actomyosin ATPase, Ca^{2+}- or K^{+} (EDTA)-activated, and Mg^{2+}-moderated myosin ATPase were not affected. This was the first example for the specific inhibition of the contractile ATPase, and it would have been important to apply this finding to isolated muscle fibers. However, neither I nor anybody else in the Weizmann Institute had any experience with fibers. I turned my interest to the characterization of the active sites of myosin. The question was: are the ATPase and actin-binding sites of myosin the same or different? Bailey and Perry [15] were the first to study the role of cysteine residues in the biological properties of myosin. They showed that a large number of –SH group reagents which destroyed the ATPase activity of myosin also destroyed its affinity for actin, and from this finding they attempted to conclude that the same site of myosin was involved in both ATP hydrolysis and actin-binding. I thought that in the presence of actin, the –SH groups around the actin-binding site of myosin would not be accessible to reagents, and planned to investigate a protective effect of

actin on the actin-binding site of myosin. These experiments required preparation of low molecular weight SH group reagents which would have no steric effect on either the actin-binding or the ATPase site of myosin. Michael Sela and Tsvi Sadeh were helping in these syntheses, and Ephraim Katchalski and Arieh Berger made valuable suggestions.

As time went on, I became unhappy with the slow progress of my research. In spite of having many friends, I remained isolated in my research endeavors. I wanted to be part of a group that was at the cutting-edge of muscle research. I contacted Szent-Györgyi in Woods Hole for a position; his answer arrived by return mail. He had no opening and suggested Hans H. Weber, the leader of European muscle biochemistry in the 1950s [16]. Indeed, Professor Weber accepted us.

It was a dilemma to go to Germany after our ordeals in Buchenwald and Auschwitz. In our correspondence with Professor Weber, we expressed our intention to eventually emigrate to America. In April of 1958, we sailed from Haifa to Marseilles.

The Weber Institute

Michael and Kate: It took almost 2 days to reach Heidelberg from Marseilles by train. Professor Weber personally waited for us. We were first accommodated in the guesthouse of the Max Planck Institute, a beautiful castle in the hills of Heidelberg. It was free. During our 2 years in Heidelberg, Professor Weber did everything in his power to compensate us for our sufferings caused by Hitler. We learned from several sources that Professor Weber was an active anti-Nazi.

The Department of Physiology of the Max Planck Institute at Heidelberg was headed by Professor Weber. Its first director was Otto Meyerhof in the early 1930s (at that time, the Institute was called Kaiser Wilhelm Institute for Medical Research). Indeed, the entire Institute resonated with the

history of muscle research. We worked in the same lab which had been used by Lipmann. Adjacent were laboratories previously occupied by Lohmann, Lundsgaard, and Ochoa. Mr. Walter Möhle, Meyerhof's technician and a co-author of several of his publications, was still working at the Institute and he told us stories about Meyerhof. One of them stands out in our mind: Meyerhof was very much impressed by Lundsgaard's discovery about muscle contraction without lactic acid formation, and he wanted to see this finding confirmed in his own laboratory. Lundsgaard was invited to Heidelberg, and as soon as he arrived in the laboratory, in the late afternoon, Meyerhof gave him twice recrystallized iodoacetate, normal saline solution, two syringes, and two frogs. Then, Meyerhof asked Lundsgaard to demonstrate the iodoacetate-induced rigor. Lundsgaard injected one frog with iodoacetate (dissolved in saline) and the other frog with saline alone, then everybody was waiting for the rigor to develop. Meyerhof's assistants were standing nearby, ready to analyze the rigor muscle and the control muscle for their lactic acid content. However, the frog did not go into rigor, and after several hours of waiting, people went to sleep. On the way home, Meyerhof told Möhle that one should never trust young investigators, and he felt vindicated in his decision to invite Lundsgaard to Heidelberg before modifying his existing lactic acid theory. It was a cold winter night in Heidelberg and the temperature in the lab was below 10°C. In the morning, when the heat was turned on in the Institute, the frog developed rigor. Two new frogs, with everything else the same, proved without doubt that the temperature must be over 20°C to allow the development of rigor.

On the same premises was the Department of Chemistry of the Max Planck Institute with Professor Richard Kuhn, who had won the Nobel Prize for establishing the structure of riboflavin (vitamin B_2). He showed us his nuclear magnetic resonance (NMR) spectrometer, with a huge magnet, and the spectrum of a compound they had just

synthesized. This was one of the few NMR instruments that existed at that time.

We discussed with Professor Weber our research program: he agreed that we should first finish the work on the active sites of myosin, and then focus on the potential of the newly discovered specific inhibitors of the actomyosin ATPase. We also asked Professor Weber's help in arranging a PhD program for Kate, with physical chemistry as major, and physics and math as minors. After repeated phone calls he found the place, in Frankfurt at the Goethe University; Professors Hermann Hartmann and Joachim Stauff became Kate's advisors (Gutachter). Her research in Hungary was included in the thesis. A young Assistant Professor, Rainer Jaenicke, had offered his generous help in all aspects of getting her degree. Since then, Rainer has remained a very good friend of our family and we are indebted for everything he has done for us.

Visiting America

Michael: Before starting the lab work, I flew to America to participate at the Symposium 'Sulfur in Proteins', Falmouth, MA, May 1958. My lecture was on the functional –SH groups of myosin and actin [17]. At the end, I outlined my hypothesis about the mechanochemistry of skeletal muscle contraction:

$$\text{ATP} + \text{F-actin-ADP} \xrightarrow{\text{myosin}} \text{ADP} + \text{G-actin-ATP}$$

$$\text{G-actin-ATP} \rightarrow \text{F-actin-ADP} + \text{P}_i$$

Accordingly, myosin in muscle acts not as an ATPase but as a phosphotransferase, carrying the terminal phosphate of free ATP to the F-actin-bound ADP; the ATP formed is bound to G-actin and it is hydrolyzed during the G → F transformation. The discussion that followed centered on the possibility of actin polymerization in skeletal muscle. A few years later, Martonosi et al. [18] demonstrated that the actin-bound

nucleotide plays no significant role in skeletal muscle contraction. (Of course, this conclusion was in agreement with the unpublished results of Biró and myself concerning cardiac muscle, as described before.) However, actin polymerization with simultaneous ATP hydrolysis has been shown in motile non-muscle cells (reviewed by Carlier and Pantaloni [19]).

At the symposium I renewed my friendship with Andrew Szent-Györgyi, and met John Gergely, Manuel Morales, and Irvin Klotz, who became my close friends. Ephraim Racker was my roommate and educated me about American biochemistry. He also gave advice about how to emigrate to America. While on the East coast, I visited Woods Hole, Harvard, the NIH, Columbia University and The Rockefeller Institute (later known as The Rockefeller University). During the return flight, I planned the experiments to be carried out in Heidelberg.

The active sites of myosin

Michael and Kate: Working conditions at the Weber Institute were phenomenal. We had two assistants who never missed a day. Minced rabbit muscle was delivered to the lab from the animal quarter within 1 h of request, the machine shop was open day and night, the library had all the major journals from volume No. 1, a full meal was served at noon, and leftovers were offered for supper. As soon as our financial situation improved, we rented an apartment within walking distance from the Institute.

In our studies on the active sites of myosin, we tested the hypothesis that in actomyosin actin protected the –SH groups of myosin that were involved in the combination between myosin and actin, whereas the –SH groups of myosin involved in the ATPase activity remained unprotected. Indeed, from actomyosins treated with iodoacetamide, we isolated myosins that had lost their ATPase activity but combined with F-actin perfectly. This is shown in Fig. 1. Thus, we demonstrated that

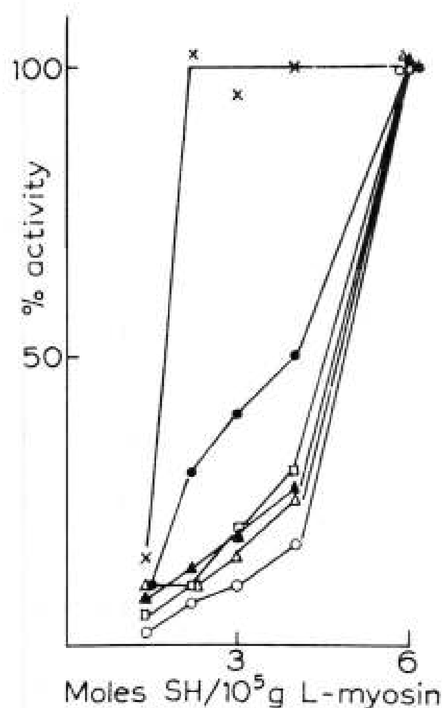


Fig. 1. Separation of ATPase activity and actin-binding ability of rabbit skeletal muscle myosin. □, myosin Ca^{2+}-ATPase activity, pH 9.1; ▲, myosin Ca^{2+}-ATPase activity, pH 6.7; △, actomyosin Ca^{2+}-ATPase activity, pH 6.7; ○, actomyosin Mg^{2+}-ATPase activity, pH 6.7; ×, actin-binding ability of myosin; ●, ATP sensitivity (reprinted from Ref. [20], with kind permission from Elsevier Science).

the sites of myosin which combine with ATP and actin are not the same [20]. Our conclusion was confirmed years later in the crystal structure of myosin subfragment 1, which revealed that the ATP-binding and actin-binding sites are located in different domains [21]. The proximity of the two sites in the crystal structure is in agreement with our finding that iodoacetamide-treated actomyosin that had lost its ATPase activity could be readily dissociated by inorganic pyrophosphate into

actin and myosin [20]. The importance of separate sites in myosin for ATP hydrolysis and actin-binding was pointed out by Morales [22] in his review on the history of muscle contraction.

Interaction inhibitors

Michael and Kate: The specific inhibition of the Mg^{2+}-activated actomyosin ATPase by polylysine could be duplicated by other polycations and, surprisingly, also by polyanions. Typically, the polyelectrolytes did not inhibit the actomyosin ATPase completely, but they reduced the high actomyosin ATPase activity to the low Mg^{2+}-moderated myosin ATPase activity. Professor Weber called them 'interaction inhibitors', because they inhibited the enzymic interaction between myosin and actin. Fig. 2 illustrates the effect of interaction inhibitors: polylysine, protamine, or heparin inhibited only the Mg^{2+}-activated actomyosin ATPase without affecting the Ca^{2+}-activated actomyosin ATPase or the Ca^{2+}-activated myosin ATPase. In contrast, cethyltrimethylammonium chloride, polyatenolol sulfonate, Germanin, Congored, or dodecylsulfate had an inhibitory effect on all these ATPase activities.

Water–glycerol-extracted psoas fibers did not contract upon addition of MgATP in the presence of interaction inhibitors. Furthermore, MgATP-contracted fibers relaxed upon addition of interaction inhibitors. Thus, evidence was provided that contraction was brought about by Mg^{2+}-activated actomyosin ATPase [23]. These polyelectrolytes were loosely bound to either myosin or actin and, therefore, their inhibitory effect on the actomyosin ATPase could be readily reversed [24].

