PROCEEDINGS OF INTERNATIONAL SYMPOSIUM

MODERN TRENDS IN PROTEIN AND ENZYME RESEARCH

当代蛋白质与酶学研究动向国际学术讨论会论文集

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PREFACE

The International Symposium on Modern Trends in Protein and Enzyme Research was held in Beijing during May 15–17, 1993 to celebrate the 70th birthday of the famous Chinese biochemist and molecular biologist Professor Chen–Lu Tsou. This Symposium was sponsored by the Institute of Biophysics and the National Laboratory of Biomacromolecules in honour of Prof. Tsou as the Chariman of the Life Sciences Division of Academia Sinica, founder and director of the National Laboratory, and also in honour of his distinguished achievements in his research on proteins and enzymes, his pioneering successes in the fields of biochemistry, his contributions in supervising many talented young people, his dedication to science and his patriotic spirit.

During his scientific career for more than 40 years his persistent and rigorous studies on the respiratory chain, total synthesis of bovine insulin, mechanisms of enzyme action, protein folding have resulted in publications of world renown, bringing him numerous national and international prizes and awards. He has also successfully trained a group of brilliant young leading scientists in the fields of biochemistry and molecular biology, many of whom are in turn making their own important contributions. Some have become members of the Academia Sinica. Now at the age of 70, Professor Tsou is still working tirelessly as ever, scaling new heights in science, heading his group engaged in the national long–term basic research project on protein folding, and deeply involved in the busy activities of promoting science and technology in China. He takes the Confucian proveb of "Knowledge is infinite"as a guideline, impelling himself to relentless studies and endeavors. This spirit of dedication and patriotism has won general admiration and respect from hia colleagues, friends and students.

More than 50 scientists from the UK, Japan, US, France, Germany and China participated in this Symposium, among whom 21 members made oral presentations reflecting their progress and perspectives in important fields of protein and enzyme studies. Teachers, friends, colleagurs and students merge their intelligence and friendship, happily celebrating Professor Tsou's 70th birthday, wishing him good health and longevity and greater successes in the many productive years to come.

This book includes 31 research articles reflecting the latest progress in this field, memoirs by some scientists on their recollections of various interactions with Professor Tsou, also Professor Tsou's autobiographical article "The Highest Grade of This Clarifying Activity Has No Limit" published in "Comprehensive Biochemistry" . The memoirs, although describing only a limited part of Prof. Tsou's research, teaching and other activities, are both inspirig and instructive. The sponsors of this Symposium presents this book together with another book "Protein and Enzyme Studies — a Collection of Chen–Lu Tsou's Publications (1949–1992)" in honour of his birthday.

We appreciate deeply with thanks the generous financial support from the State Commission of Science and Technology, National Science Foundation of China and Academia Sinica for this International Symposium.

February 1, 1993

Dr. C. L. Tsou
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China

To whom it may concern,

I would like to celebrate the 70th birthday of Dr. C.L. Tsou on account of his remarkable contribution to research on the one hand, and because of my close personal contact with him over the years on topics of bioenergetic interest.

C. L. Tsou has made historic progress in the fields of bioenergetics and protein structure and is world reknown for his efforts therein. Equally important is that he has maintained close contact with the fields as they have developed and has at the same time trained remarkable students with whom I have had the pleasure to work.

Finally and perhaps most important, is that he represents the resilience and elegance of Chinese science. His works will live on as a lighthouse to the young students that follow and who will bring Chinese science to the forefront of the world as it properly should be.

Very sincerely yours,

Britton Chance

Britton Chance

序　言

为了庆贺我国著名生物化学家、分子生物学家、中国科学院生物学部主任、生物大分子国家重点实验室创立者邹承鲁教授七十华诞和他在蛋白质与酶学研究领域取得的丰硕成就，感谢他为我国生物化学、分子生物学的开拓和发展及优秀人才培养所做的贡献，弘扬邹承鲁教授献身事业、热爱祖国的高尚精神，中国科学院生物物理研究所和生物大分子国家重点实验室于 1993 年 5 月 15 日在北京召开了“当代蛋白质与酶学研究动向国际学术讨论会”。