It remained to be shown that interaction inhibitors work in living muscle in the same way as in vitro. We found that at least four charges on a molecule were needed for the interaction inhibitor effect; however, such charged compounds did not permeate through the plasma membrane of excised frog muscles. After weeks of frustration, we raised the question:

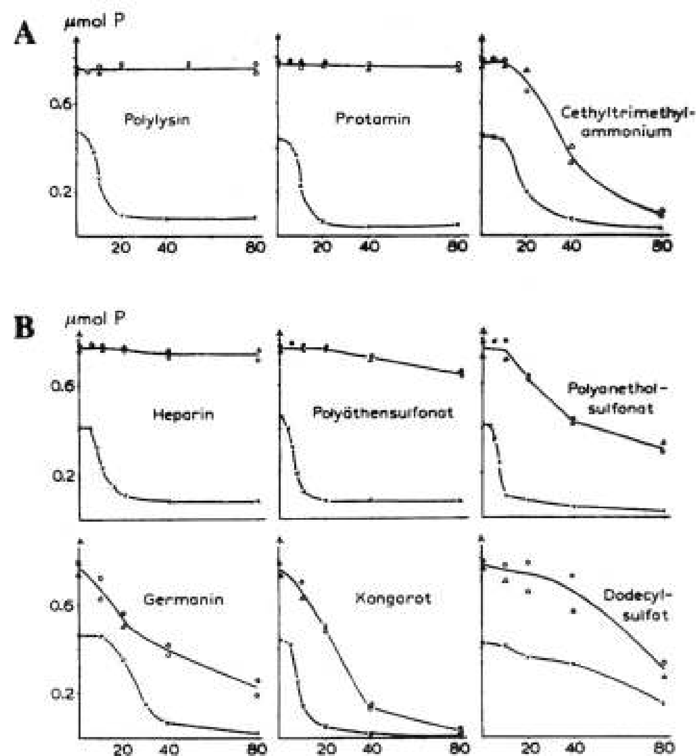


Fig. 2. (A) The effect of high molecular weight organic cations on the ATPase activity of actomyosin and myosin. (B) The effect of high molecular weight organic anions on the ATPase activity of actomyosin and myosin. Abscissa shows the number of charge equivalents of the compounds added per 100 000 g of actomyosin or myosin. ×, Mg^{2+}-activated actomyosin ATPase; ○, Ca^{2+}-activated actomyosin ATPase; △, Ca^{2+}-activated myosin ATPase (reprinted from Ref. [24], with kind permission from Elsevier Science).

what can readily enter into the intracellular muscle water? Three substances were described in the literature: O_2, H_2O, and urea. Accordingly, we tested the effect of urea on actomyosin and myosin ATPase activities. To our delight, 1.0 M urea transformed the Mg^{2+}-activated actomyosin ATPase to Mg^{2+}-

moderated myosin ATPase, without inhibiting the Ca^{2+}-activated actomyosin or myosin ATPase. (Under the same conditions, the control compound acetamide – 'half urea' – was much less efficient on the Mg^{2+}-activated actomyosin ATPase activity.) MgATP-contracted psoas fibers relaxed when 1.0 M urea was added to the bath, and their resistance to stretch dropped to zero indicating complete dissociation of actomyosin into actin and myosin. The urea inhibition of contraction was fully reversible after the urea was washed out from the fibers.

In agreement with in vitro studies we found: (1) urea inhibited contraction of living frog muscles – sartorius, rectus and gastrocnemius – upon electrical stimulation; (2) the intracellular urea concentration ranged from 0.5 to 0.9 M in the muscles when contraction was inhibited; and (3) the inhibition was fully reversible [25]. Furthermore, urea inhibited drug-induced contractures of frog muscles; 11 different agents gave the same pattern. At the same time, resting membrane potential and action potential in muscles treated with 1.0 M urea remained practically unchanged.

The paper published on the effect of urea on live muscle [25] concluded: (1) the mechanism of contraction in intact muscle is the same as in isolated contractile systems; and (2) in both, contraction–relaxation is controlled by the interaction of actin and myosin filaments and ATP: in contraction, the two types of filaments are associated and ATP is hydrolyzed by the actomyosin ATPase; in relaxation, the filaments are dissociated and ATP is hydrolyzed by the myosin ATPase. In 1960, the role of Ca^{2+} in excitation–contraction coupling was not known, leaving open the possibility that in the intact muscle, the urea effect also involves the pumping activity of the sarcoplasmic reticulum. However, the ultimate reaction in the contractile event – the interaction of actin, myosin and ATP – was clearly elucidated by our studies. Introducing urea, a substance of known in vitro function, into live muscle opened a new avenue in muscle research. We used such an approach for studying the molecular mechanism of contraction [26–29].

Leaving for America

Michael and Kate: In the Fall of 1959, Wilfried Mommaerts visited the Weber Institute. He informed us about the establishment of a new muscle research institute in New York City, directed by Dr. Ade T. Milhorat. We inquired about the possibility of positions. Dr. Milhorat's answer arrived in 2 weeks: he offered Michael the headship of a new department, called Contractile Proteins. The position provided an annual salary of $10 000; with the exchange rate of 4.25 German mark per US dollar in 1959, the salary appeared to be generous. We gladly accepted.

Before going to America we visited A.V. Hill, Douglas Wilkie, and Hugh Huxley in London, Dorothy Needham and Max Perutz in Cambridge, Victor Perry in Birmingham, Fritz Buchthal in Denmark, and Paul Edman in Sweden. In Uppsala, we visited the Svedberg museum and observed with wonder a hole in the wall caused by the explosion of an early version of the ultracentrifuge rotor. Returning to Heidelberg, we learned from Wilhelm Hasselbach the status of Ca^{2+} uptake by the sarcoplasmic reticulum and from Hartmut Hoffmann-Berling the recent advances in non-muscle cell motility. We had a general idea where muscle research stood in Europe at the beginning of 1960.

Professor Weber invited us to his house for a farewell dinner and also organized a farewell party with the participation of the entire Institute. We crossed the Atlantic Ocean from Hamburg to New York in 8 days. Caspar Rüegg succeeded us at the Weber Institute. In 1966, on the occasion of Professor Weber's 70th birthday, we edited in his honor a special issue (volume 345, No. 1) of the *Biochemishe Zeitschrift*, with contributions of the leading muscle researchers in the world. He sent us a handwritten thank you letter.

Institute for Muscle Disease

Michael and Kate: Our salary was not enough to rent an apartment in the neighborhood of the Institute in Manhattan; therefore, we settled in the outer borough of Queens. The round trip to the Institute by subway took 2–3 h per day. Less time was needed for traveling by car, if one was willing to beat the traffic by leaving between 5 and 6 a.m. and returning after 8 p.m. Routinely, we drove to the city together early in the morning, and Kate returned home by subway in the afternoon. Due to the 'nepotism rule', Kate's appointment was in the Physical Chemistry Division, under the leadership of Hans Oppenheimer. With two salaries, we could afford a housekeeper–governess living with us to watch the children.

The Institute was located in the immediate neighborhood of Cornell University Medical College and New York Hospital, a few blocks away from The Rockefeller Institute and Memorial Sloan Kettering Institute. This was one of the leading medical centers of the US in the 1960s. Dr. Milhorat, a neuromuscular disease professor of Cornell Medical College, initiated the establishment of the Institute for Muscle Disease. He conducted research on the cause and cure of muscular dystrophies in New York Hospital. The Institute was built between 1956 and 1960. It was a 10-story glass building, glamorous both on the outside and inside. It was financed by the Muscular Dystrophy Association (MDA) whose money originated from private donations and from the substantial income of the Jerry Lewis Labor Day Telethon (Jerry Lewis was the National Chairman of the Association). The top floor housed a small hospital for children suffering from hereditary muscular dystrophy (Duchenne disease). Dr. Milhorat was the head of the hospital, but a physician from New York Hospital was in charge of the daily routine. Several nurses were always in attendance, and technicians analyzed the blood and urine of the patients. A dietician ran the kitchen. An entire floor was devoted to breeding and housing experimental animals,

including chickens and mice with hereditary muscular dystrophy. The other floors were each occupied by one or two basic research departments. The largest department, Physiology, was headed by Alexander Sandow, the pioneer of excitation–contraction coupling.

When we arrived, we handed over a list of our needs to the business manager and politely asked him how much of it could be provided? He answered: you can get everything you need. Soon we learned that 'money is no problem', the Institute received \$1.2M from MDA per year, and if this was not enough, Dr. Milhorat asked for more. In setting up our laboratories, we received help from Annemarie Weber (the daughter of Professor Weber) who had emigrated to the US earlier and worked in the Physiology Department. Andreas Chrambach was the first PhD who joined us, and when he left, Eric Gaetjens came from Dan Koshland's lab. Then, Gary Bailin came from Long Island University, followed by Tom Conover from Ephraim Racker's lab. At full capacity, the Department of Contractile Proteins had six PhD associates, two technicians, one cleaning woman, and one secretary. We were working in the lab with some technical help all of the time; it has been our philosophy that the best ideas are born during experimentation. Since the Institute did not require writing grants for external support, our research could be planned on an ad hoc basis, i.e. the experiment of tomorrow was based on the result or idea of the day. As Szent-Györgyi put it, 'how can I write a long range proposal, when I even don't know what I will do tomorrow'.

Myosin in muscular dystrophy

Michael and Kate: We were hopeful that we could contribute to the understanding of the biochemistry of muscular dystrophy, and thereby fulfill our obligation to the Institute for Muscle Disease. Milhorat and collaborators hypothesized that the wasting of muscle in muscular dystrophy was caused by the

accumulation of proteolytic enzymes (cathepsins). Furthermore, Oppenheimer et al. [30] found a rather selective decrease of myosin in mice with hereditary muscular dystrophy. We thought that myosin with improper structure was synthesized in muscular dystrophy, and such a myosin would be more susceptible to proteolytic enzymes than myosin from healthy muscle. This should be reflected in the amino acid composition of myosin from dystrophic muscle versus that from healthy muscle.

Semi-automatic amino acid analysis had been introduced by Moore and Stein at the nearby Rockefeller Institute a few years earlier. We consulted Moore and Stein and received much helpful advice. A few amino acid analyzers were commercially available and we purchased one from Technicon. The analyzer was assembled from several units and required about 20 feet of floor space. Ninhydrin was prepared in 6.5 gallon quantities, and was kept under nitrogen in a black-painted glass bottle. Proteins were hydrolyzed for 24, 48, and 72 h to correct for acid-vulnerable or acid-resistant amino acids by extrapolating their concentration to zero time hydrolysis. It took a full day to complete a single analysis. The amino acid peaks had to be integrated manually. Seventeen amino acid peaks were evaluated on the analyzer (asparagine and glutamine appeared together with aspartic and glutamic acid). Tryptophan was determined spectrophotometrically. Although determination of the amino acid composition of a protein on the Technicon analyzer in the early 1960s appears to be unacceptably long by today's standards, the procedure was extremely accurate and the results were very reproducible.

We isolated myosin, homogenous in the analytical ultracentrifuge, in a good yield from the breast muscle of both dystrophic and healthy chickens. The amino acid composition of the myosin from the dystrophic muscle was identical with that prepared from the healthy muscle [31]. Homogeneous myosin was also isolated from the hind leg muscles of mice

with hereditary muscular dystrophy. No differences were found in the amino acid composition of myosins from muscles of dystrophic and healthy mice either. Furthermore, various ATPase activities and actin-binding abilities of myosins from dystrophic and healthy muscles were the same [31], suggesting no difference in their active sites. Thus, the results did not support the concept that hereditary muscular dystrophy is a molecular disease of myosin. About 20 years later, it turned out that in muscular dystrophy the affected protein is not the most abundant one, i.e. myosin, but rather a trace protein, dystrophin [32].

Myosin ATPase activity correlated with speed of muscle contraction

Michael and Kate: In contrast to the absence of any differences in amino acid composition and biological activities of myosins from dystrophic and healthy muscles, differences were readily found among myosins from cardiac and skeletal muscles of rabbit [33]. Various ATPase activities of cardiac muscle myosin were lower than those of skeletal muscle myosin. (For the Ca^{2+}-activated ATPase, this had been shown by Bailey [34].) Even larger differences were found between the ATPase activities of chicken breast and chicken gizzard myosin [35]. Inadvertently, we had become comparative biochemists, and remembered Szent-Györgyi's remark about this kind of scientist: 'The true biochemist prepares myosin from rabbit, chicken, and frog, and shows that each of these myosins hydrolyzes ATP. The comparative biochemist isolates myosin from the grasshopper and shows that it also hydrolyzes ATP.' Professor Ernst Gutmann, a visiting scientist from Prague, called our attention to the existence of fast and slow muscles. With our standard method, we prepared myosin from the fast, extensor digitorium longus, and the slow, soleus, muscles of rabbit and found that the ATPase activities of myosin from the fast muscle were 2–3-fold elevated over those of myosin from

the slow muscle [36]. Professor Gutmann also told us that muscles of newborn mammals are physiologically slow, and we found that the ATPase activities of myosin of 2-day-old rabbits were about one-half of those of myosin of adult rabbits [37]. In the case of invertebrate muscles, actomyosin from the fast striated scallop adductor had about twice as high Mg^{2+}- and Ca^{2+}-activated ATPase activities as those of actomyosin from the translucent part of the smooth scallop adductor (intermediate speed), and about five times as high Mg^{2+}- and Ca^{2+}-activated ATPase activities as those of actomyosin from the opaque part of slow smooth scallop adductor [38]. A relationship between ATPase activity of myosin and speed of muscle contraction started to emerge.

In the Spring of 1965 Michael spent some time in Professor Gabriel Hamoir's laboratory in Liege as a Fellow of the Belgian National Scientific Council. Professor Michel Goffart at the adjacent Physiology Department had a sloth farm. While viewing the sloths hanging on the trees, it occurred to Michael that the ATPase activity of the sloth muscle myosin may be low. Upon discussing this idea with Professor Goffart, it turned out that their laboratory determined the speed of contraction of several sloth muscles and compared it with the corresponding cat muscles. A collaboration was arranged in which sloth and cat muscles, with known contraction times, were dissected in Liege, transferred into glycerol at − 20°C, and shipped to New York by air in insulated polyfoam containers. We prepared myosins from the glycerinated sloth and cat muscles, showed them to be homogenous in the analytical ultracentrifuge, and subjected them to various assays. Actin-, Ca^{2+}-, and K^{+} (EDTA)-activated ATPase activities of myosins from cat muscles were two to four times higher than those of the myosins from the same muscles of the sloth. The difference in Ca^{2+}-activated ATPase activity was found in the pH range of 5.5–10.0, and in the KCl concentration range of 50–500 mM, at pH 7.0. The actin-activated ATPase activity of the cat and sloth myosins was found to be inversely proportional to the

contraction time of their respective muscles, i.e. the less time was required to reach half of the maximal isometric tension, the higher was the myosin ATPase activity, and vice versa [39]. These experiments demonstrated a correlation between contraction time of muscle and ATPase activity of myosin from the same muscle.

We were interested to find out to what extent this correlation holds in the animal kingdom. With the help of Professor Ladder Prosser from the University of Illinois at Urbana, we collected (in Woods Hole) muscles from invertebrates with up to 300-fold differences in contraction time. The ATPase activities of the myosins from these muscles were also inversely proportional to the isometric contraction times of their muscles [40].

Since the isometric twitch contraction time is dependent upon the properties of the series elastic component, the duration of the active state, and the force–velocity properties of the contractile component (the only one that can be related to the ATPase activity of the myosin), we compared the ATPase activities of several myosins with the maximal speed of shortening (V_0, intrinsic speed) of their muscles, that is a true property of the contractile material. The muscles used were of mammals, lower vertebrates, and invertebrates. Actin- and Ca^{2+}-activated ATPase activities of the myosins were generally proportional to the speed of shortening of their respective muscles, i.e. the greater V_0, the higher the ATPase activity [40]. This relationship was found when the speed of shortening ranged from 0.1 to 24 muscle length/s; it is depicted in Fig. 3. In contrast to the ATPase activity of myosin, which varied according to the speed of contraction, the F-actin binding ability of myosin from various muscles was rather constant.

When this work was scheduled for presentation at a New York Heart Association symposium, just before the lecture a fire broke out in the hotel and the audience left the hall. Thus, the results were not known to the scientific community for a while. After publication of the symposium's proceedings,

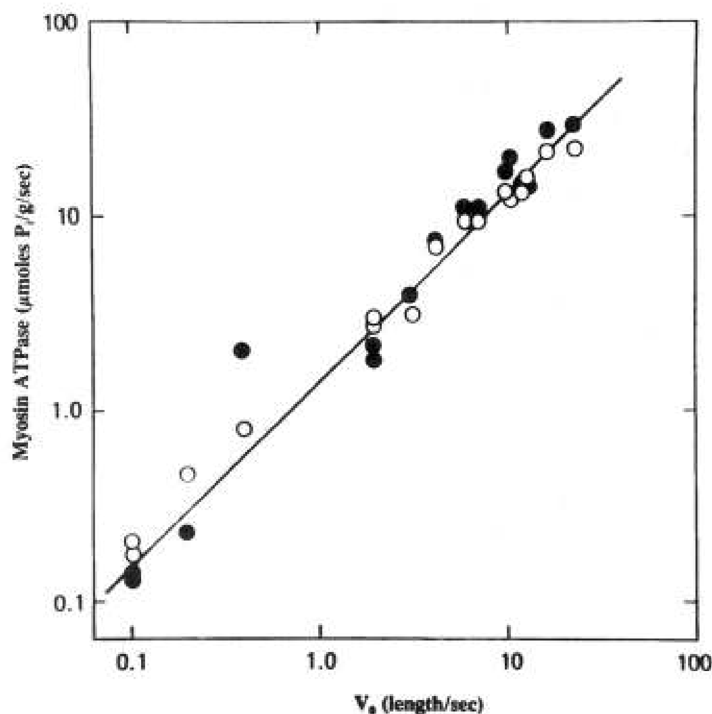


Fig. 3. Relationship between speed of muscle contraction and ATPase activity of myosin. ●, actin-activated ATPase; ○, Ca^{2+}-activated ATPase (modified from Ref. [40]).

Michael was invited to a symposium of the 7th International Congress of Biochemistry in Tokyo and to a Gordon conference. In 1984, *Current Contents* counted over 725 citations of the work correlating myosin ATPase activity with speed of muscle contraction, and it was one of the five most cited papers for the *Journal of General Physiology*. In 1986, it was included in a volume of *Contemporary Classics in the Life Sciences* [41]. As of 1997, a total of about 1700 citations were registered.

Myosin ATPase activity in cross-innervated muscle

Michael: One of the major discoveries in neurophysiology was the observation that cross-innervation transforms mammalian fast muscle into slow muscle and vice versa [42]. In spite of numerous physiological studies on this phenomenon, the underlying biochemical changes were essentially unknown. Victor Dubowitz, a visiting scientist from England, repeated the experiments of Buller et al. [42]. He had found, by histochemical techniques, that cross-innervated fast muscles in kittens 18–20 weeks after the operation exhibited enzymic reactions characteristic of normal slow muscles. To a limited extent, the opposite was also true, a few of the cross-innervated soleus muscles exhibited patterns characteristic of normal flexor digitorum longus [43]. Victor and I started to collaborate on biochemical characterization of myosin in cross-innervated kittens, but he was called back to London. At that time, several papers were published on changes in enzymic profiles upon cross-innervation [44–46] including the enzymic properties of myosin [47]. I was pleased when I received a letter from Russell Close, Canberra, Australia, asking me to join him in studies on the biochemical and physiological characterization of cross-innervated rat extensor digitorum longus (EDL) and soleus (SOL) muscles.

Our collaboration through the New York–Canberra distance followed the same pattern as that between New York–Liege, described before. Muscles were dissected from cross-innervated, self-innervated, and control rats, 380–420 days after the operation, and the characteristics of isometric twitch and tetanic contractions were determined. The muscles were then transferred into glycerol and shipped to New York for analyses of properties of myosin and actomyosin. The ATPase activities of myosin and actomyosin of cross-innervated EDL decreased to the level of those of normal or self-innervated SOL, and the ATPase activities of cross-innervated SOL approached the about 2-fold higher level of normal or self-innervated EDL

[48]. The changes in ATPase activities of myosins of cross-innervated muscles were related to changes in force–velocity properties of these muscles. Of the various ATPase activities, the actin- and Mg^{2+}-activated ATPase activity of myosin and the Mg^{2+}-activated ATPase activity of actomyosin showed the highest degree of correlation with the V_0 of the muscles. This provided strong evidence in support of the hypothesis that neural influences determine the fundamental dynamic properties of the contractile material through an effect on the ATPase site of myosin.

Changes in the structure of myosin caused by cross-innervation were also detected by methods other than ATPase activity determinations. Sreter et al. [49] and Samaha et al. [50] described that slow muscle myosin ATPase is labile to alkali and stable to acid, whereas for fast muscle myosin ATPase activity the opposite is true, i.e. alkaline stability and acid lability. We found that the pH profile curve of cross-innervated SOL myosin ATPase followed the profile of normal and self-innervated EDL myosin ATPase, whereas cross-innervated EDL myosin ATPase exhibited the pattern of normal and self-innervated SOL myosin ATPase activity [48]. Previously, we had shown that the ATP-mediated changes in the dinitrophenylation of slow muscle myosin were less pronounced than those of fast muscle myosin [51]. When this test was applied to cross-innervated SOL, the pattern imitated that of fast muscle, whereas cross-innervated EDL behaved like a slow muscle [48]. Thus, different kinds of evidence indicated that the 13–14 month cross-innervation period resulted in a major transformation of the myosin molecule.

The long incubation time necessary for the cross-innervation to be manifested in a new type of myosin suggested de novo myosin synthesis. This led to the hypothesis that innervation controls gene expression of contractile proteins in adult muscle. Later, it was found that chronic electrical stimulation of muscle at low frequency also transformed fast muscle myosin into slow muscle myosin [52]. A plethora of publi-

cations appeared on the effect of neural interventions (cross-innervation, chronic stimulation, denervation) to the expression of fast and slow muscle myosin isoforms. The very active research in this field revealed neural influences on the distribution of the components of the troponin complex [53], on the composition of tropomyosin subunits [54], and on muscle energetics [55]. Apparently cross-innervation transformed not only myosin but the entire muscle cell.

Conformational changes in myosin

Michael and Kate: The correlation between myosin ATPase activity and speed of muscle shortening initiated research on the molecular mechanism of muscle contraction. The power output of a muscle equals force times speed. The force component, the combination of actin with myosin, does not vary significantly in the animal kingdom. Therefore, the myosin ATPase activity controls muscle power. The nature of the muscle engine was a primary interest of biochemists ever since the discovery of Engelhardt and Lyubimova in 1939 [56] that myosin, the contractile protein, hydrolyzes ATP, the energy-yielding substance of the muscle. In classroom-teaching of muscle energetics, the muscle engine is usually compared with man-made engines. In a heat engine, the combustion of the fuel performs the work without changing the structure of the engine. Meyerhof postulated that in the biological engine, the chemical process that supplies the energy acts on the structure of the machine [57]. However, Meyerhof did not carry out experiments to test his theory. Based on the hypothesis of Dan Koshland [58] that ligands induce conformational changes at the active sites of enzymes, we thought that binding and/or hydrolysis of ATP might change the structure of myosin. Our strategy was to permeate into live muscle highly reactive reagents that could form covalent linkages with myosin, then establish the location of the reagent in the myosin molecule, and finally compare the reac-

tivity of the reagent with myosin in contracting muscle versus resting muscle.

Our first choice was 1-fluoro-2,4-dinitrobenzene (FDNB), the reagent used by Bob Davies and collaborators to demonstrate ATP hydrolysis during contraction of intact muscle [59]. (This is the same reagent that was used by Sanger to determine the amino acid sequence of insulin.) The tritiated form of FDNB was available commercially; this allowed an easy determination of the extent of myosin reaction with the reagent. In vitro studies on the incorporation of [^{3}H]FDNB into myosin at physiological ionic strength showed that free ATP increased the incorporation whereas MgATP or actin decreased it [60]. Most of the label was in the head part of myosin (cysteine, tyrosine, lysine, and histidine residues were dinitrophenylated). Subsequently, live frog muscles were exposed to [^{3}H]FDNB: one of the paired muscles was electrically stimulated for about 1 h while the other muscle remained resting. Myosin and sarcoplasmic proteins were isolated from the stimulated and resting muscles; the incorporation of reagent into myosin was decreased during isotonic contraction, but incorporation into the sarcoplasmic proteins did not differ [26]. Furthermore, the incorporation of [^{3}H]iodoacetate into myosin or sarcoplasmic proteins during contraction did not differ from that into myosin or sarcoplasmic proteins of the resting muscle.

Out of the various radioactive reagents tested to further probe structural alterations in myosin in vivo, ^{14}C-labeled *N*-ethylmaleimide ([^{14}C]NEM) was selected because of its high incorporation into myosin, which allowed determination of the distribution of the label in the substructure of myosin. Most of the [^{14}C]NEM was incorporated in the head part of myosin in the live muscle, and only a small amount of label was found in its rod-like part [27]. The entire difference in incorporation between contracting and resting muscle (0.5–0.7 mol label per mol of myosin) occurred in the globular head (Fig. 4). This indicated clearly that the change in reactivity during

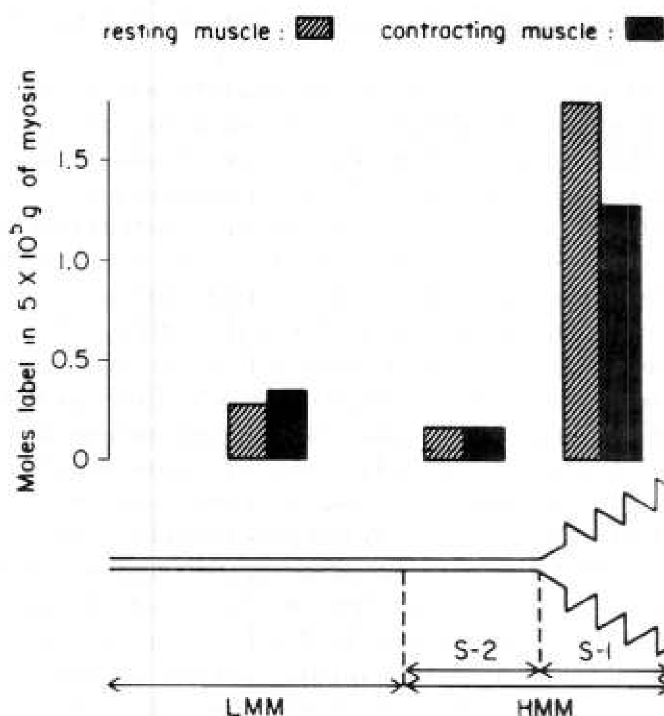


Fig. 4. Distribution of [^{14}C]NEM-label in the substructure of myosin of resting and contracting frog muscle. LMM, light meromyosin; HMM, heavy meromyosin; S-2, myosin subfragment 2; S-1, myosin subfragment 1 [62].

contraction was localized to the crossbridges, whereas the backbone of the myosin filament and its linkage with the crossbridge remained unaffected. Further information was obtained by proteolytic digestion of the myosins from contracting and resting muscles. Analyses of the peptides showed that most of them were similar but a number were different. The multiplicity of differences suggested that during contraction of live muscle, a conformational change occurs in a certain area of the crossbridges, rendering this area inaccessible for reaction with [^{14}C]NEM.

Using frog semitendinosus, a muscle that can be stretched without injury, we modified the interaction of actin, myosin and ATP in the intact muscle [61] as follows: (1) actin was prevented from interacting with myosin by stretching the muscle to 140% of its rest length, so that no tension developed upon stimulation; (2) ATP was eliminated by inducing rigor with iodoacetate, thus allowing maximal combination of the actin filaments with the crossbridges; or (3) the interactions of myosin with both actin and ATP were blocked by treatment of the muscle at 140% rest length with iodoacetate. By comparing the reactivity of myosin with [^{14}C]NEM in the modified muscles with that of the control muscles, the requirements for the changed reactivity of myosin during contractions could be established. The results demonstrated that the simultaneous interactions of myosin with actin and ATP, corresponding to isotonic or isometric contraction, were required for the maximal decrease in the incorporation of [^{14}C]NEM into myosin. Since under these conditions the incorporation of [^{14}C]NEM into sarcoplasmic proteins, myosin light chains, tropomyosin, troponin, actin, and other proteins did not change [61], we proposed that the conformational change in the myosin head is the driving force for generating movement and tension [61,62]. This proposal, derived from experimentation on live muscle, is essentially the same that was derived 20 years later from X-ray crystallography of the myosin head [63].

Our proposal that the concerted interaction of actin and ATP on myosin produces force and movement is not incompatible with the existence of the myosin·ADP·P intermediate [64] that plays a central role in current theories of muscle contraction. It is possible that, instead of ATP and actin, the ADP·P product and actin change the conformation of the myosin head during intact muscle contraction. However, it should be pointed out that we could not find myosin-bound ADP in resting frog muscle [61,62], and therefore it remains a challenge to current muscle researchers to prove that myosin·ADP·P indeed occurs in live muscle.

We further developed the technique of protein labeling in vivo. Tritiated water, added into the Ringer's solution bathing the frog muscles, rapidly equilibrated with the intracellular water. The tritium easily exchanged with the hydrogens of the protein side chains, and much more slowly with the peptide N–H protons. The rate of incorporation of tritium into the slowly exchangeable peptide protons of myofibrils decreased as a result of muscle stimulation [28]. We worked out procedures for fractionation of myofibrillar proteins in anhydrous media. The results indicated conformational changes in the peptide backbone of myosin and a weakening of binding between regulatory proteins and actin during muscle activity. These observations foreshadowed the currently well-accepted protein movements in the thin filaments during contraction.

Myosin light chains

Kate: Parallel with our studies on the ATPase activity of myosin, we decided to take a new look at the structure of myosin. Previous studies with guanidine hydrochloride and urea, agents which break hydrogen bonds, demonstrated dissociation of myosin into subunits with MW of about 200 000. Hans Oppenheimer used a milder approach: succinylation of myosin converted the positively charged lysine residues to negatively charged residues, thereby introducing an electrostatic repulsion within the myosin molecule. The succinylated myosin exhibited polydispersity in the analytical ultracentrifuge: behind the main peak, a much slower sedimenting component appeared [65]. The sedimentation coefficient of the main peak was in the range of the native myosin. Thus, we concluded that succinylation, unlike guanidine hydrochloride, does not dissociate the myosin molecule into identical polypeptide chains, but releases a much smaller subunit [66]. Independent studies by Paul Dreizen and collaborators at Downstate Medical Center in New York also found polydispersity of myosin in the analytical ultracentrifuge; the

terms heavy and light chain were suggested for the faster and slower sedimenting components, respectively [67]. We localized the light chain component to the heavy meromyosin part of the myosin molecule [68]; this was the first indication that the light chain may be involved in the functional properties of myosin. Succinylated myosin showed multiple bands in polyacrylamide gel electrophoresis [66]. The purified light chain fraction was resolved into three components by DEAE chromatography, with each fraction migrating as a single peak on gels [69]. This study of succinylated myosin and meromyosins was the prelude to the current research on the light chains of myosin.

Actin

Michael: Straub described in his classic studies that actin contains bound calcium [70]. We confirmed the presence of Ca^{2+} in purified actin preparation, 1 mol of Ca^{2+} per G-actin monomer, and showed that the ratio of bound Ca^{2+} to bound nucleotide is close to 1 [71]. The bound Ca^{2+} of actin readily exchanged with free Ca^{2+} in G-actin but not in F-actin; Ca^{2+} did not exchange in F-actin when synthetic actomyosin hydrolyzed ATP in the presence of Mg^{2+} [72]. Based on the discovery of Asakura [73] that under ultrasonic vibration F-actin catalyzes the hydrolysis of added ATP, we used ultrasonic vibration to exchange the bound Ca^{2+} or nucleotide of F-actin [74–76]. Furthermore, we used ultrasonication to remove either the bound Ca^{2+} or the bound ADP from F-actin. Unexpectedly, the Ca^{2+}-free or ADP-free F-actin combined with myosin and activated the ATPase activity of myosin as normal F-actin did. It became clear that only the amino acid side chains of actin are involved in its interaction with myosin. With Eric Gaetjens, we initiated the structural analysis of actin; the sequence of the amino terminal was found to be *N*-acetyl-Asp-Glu-Thr [77]. Later it was found that the amino terminal region of actin is rich in aspartic and glutamic acid residues and this negative

cluster participates in the combination of actin with myosin ('the weak binding site').

The mechanism of ATP cleavage during the G → F transformation of actin remained still open. In the 1950s, Dan Koshland studied the mechanism of ATP hydrolysis by myosin using $H_2{}^{18}O$ water. He was senior investigator of the Biology Department at Brookhaven National Laboratories, 40 miles from New York City, and he visited me occasionally when he came to the City. We discussed a joint project to study ATP hydrolysis by actin in $H_2{}^{18}O$. This was the largest preparative experiment of my scientific career. A few details are described. Forty adult rabbits were used for preparation of the acetone-dried actin powder. Purification of actin required a full month of work by myself and two diligent assistants. For ^{18}O analysis, 11 750 mg of F-actin, containing 185 μmol of bound ADP and 164 μmol of P_i, was dissolved in 1766 ml of $H_2{}^{18}O$. Until this stage, we had worked in New York City, but now it was time to drive to Brookhaven and continue the work with Dan's assistant. We were tense and it happened that one of the two P_i samples was lost. Fortunately, the remaining P_i sample contained the incorporated ^{18}O, whereas the phosphates of the two ADP samples were free of isotope. This indicated unambiguously that during polymerization of actin in $H_2{}^{18}O$, the nucleophilic attack was on the terminal phosphorus atom of ATP [78]. In other words, despite its significant difference from ATPases, actin caused a similar cleavage of the terminal phosphorus–oxygen bond.

In the 1970s, evidence accumulated for the role of actin in non-motile cells. I wished to contribute to this new actin chapter. In collaboration with the Holtzers, we showed that chondroblasts, cells notoriously sessile, contained considerable amounts of a protein that had bound ADP in a manner which was indistinguishable from actin [79].

Raising children

Kate: On the occasion of Francis' graduation from The Rockefeller University in June 1981, we had the honor of having lunch with the Nobel Prize winner Joshua Lederberg, the President of The Rockefeller University. We had a lively discussion about nature versus nurture in raising a successful individual. Here I will briefly describe my ideas about nurturing.

I nursed both of my children for 9 months, strictly on a schedule as recommended by a well-known Hungarian pediatrician in her book. I thought punctuality would be in their blood, but that was not realized. I cuddled with them, talked to them, sang to them, and always safeguarded their well-being. I was reading the experts about how to avoid jealousy and resentment among siblings. I spent time alone with each of the boys every single day in order to know what was going on in their world. Toys were carefully chosen to fit the interests of each of the boys [80].

In 1958, when we lived in Heidelberg, I hired a lovely housekeeper–governess. She came with us to New York. We had a mutually satisfying relationship: she took care of the children according to my instructions and we took her to various cultural activities. After her return to Germany, her friend took her place, then her sister, and finally another friend. In summary, we were very lucky concerning childcare.

When we emigrated to the US, we lived in an outer borough. Michael and I left the house very early when the boys were still sleeping. I telephoned them every morning. I returned from work in the early afternoon so I could be with them. I developed in the boys habits of learning and specific ambitions. I was careful that they should neither be bored nor frustrated. They had natural abilities which were honed and polished within the family circle and in schools. One of our mottos was, 'Mens sana in corpore sano'. Sports were cultivated. For example, I prepared a poster about 17 good reasons

for doing push-ups and the boys did their push-ups routinely after getting out of bed every morning. We loved classical music, and we regularly attended operas, ballets, and concerts. The boys learned to play the piano.

One family tradition was a system of 'raisin accounting'. The boys loved raisins in their breakfast cereal. My younger son Francis in his first school year happened to be in group 2 for reading. He explained that not everybody can be in group 1, some children must be in group 2, which is not the worst. Anyway, in group 2 he was the best. I promised him 50 raisins for working his way up to group 1. He achieved this in 3 weeks. My older son George joined the raisin system. Eventually, the boys lost raisins for failing to do something and learned about negative numbers.

In 1965, I was invited to a Conference and had to prepare for that. I skipped our daily special time with the boys. George had a temper tantrum and I scolded him. He retorted, 'A mother who is successful in her profession but did not bring up her child in a way she wanted is just a failure'. From this time on, I never shortchanged my children.

In 1966, we moved to Manhattan in the vicinity of the Institute for Muscle Disease. We had good schools nearby. Michael and I walked to work; the boys walked to school and they were getting more and more independent. George attended a 2-year Special Program in Junior High School. This allowed him to progress beyond the usual curriculum for a given grade. As a matter of fact, by the end of his 9th grade, George took also the 10th and 11th year math Regents exams; he learned a lot as a member of the Math Team and I also coached him. He was the top scorer in the citywide math competition. I was delighted. However, this Special Program in New York City was criticized as elitist and was discontinued. Throughout the years, I was very active in the Parent–Teacher Association. With the Association we succeeded in reinstating the 2-year Special Program; thus, Francis also became the beneficiary of this outstanding program. Both boys were accepted to a magnet

science high school, Stuyvesant, where the students were stimulated not only by superior teachers but also by the challenge of other exceptional peers.

Michael was completely devoted to his research and worked all the time. Michael and I always discussed the principles of child-rearing and he fully supported my activities. As the boys got older, Michael enjoyed playing a game of tennis or chess with them, and he was rooting for them at various competitions. Actually, George won the New York City high school tennis championship. Once, Francis won $32 at a chess tournament in New York City. To avoid mugging, he hid his prize money in his sneakers while walking home across Central Park.

When we lived near to the Institute, the boys came in regularly and naturally became interested in research. First, they learned some techniques and the use of our equipment. George asked for his own research project and Michael let him work on frog myosin. Once George ran myosin ATPase assay at 37°C. The bath was on the top of the bench and in order to reach it, George had to stand on a stepstool. He was pipetting samples from the reaction mixture into centrifuge tubes (containing trichloroacetic acid) so energetically that the stepstool was swaying. Michael was afraid that George would fall; he told him that there was no need to take an aliquot from the bath every half minute but a five-minute interval would be sufficient. George turned to Michael and told him, 'Dad, I know what I am doing'. Francis selected the Venus' flytrap for his own research. He wanted to isolate the enzyme from the plant that 'eats the meat'. With Sephadex chromatography, he isolated a fraction from the plant extract that hydrolyzed both myosin and hemoglobin. Enormous quantities of Venus' flytraps were needed for the isolation of the enzyme. After wasting lots of time to catch flies for feeding the traps, Francis discovered that the traps eat hamburgers as well.

The boys won numerous awards at science and math fairs.

George and Francis have always been disciplined, diligent, conscientious, motivated, and above all honest, kind and good human beings. George was accepted at The Rockefeller University directly from high school at the age of 16 and received his PhD at the age of 22. He was selected as one of 'America's 100 brightest scientists under forty' [81]. Currently, he is Distinguished Professor of Chemistry at the University of Minnesota. Francis was a national finalist of the Westinghouse Science Talent Search and his picture was featured on the front page of the New York Times Magazine as 'Lord of the Venus Flytrap'. The accompanying article was very complimentary to the whole family [82]. Francis received his PhD from The Rockefeller University at the age of 24. Currently, he is Professor of Microbiology at Cornell University Medical College.

Closing the Institute for Muscle Disease

Michael and Kate: Our director, Dr. Milhorat, reached retirement age. MDA conducted a nationwide search and found a new director from a leading university. As we recall, he was wearing a bow tie, had a pleasant smile, and let us do whatever we wanted. It was his philosophy that any good research (not necessarily muscle research) may lead to a major discovery in hereditary muscular dystrophy, since the cause of the disease was unknown. To our surprise, he refused to participate in the Jerry Lewis Telethon, because – in his opinion – it was not the job of the director of the Institute for Muscle Disease to help MDA in fundraising. Soon we heard rumors that MDA was establishing Jerry Lewis research centers throughout the USA and even abroad. The argument was: MDA's research dollars would be better invested in cost-effective university environments than in an expensive private institute.

In the middle of July 1973, MDA announced the closing of the Institute for Muscle Disease effective 30 June 1974. In

order to help the scientific staff to continue their careers, MDA offered to continue paying their salaries at their new locations for 3 years, and to allocate research grants. Department heads who could find a position before the closing date could transfer the unused part of their budget to their new institution and MDA was willing to pay for shipping of their equipment. Michael's yearly budget was \$200 000; we realized that even a small fraction of this money would be invaluable at any new place. Shocked by losing our jobs so abruptly, we were looking for tenured university positions. János Molnár, our previous colleague in the Straub Institute, and Harold Feinberg, our good friend from the Federation Meetings, both on the faculty of the University of Illinois College of Medicine in Chicago, informed their dean of our availability. Dean Truman Anderson was looking for research-oriented people and our background suited well into his plans. On 1 February 1974, Michael moved to Chicago as a tenured Professor of the Department of Biochemistry, while a few months later Kate was recruited as a tenured Associate Professor of the Department of Physiology. Francis enrolled at the University of Illinois at Chicago and George moved to the dormitory of The Rockefeller University. Francis got a BA in chemistry in 2 years and then joined George as a graduate student of The Rockefeller University.

University of Illinois at Chicago

Magnetic resonance spectroscopy

Michael: When I arrived at Chicago, I was pleased to learn about a nuclear magnetic resonance (NMR) spectrometer that was housed in the Research Resources Facility of the Medical Center. I was fascinated by viewing the phosphorus (^{31}P) spectra of human red blood cells recorded by Tom Henderson and Aki Omachi [83]. This was the second ^{31}P NMR spectrum published in the US; the first was recorded

by Moon and Richards [84] on rabbit red blood cells at Caltech. It was surprising to me that one could see resonances in a cell suspension, since I had always thought NMR was applicable only for solutions. I thought that if one could record ^{31}P spectra of red blood cells one could also record the spectrum of muscle, a tissue much richer in phosphorus metabolites. Thus, I sacrificed a large frog (in the NMR room), quickly dissected the gastrocnemius muscle, placed it into the 10 mm NMR tube, pressed the muscle to the bottom of the tube with a Teflon plug, and then handed it over to Tom Glonek, the NMR spectroscopist. Tom inserted the reference capillary through the plug into the muscle sample, put the tube between the two poles of the visible magnet, and started to spin the muscle. Due to the primitive computer techniques in 1974, we had to wait until all data were collected (90 min) before Tom could work up the spectrum [85]. This is reproduced in the upper part of Fig. 5. The spectrum revealed the major phosphate compounds, phosphocreatine, ATP, inorganic phosphate, and sugar phosphates, all known to be present in muscle. The inorganic phosphate peak was much larger than expected from analytical determinations; this was explained by the breakdown of phosphocreatine during the prolonged incubation of the thick gastrocnemius muscle that could not be saturated with atmospheric oxygen. Importantly, in the center of the spectrum, two resonances were noted which could not be correlated with the known phosphates of muscle tissue listed in textbooks.

At that time, I was invited to a winter conference of the University of California to speak about structural changes in myosin during muscle contraction. However, I talked about our ^{31}P NMR studies. At the end of my presentation, the audience applauded enthusiastically and a vivid discussion followed. Professor Andrew Huxley was among the discussants and he mentioned that already George Radda and collaborators, in Oxford, reported the ^{31}P NMR of rat muscle [86]. That paper was published in *Nature* at the end of 1974, and due

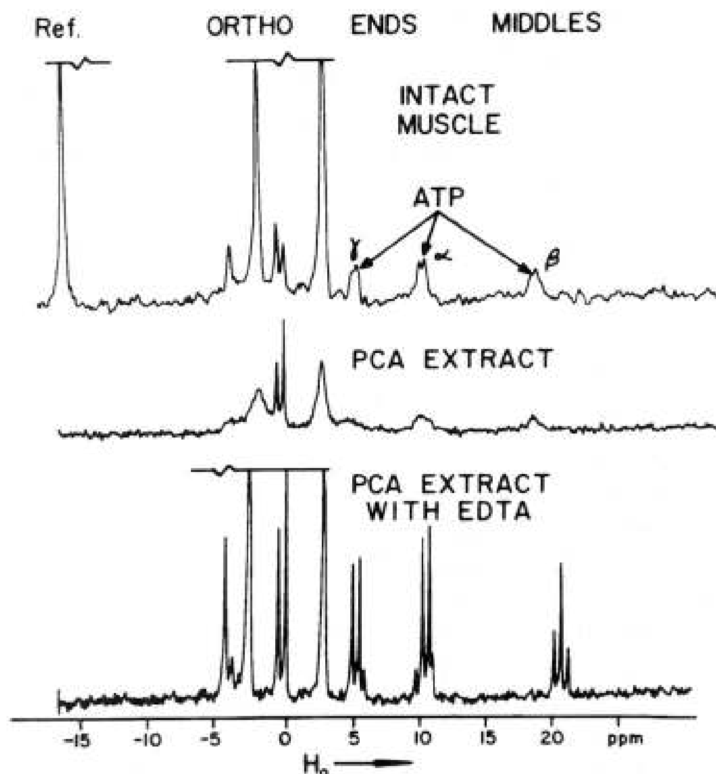


Fig. 5. [31]*P NMR spectra from intact frog gastrocnemius muscle, a perchloric acid (PCA) extract of minced gastrocnemius muscle, and the same PCA extract in the presence of EDTA. Ref. is the position of resonance reference, methylenediphosphonate; ORTHO corresponds to (from left to right) sugar phosphates, orthophosphate, two unidentified peaks, and phosphocreatine; ENDS are the terminal phosphates of ATP, γ and α, MIDDLES from the middle phosphate, β, of ATP. The prominent resonances have been truncated for the purpose of illustration [85].*

to the Christmas mail traffic, was not available here in January of 1975. Thus, this was the first time we heard about Radda's work.

Tyler Burt, my postdoctoral associate, joined the ^{31}P NMR

project. Tyler, Tom, and I quantified the concentrations of phosphate metabolites, measured the intracellular pH, and established the complexation of ATP with Mg^{2+} in various intact muscles [87]. The ^{31}P spectra also showed the presence in muscles of unknown phosphate peaks that resonated in the phosphodiester region. We were excited when it turned out that one of the peaks was a metabolite of the dystrophic chicken pectoralis muscle, but not of the normal pectoralis [87]. Isolation and identification of the compounds that gave rise to these resonances became our primary goal. The compound showing resonance at 0.13 ppm was purified and identified as glycerol-3-phosphorylcholine (GPC) [88]. The phosphodiester which resonated at 0.4 ppm was purified by Joe Chalovich, an outstanding new graduate student (now Professor of Biochemistry at East Carolina University); it was identified as serine ethanolamine phosphodiester (SEP) [89]. We extended our research with several new collaborators: Sheila Cohen, the NMR spectroscopist of our Chemistry Department, Rich Labotka and Mark Vuolo, medical students, and Morris Danon and Brian Huncke, clinicians working on human muscle diseases. As human muscle biopsy samples became available, we showed that GPC was absent in the muscles of children suffering from the Duchenne hereditary muscular dystrophy [90,91]. It appeared that lipid derivatives would play a major role in the search for clues to the nature of hereditary muscular dystrophy. Therefore, with Louis Schliselfeld and Joe Chalovich we characterized the enzymes involved in GPC and SEP metabolism [92–94]. This research was supported generously by MDA.

In 1977, we wrote the first review on biological ^{31}P NMR in *Science* [95]. Subsequently, I received many letters. The biologists realized that with ^{31}P NMR, one could obtain chemical information without destroying the tissue. The publishers thought they could get rich by selling ^{31}P NMR books and we got offers to write them. Our pioneering work was acknowledged by an invitation to write a chapter for the next *Annual*

Reviews of Biophysics and Bioengineering [96]; other prestigious invitations followed [97,98].

At the end of the 1970s, research on biological NMR exploded: a large number of investigators entered the field and high resolution instruments appeared on the market. Our existing 90 MHz Bruker instrument became outdated. For us to remain competitive, there was an absolute need to get a spectrometer of much higher field strength. Unexpectedly, the administration of the university did not support our grant application for a better NMR machine, but preferred the alternate proposal of a newly hired department head. My senior associates, Tom Glonek and Tyler Burt, were disappointed and they soon left the university for better facilities elsewhere. Don Doyle, a recent PhD in physiology, joined me from our sister campus at Urbana (160 miles south from Chicago) and called my attention to a new 360 MHz machine in the Urbana Chemistry Department. However, since this instrument had no ^{31}P probe, I decided to try ^{13}C NMR. We traveled from Chicago to Urbana with the surgical tools in our bag, sacrificed the chicken or frog in the Physiology Department, carried the dissected muscles in ice to the Chemistry Department, and worked overnight to record carbon spectra. Steve Ulrich, the NMR spectroscopist, kindly taught us the use of the spectrometer. We reported the first natural abundance ^{13}C NMR spectra of intact muscle [99], which showed resonances from carbons of lactic acid, creatine/phosphocreatine, and lipids. Next we quantified lactic acid production in caffeine-treated frog muscles [100] and also showed the mobilization of phospholipids in frog muscle upon caffeine contracture [101]. My interest in ^{13}C NMR continued for several years: we compared the metabolite composition of normal human muscle with that of diseased muscle [102], separated phosphocreatine from creatine in frog muscle [103], detected increased mobility of phospholipid resonances in brain of rats anesthetized with halothane [104], and assigned 55 resonances in rat brain [105]. The

last mentioned of these contributions became a reference of biological ^{13}C NMR spectra.

At the beginning of the 1980s, in response to the great demand for high resolution spectrometers, NIH established NMR centers, which were free to qualified investigators whose proposal was approved by the director of the center. Purdue University had the center nearest to Chicago and I was glad when John Markley accepted me as a user of their center. Our long collaboration was most fruitful, and I could also help John in the renewal of the center grant at Purdue. The Purdue NMR facility housed a 470 MHz instrument, the highest field strength in the Midwest at this time. Carlos Arús came to work with us from Spain and he was anxious to record ^{1}H NMR of intact muscle. This was a very ambitious goal, because it required suppression of the about 90 M protons in the total muscle water (about 81% of the skeletal muscle volume is water) in order to see the protons in metabolites, present only in mM concentrations. Plateau and Guéron [106] had just published a pulse sequence for water suppression in the *Journal of the American Chemical Society*, and using their sequence in the 470 MHz machine Carlos was able to record the first ^{1}H NMR spectrum of an intact tissue, the freshly dissected tibialis posterior and sartorius muscles of the frog [107]. It took us a while to identify all of the proton resonances in muscle and brain [108,109]; these became reference papers in ^{1}H NMR spectroscopy. This research attracted two experienced spectroscopists: Yen-Chung Chang from Iowa State and Palamadai Venkatasubramanian from Texas. We used ^{1}H NMR to characterize the metabolite pattern of intact tissues [110], and the pattern of normal versus diseased human muscles [111,112], and to measure the temperature in brain slices [113].

Finally, our own institution acquired high resolution spectrometers, and our research could be continued in Chicago. Here we worked out a procedure for NMR measurements of intra- and extracellular sodium in intact tissues [114].

In the 1980s, NMR technology advanced with the development of the large bore magnets which first accommodated live animals, from rat to rabbit, and subsequently live human beings. The name of biological NMR was changed to MR, and its goal was oriented to human disease diagnosis by magnetic resonance imaging (MRI). The visualization of live tissue structures by MRI provided better resolution than seen in anatomical handbooks; hospitals were rushing to purchase MRI machines. Siemens, Philips, and several other electronics companies focused on production of MRI imagers, and sometime later, General Electric (GE) entered the race. In order to attract attention, GE introduced a high resolution machine, called Signa, that operated at 1.5 Tesla (T) field strength (about 64 MHz), as compared with the 0.5 T of the other manufacturers. At 1.5 T, magnetic resonance spectroscopy (MRS) was feasible with the large bore magnet, and Signa included special software for spectroscopy. GE built its main MR plant 90 miles north of Chicago, and we established contact with them. The University of Illinois Hospital received its Signa in 1986. To better understand the equipment, I was working with the GE technicians at the installation. Since during the day Signa scanned our patients, I worked at night to set up human spectroscopy using model solutions. The medical students in my biochemistry class volunteered so that I could record the spectra of their normal tissues; one of our first ^{31}P spectra of human gastrocnemius muscle is reproduced in Fig. 6 [115]. The overwhelming peak was from phosphocreatine (PCr), and the three phosphate peaks of ATP (upfield of PCr) were relatively small, similarly to the inorganic phosphate (P_i) peak (downfield of PCr). This spectrum indicated clearly that in normal resting human muscle, the energy reservoirs were completely filled, and there was only a small amount of unesterified, free P_i present. This information highlights the non-invasive nature of MRS, since phosphate analyses in extracts of human muscle biopsies show much higher P_i concentration due to the inevitable breakdown of

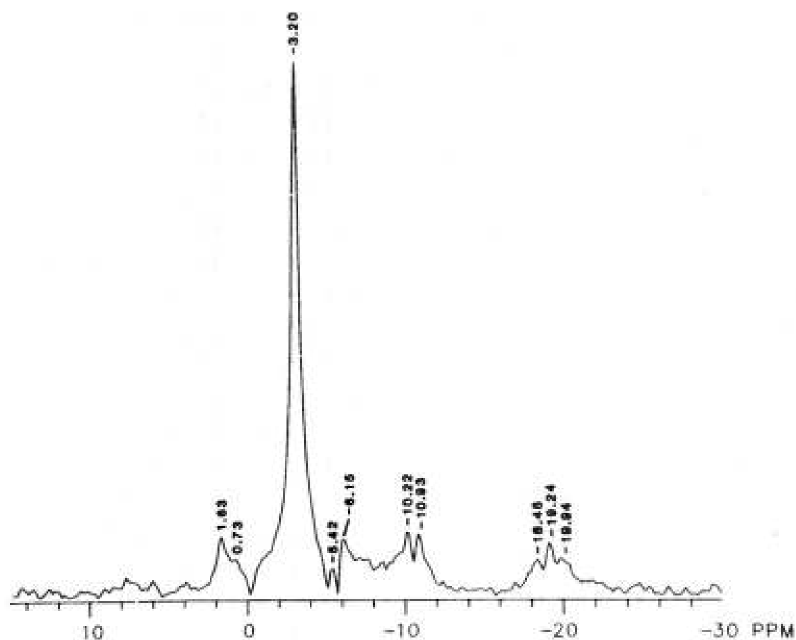


Fig. 6. ^{31}P MR spectrum of normal human gastrocnemius muscle. Peaks (from left to right): P_i, PCr, the γ-, α-, and β-phosphates of ATP. The parts per million (ppm) values are indicated on the peaks [115]. Following the recommendation of the International Union of Pure Applied Chemistry, downfield shifts are shown as positive and upfield shifts as negative. This is opposite to the old convention used in Fig. 5.

PCr during sample preparation. The ^{31}P MR pattern changed dramatically in muscles of patients with neuromuscular diseases [116]: the level of PCr decreased, that of P_i increased, and resonances not present in normal human muscle appeared. In addition to ^{31}P, we also recorded the ^{1}H spectra of normal and diseased human muscle, brain, liver and heart [117–119]. I was invited to a number of international and national symposia to present our work.

My work in human MRS caught the attention of private institutions in the Chicago area; they wanted to offer this

new technique to their patients. I was invited to run their spectroscopy. They provided a large amount of money for upgrading their Signa to produce high resolution spectra. My assignment was to record the ^{1}H or ^{31}P spectra of lesions identified by MRI on the patients, then submit the spectra to the radiologists with an evaluation of the pathology that the spectra detected. My first mention of *N*-acetylaspartic acid made the radiologists uneasy, because they never heard this name before. However, as time went on they learned biochemistry and I learned radiology. We evaluated each case together and I have found great satisfaction in helping the fight against human diseases. Frequently I could differentiate cancer from non-cancer, assess the effect of drugs on the progression of diseases, or estimate when the patient will need a wheelchair.

MRS raised high hopes in the medical community. The possibility of getting chemical information about the physiological or pathological status of live human tissues was unprecedented in medical history. However, MRS is not a very sensitive method; relatively large volumes of tissues are needed to get accurate information. Therefore, the initial promise of MRS has not been realized. As time went on, the rooms assigned to MRS at radiology meetings became smaller and smaller, and the time that various institutions were willing to assign to MRS became shorter and shorter. Moreover, when permission was granted to inject contrast enhancing agents into human patients, the resolution of MR images was increased to an extent that pathological lesions could be detected in about a cubic millimeter. The radiologist did not need the spectroscopist any more to make the diagnosis. Considering my limited possibilities for the future, I decided to leave the field at the end of 1989. Kate was happy when I joined her research on protein phosphorylation.

Protein phosphorylation

Kate and Michael: At the end of the 1960s, Michael served on the

Grant Committee of the American Heart Association along with Ed Krebs, who later received the Nobel Prize for his work on protein phosphorylation, together with Eddie Fischer. Already at that time, Krebs suggested that we should work on protein phosphorylation in muscle; some years later at the University of Illinois, we submitted an NIH grant application on protein phosphorylation and received the award. In our physiological approach to biochemistry, we injected a live frog with [^{32}P]orthophosphate and after 2 days of incubation, we analyzed the skeletal muscles for protein phosphorylation. Tropomyosin was the major radioactive peak on the gels, and the [^{32}P]phosphate remained in the purified tropomyosin which migrated as a single peak [120]. In collaboration with Larry Smillie, we found serine-283 (penultimate to the COOH-terminal end) of frog tropomyosin to be phosphorylated [121]. The same phosphorylated peptide was recovered in low yields from both rabbit skeletal α and cardiac tropomyosin. Larry proposed a model for the head-to-tail overlap of α-tropomyosin molecules, in which one phosphorylated serine-283 residue could form a salt linkage with lysine-6 on one side of the overlap region and another phosphorylated serine-283 with lysine-12 on the other side.

We were interested in the phosphorylation of tropomyosin during muscle contraction. Experimentally, one of the paired muscles of the [^{32}P]phosphate-injected frog was tetanized and, while contracting, the muscle was immersed into isopentane chilled by liquid nitrogen. The paired resting muscle was frozen under identical conditions. The muscles were pulverized over a surface of frozen 5% perchloric acid so that all muscle proteins were denatured when the muscle thawed. The extract was then homogenized, centrifuged, and washed with 3% perchloric acid several times. The final pellet was dissolved in SDS solution, and subjected to electrophoresis on SDS-polyacrylamide gels. This procedure assured that no enzymic reaction could take place in the muscles after the time of freezing. Thus, the state of protein phosphorylation on the

gel was identical to that in the live contracting or resting muscle. The results showed no change in the phosphorylation of tropomyosin but a major change in the phosphorylation of the 18 kDa myosin light chain during muscle contraction [122]. The same results were obtained when the muscles were contracted by stimulation with caffeine. Phosphorylation of the myosin light chain in vitro was first described by Victor Perry and collaborators [123] and we provided the first evidence for phosphorylation of the light chain in contracting live muscle. Light chain phosphorylation in stimulated frog semitendinosus muscles stretched beyond the overlap of thick and thin filaments was identical to that in muscles stimulated at standard rest length. This indicated that the stimulation of the muscle per se, and not the mechanical event, initiated the phosphorylation [124]. Localization of light chain phosphorylation to the activation of muscle raised the question of what is the role of this phosphorylation in the contraction cycle. We pointed out that phosphorylation of a serine residue in the light chain adds 1.8 negative charges to one myosin head at physiological pH. This may lead to about 11 negative charges per crossbridge and this electrostatic force could play a role in crossbridge movement and/or configuration [124]. This idea is quoted in the current literature, although sometimes it is 'rediscovered'.

Our work on protein phosphorylation attracted several graduate students, George Sarmiento, Fred Homa, and Scott Sayers, and postdoctoral fellows, Steve Hager, Dave Vander Meulen, Ron Ledvora, and Steve Kopp. We extended our studies from the live frog to excised bird and mammalian skeletal muscles. An automatic freezing apparatus was constructed to follow the kinetics of contraction and light chain phosphorylation. Both in chicken and rat muscles the rate of phosphorylation was slower than the rate of contraction [125], indicating that the contractile event was not strictly coupled to light chain phosphorylation. We also found light chain phosphorylation in cardiac muscles. The hearts from

turtle, rat, cat, dog, adult chicken, and 1-week-old chicken showed major differences in their phosphorylated light chain content. At the extremes, 76% of the total 19 kDa light chain was phosphorylated in turtle heart and 10% in the heart of adult chicken. Phosphorylation was correlated with the ratio of myosin light chain kinase to myosin light chain phosphatase activity [126]. Diphosphorylation of the light chain was also reported for the first time [127]. In rat heart perfused with positive and negative ionotropic agents, a correlation was found between ionotropy and light chain phosphorylation [128] but the extent of phosphorylation was low. We confirmed John Solaro's finding that phosphorylation of the inhibitory component of troponin was correlated with the force produced by the heart [129]. Our contributions to muscle protein phosphorylation were acknowledged by invitations to write a chapter in *Annual Review of Physiology* [130], to write a review in *American Journal of Physiology* [131], and to lecture at a *Cold Spring Harbor Symposium* [132] and at Federation Meetings [133,134].

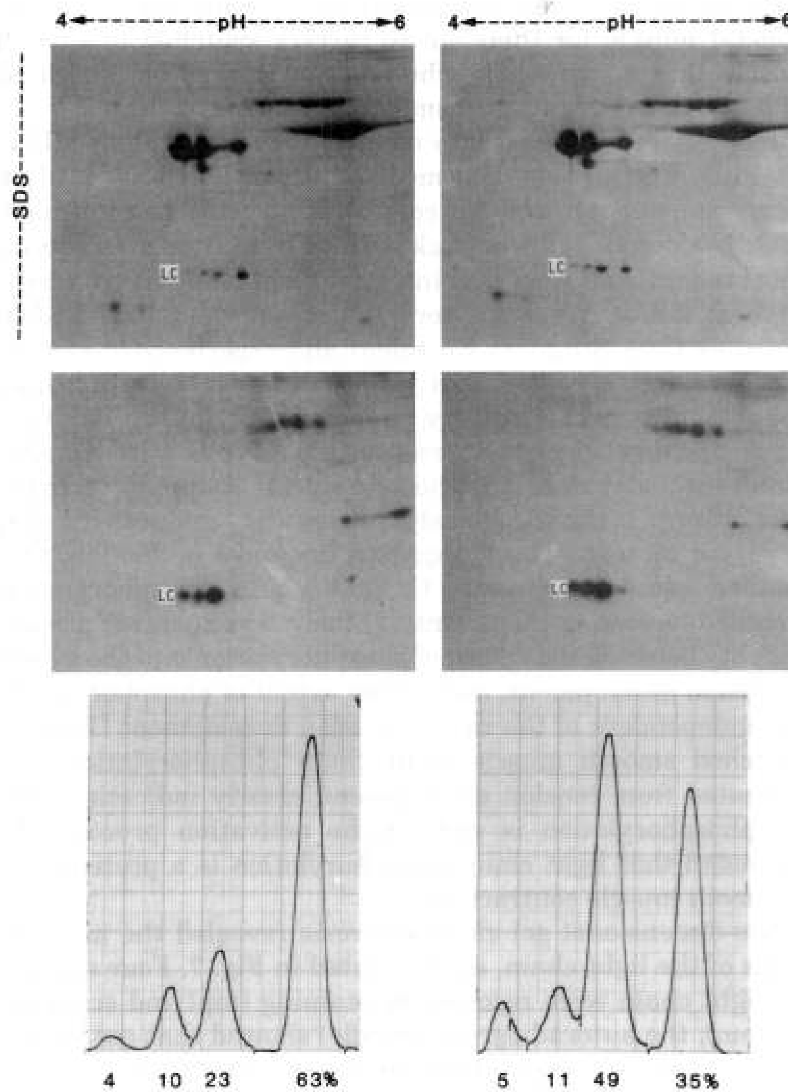
The discovery that phosphorylation of the 20 kDa light chain of smooth muscle myosin increases its actin- and Mg^{2+}-activated ATPase [135] directed interest on this phosphorylation in intact smooth muscle. Luckily, John Barron, a recent PhD, joined us from Joe Di Salvo's laboratory in 1978. John had enormous experience in smooth muscle research, knew all of the slaughterhouses in the Chicago metropolitan area, and also knew the glassblowers who made smooth muscle chambers. John has been a dedicated scientist, full of ideas, and very energetic. Soon after his arrival, we submitted our communication on the phosphorylation of the 20 kDa myosin light chain (light chain) in porcine carotid arterial smooth muscle contracted by norepinephrine or KCl [136]. In our full paper, we described: (1) the phosphorylation–dephosphorylation of the light chain during contraction–relaxation of the arterial muscle; (2) the central role of Ca^{2+} in regulation of the phosphorylation; and (3) the inhibition of

phosphorylation by inhibitors of calmodulin [137]. This work initiated extensive studies on light chain phosphorylation in all kinds of smooth muscles, stimulated by many different agents (enumerated in Ref. [138]). Smooth muscle took over the lead in muscle research; many people who had worked on skeletal muscle for their entire careers switched to smooth muscle, and many people who had not worked on muscle at all became investigators of smooth muscle.

John Barron enrolled into medical school to add an MD to his PhD. Fortunately, the medical school was only a block away from our lab, and our collaboration could be continued. Vassilis Mougios came to work with us from Greece as a graduate student, and Alex Csabina came from Hungary as a postdoctoral fellow. We were very fortunate when Feri Erdödi joined us from Hungary; his talent and experience in protein phosphorylation contributed to our progress greatly. Aniko Rokolya, also from Hungary, helped in setting up our two-dimensional gel electrophoresis system (16 gels were running simultaneously) and our phosphopeptide mapping (8 maps were running simultaneously). Scientific productivity was promoted by using the Hungarian language in the lab. Our detailed studies showed: (1) light chain phosphorylation preceded tension development; (2) there was no direct proportionality between the extent of phosphorylation and the extent of tension development; and (3) the extent of phosphorylation was independent of the rate of tension development [139]. In stretched smooth muscle, light chain phosphorylation was separated from tension development, clearly indicating that the phosphorylation is part of the activation process. We concluded that light chain phosphorylation is a prerequisite of smooth muscle contraction.

Two-dimensional gel electrophoresis revealed the multiple forms of the light chain, as illustrated in Fig. 7. Four spots of the light chain were resolved by staining (top) and scanning (bottom); the autoradiogram (middle) showed that out of four spots, three contained phosphorylated light chain, whereas

the fourth (most basic) spot was non-phosphorylated. We provided evidence that the multiple spots originate from different isoforms. We showed that fully dephosphorylated arterial and uterine muscles contained two and three light



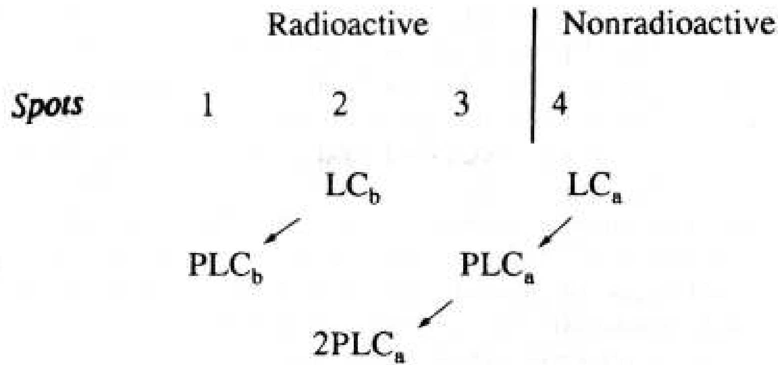


Fig. 8. Scheme for the explanation of four stained and three radioactive arterial light chain spots on a two-dimensional gel electrophoretogram. LC_a is the major and LC_b is the minor light chain isoform, and PLC and 2PLC are the mono- and diphosphorylated light chains [158].

chain spots, respectively [140,141]. Based on the existence of two non-phosphorylated isoforms that can be mono- or diphosphorylated [142,143] the four spots shown in Fig. 7 were explained by the scheme of Fig. 8. As indicated, *Spot* 2 contained both diphosphorylated and non-phosphorylated isoforms, which was the reason for the four stained and three radioactive spots. The scheme of Fig. 8 became more complicated with three non-phosphorylated light chain isoforms, but it was accessible to a simple mathematical analysis [142].

At the beginning of the 1980s, it was well-accepted that the light chain is phosphorylated by its specific enzyme, myosin light chain kinase. Surprisingly, somewhat later it was reported that protein kinase C also phosphorylated the light

Fig. 7. The multiple forms of porcine carotid arterial 20 kDa myosin light chain, separated by two-dimensional gel electrophoresis. The upper panel shows the Coomassie Blue staining patterns of arterial proteins, the middle panel shows the corresponding autoradiograms, and the bottom panel shows the densitometric scans of the light chain. Left, resting; right, contracting [158].

chain [144]. Subsequently, several laboratories investigated the possibility of protein kinase C involvement in light chain phosphorylation in intact smooth muscle. A simple method was needed that could unequivocally demonstrate the participation of protein kinase C. We found that the phosphopeptide map of light chain phosphorylated by myosin light chain kinase was entirely different from that phosphorylated by protein kinase C [145]; this is shown in Fig. 9. We applied this technique to porcine carotid arteries contracted with phorbol dibutyrate, an activator of protein kinase C, and expected a phosphopeptide map characteristic for protein kinase C-catalyzed light chain phosphorylation. However, two-thirds of light chain phosphorylation was attributable to myosin light chain kinase, and only one-third to protein kinase C [146,147]. It is an old proverb that 'in a biochemical lab, a 25% activity should be considered as no activity, and a 75% activity as full activity'. Since our 33% value did not fall into any of these categories, we did new experiments. It turned out that in physiologically contracted arterial muscle, the protein kinase C-induced light chain phosphorylation was negligible.

In our early experiments with arterial muscle, we noted that

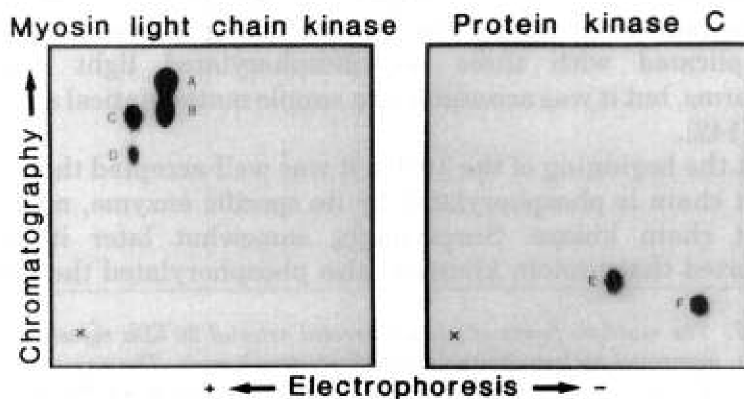


Fig. 9. Autoradiograms of phosphopeptide maps of light chain phosphorylated by either myosin light chain kinase or protein kinase C [158].

passive stretch causes phosphorylation of the light chain [136]. A follow up of this observation led to the discovery of stretch-induced light chain phosphorylation both in arterial [148,149] and uterine [150] smooth muscles. When fully stretched arteries or uterine strips were released, active tension developed without any exogenous stimulating agent. Thus, this was a novel mechano-chemical coupling: the external work performed on the muscle was transformed into a chemical activation of the contractile protein, myosin. The stretch-induced light chain phosphorylation was Ca^{2+}-dependent. We also showed by phosphopeptide mapping that stretching activated myosin light chain kinase in the intact muscle [151]. These results provided the biochemical basis for Bayliss' proposal from 1902 [152] that 'Stretch is a stimulus for blood vessel constriction'.

Since the discovery that light chain phosphorylation is associated with smooth muscle contraction, it was assumed that light chain dephosphorylation is associated with relaxation. Indeed, experiments in various laboratories supported this concept. However, we observed smooth muscle relaxation without light chain dephosphorylation, when the muscle was contracted with agonists and relaxed with agents in the presence of the same agonists [138]. Under those conditions, the agonists continued to release Ca^{2+} from the intracellular store of the muscle. Consequently, myosin light chain kinase remained activated and the light chain remained phosphorylated. Accordingly, mechanisms other than light chain dephosphorylation were postulated to be involved in smooth muscle relaxation. Caldesmon and calponin, the thin filament proteins that inhibit the Mg^{2+}-activated smooth muscle actomyosin ATPase, are candidates to induce relaxation, since these proteins underwent conformational changes during contraction of intact smooth muscle [29]. Caldesmon and calponin in smooth muscle are generally considered the equivalents of the troponin system in skeletal and cardiac muscle. These proteins are implicated in various hypotheses

about regulation of the contraction–relaxation cycle of smooth muscle [153]. Many researchers believe that nature used similar principles in designing the basic mechanism of contraction in skeletal, cardiac and smooth muscle.

Our contributions to the smooth muscle field were acknowledged by invitations for book chapters [139,154] and symposia lectures [142,155].

Teaching

Kate: In 1974 the University of Illinois at Chicago had the largest, most comprehensive academic health science center in the US. There were 275 students admitted to the freshman medical class, 200 each in the pharmacy or nursing classes, 150 in the dental class, and a large number of students in the College of Associated Health Professions that were taught in seven different disciplines such as occupational therapy, or human nutrition and dietetics. In addition, the university had an extensive graduate program. Both Michael and I were assigned to teach medical and graduate students. In addition, I was teaching pharmacy and dentistry students. Since we had no teaching experience in America, the interruption of lectures by questioning students and the construction of exams with multiple choice questions were a challenge for us. However, the main problem was our Hungarian accent. Complaints from the students were quite varied and ranged from those that were severe enough that they reached the Dean's office to the more mild which included the suggestion that we attend multiple screenings of 'My Fair Lady' to attain flawless English. We compensated for the accent by preparing extensive handouts in which all of the slides were reproduced and summaries were included. The doors of our offices were always open for students, and before the exams, we conducted review sessions.

The efficiency of our techniques could be assessed at three different levels: the grades we received from the students at

the end of the course, how much the students teased us at their yearly skits, and their writings in the 'Student to Student' book (freshman students to the incoming students). Eventually, I became a successful teacher and received numerous teaching awards. During our long stay in Chicago, we educated several thousand students, and a large number of them remained in the city. We are greeted all the time by our former students. Our family doctor, dentist, and pharmacist are all former students.

We quickly became accustomed to teaching small graduate classes of about 25 students. We introduced here an advanced course, 'Physiology and Biochemistry of Muscle Contraction'. The lectures included historical notes, anecdotes, the latest scientific findings, problem solving, and demonstrations. This course became quite popular and was also attended by postdoctoral fellows and physicians. Our devotion and enthusiasm have been appreciated. At the end of the semester, I give a party with Hungarian cakes and cookies. Students from the previous years are also invited, and the party is like a family reunion. Several of our students have become department heads, or esteemed members of research institutions. They remain our good friends, and their cards make up the bulk of our Christmas mail.

Service

Kate: Serving on committees is an integral part of university life. In committees, people share their knowledge and intentions, but actions are taken by individuals. Michael and I have an extensive record of committee work.

I have been a member of a special committee, the Chancellor's Committee on the Status of Women, for over 20 years. I successfully campaigned for a child care center, for flexible tenure policies compatible with family responsibilities, for career guidance to women returning to school after raising children, and for female-friendly facilities (e.g. providing

more ladies rooms). I participated in urging our administrators to change policies that discriminate against women; now many of these changes have been enacted.

At the national level, together with Bob Davies from the University of Pennsylvania, we were involved in forcing the insurance companies to eliminate pension inequities between males and females.

In 1996, I was named 'Woman of the Year'. There was a splendid celebration with a large crowd, including my children and grandchildren. To illustrate the situation of the women at our university, I recalled a story from my own experience: I was teaching the medical students and at the beginning of the course the students respectfully called me Dr. Bárány. Subsequently Michael gave a few lectures, and when I returned to the class, the students called me Mrs. Bárány. I asked them 'is this a promotion or a demotion?' The male students answered 'a promotion, you got a husband'. The female students answered 'a demotion, you were stripped from your Doctor title and became an appendix to your husband'.

Retirement

Michael: In my 50 years in science I have attended several important symposia. The closing of the meetings always piqued my interest. I remember what Doug Wilkie said: 'In biology everything comes to an end, so did this symposium.' On 1 September 1992, I became Professor Emeritus. However, after 2 months in retirement, my department head rehired me and accordingly my title was changed to Professor Emeritus/Rehired. With two more years to go on my NIH grant, I continued my research. However, thereafter my grant was not renewed.

I now collaborate with John Barron, in the neighboring Rush Medical College, to investigate lipid metabolism in smooth muscle. This project requires determination of water produced by the muscle from substrates added into the physio-

logical salt solution bathing the muscle, or from the endogenous substrates in the muscle. Using ^{3}H-labeled substrates, I found that the $^{3}H_2O$ formed can be quantified by any column chromatography, since the $^{3}H_2O$ passes through the column while ^{3}H substrates remain bound to the appropriate resin. We have published two papers using this method [156,157], and we have presented our results at the Experimental Biology Meeting (previous Federation Meeting).

I edited the book *Biochemistry of Smooth Muscle Contraction* for Academic Press in 1996. The book was reviewed favorably in *Science*, *Nature*, and *Trends in Biochemical Sciences*; it became a textbook of the field. The statistical program of my computer reveals that the Web site for the book has 5–20 visitors every day. I was also glad when my peers elected me as the first president of the Society of University Scholars of the University of Illinois at Chicago, which kept me busy.

At the age of 77, I am still working in the department virtually full time. I continue teaching graduate students and advising medical students. I benefit from the friendship of the faculty and from the help of our secretaries. In 1996, Don Chambers, Head of the Biochemistry Department, and John Solaro, Head of the Department of Physiology and Biophysics, organized a symposium entitled 'Biochemistry and Pathophysiology of Muscle' in tribute to Michael and Kate Bárány. This event was very well attended. Both of our sons, George and Francis, were invited speakers, and were complimented by the audience. In my thank you remarks, I recalled my father, a simple farmer who could not write but had extensive experience. He told me, 'One can have everything in life with one exception: one can't have enough friends.'

Being retired allows me to spend more time with my family. I especially enjoy reading the scientific papers of our sons, and viewing the science-fair posters of our grandchildren.

Concluding remarks

Michael and Kate: 'Man, Strive and Hope!' is the concluding sentence of the 15-scene drama entitled 'The Tragedy of Man' written by the Hungarian playwright Imre Madách in the second half of the nineteenth century. The play reveals the triumphs and tragedies of the human being. Our life has also been a drama which mixed struggle and success. Certainly, at times our situation has been desperate, but at no time did we despair. Indeed there were interludes where hope was our only possession and suffering, our birthright. Our hope has been sustained and we have achieved much of what we pursued. We have contributed to science through our efforts in the laboratory, the classroom, and the encouragement of our children. As we near the end of the twentieth century, we see how science has expanded knowledge and thereby improved the quality of human life. We hope that the power of science in the next century will also contribute to build a better society of humankind.

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