邹承鲁教授从事科研和教学已四十多年，他数十年如一日，以严谨的科学态度，一丝不苟的求实精神，从事科学研究，在他所从事的呼吸链、胰岛素人工合成、酶作用机理、动力学和新生肽链与蛋白质折叠等研究领域都取得了举世瞩目的成就，曾多次荣获国际、国家和中国科学院的科学奖，享誉国内外。多年来他悉心培养了一批具有国际水平的生物化学、分子生物学学术带头人，他们在科研岗位上发挥着重要作用，有的已成为中国科学院的学部委员。现在，邹承鲁教授虽已七十高龄，却仍执著追求，奋力攀登，他以充沛的精力，亲自指导国家重点实验室工作和“新生肽链和蛋白质折叠”国家攀登项目研究，同时活跃于发展我国科学事业的繁忙活动中。他以孔子“学无止境”为座右铭，鞭策自己学到老、奋斗到老，他的壮心系科学，孜孜为国昌的精神，深受大家的尊敬和爱戴。

此次学术讨论会，我们邀请了英国、日本、美国、法国和德国以及国内著名科学家 50 余位参加，有 21 位科学家做了大会报告，就当代蛋白质和酶学研究若干重要领域的现状、进展和前景进行了交流和讨论。会上，师长、朋友、同事和学生间融学术和情谊于一堂，都以欣喜的心情，恭贺邹承鲁先生 70 华诞，并祝愿他健康长寿，为我国科学事业做出更大的贡献。

本论文集收编了 31 篇学术论文，反映了这一研究领域的最新成果与进展。同时汇集了部分科学家从各个侧面回忆邹承鲁教授的纪念性文章，以及邹承鲁教授亲自为国际丛书“综合生物化学”撰写的自传性文章（“The Highest Grade of This Clarifying Activity Has No Limit”−Confucius）等，这些回忆文章虽然仅能展示邹承鲁教授四十余年科研、教学和社会活动以及他卓越成就的几个侧面，但也能使我们从中受到启迪与教益。会议组织者谨以此论文集和“蛋白质与酶学研究—邹承鲁文选（1949—1992）”两本书作为献给邹承鲁教授七十寿辰的贺礼。

这次国际学术讨论会，得到国家科委、国家基金委和中国科学院的赞助，借此表示感谢。

当代蛋白质与酶学研究动向
国际学术讨论会组织委员会

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of Biochemistry: Personal Recollections, III.
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Chapter 9

'The Highest Grade of This Clarifying Activity Has No Limit' – Confucius

CHEN-LU TSOU

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Confucius said: *'At fifteen I wanted to learn. At thirty I had a foundation. At forty, a certitude. At fifty, knew the orders of heaven. At sixty, was ready to listen to them. At seventy, could follow my own heart's desire without overstepping the t-square' [1].*

From chemistry to biochemistry – days in Cambridge with David Keilin

Although I studied chemistry in the National Southwest Associated University in Kunming in the interior of China during World War II, I had always been attracted by the mystery of life's processes and believed firmly that the best approach to solve these mysteries would be a chemical approach. My university years ended with a brief spell in the army until the end of the War in 1945. After two years' work in organic chemistry I finally won a keenly contested examination for a scholarship to do postgraduate study in England: I naturally selected biochemistry as my subject. I was very much disappointed when I was sent, in autumn 1947, to the Department of Chemistry at the University of Birmingham which no doubt was an excellent department, especially in the field of carbohydrates, with

the Nobel laureate Sir Norman Haworth at its head. Fortunately, before I left China, Ying-Lai Wang had just returned from Cambridge to take up a Professorship at the National Central University at Nanking. Armed with a recommendation letter from Wang to Prof. David Keilin, I went to Cambridge for an interview and eventually moved to Cambridge after only a few months working on the 'sweetest of all sugars, inulin'. Coincidentally, I was later destined to spend a large amount of my life's work on a protein with a very similar name, insulin. However, my background and training in organic and physical chemistry did me much good with my work on proteins and enzymes.

I started my scientific career as a Ph. D. student with the late Prof. D. Keilin in the beginning of 1948 in the Molteno Institute of Parasitology. I believe very few biochemists today know the Molteno Institute and may wonder why I should go to an Institute of Parasitology to study biochemistry. David Keilin was very well known in both fields and as the Director of the Molteno Institute made numerous important contributions to both parasitology and biochemistry and was in fact the founder of the discipline biochemical parasitology. In those days Keilin's fame as the discoverer of the cytochromes made the Molteno Institute the mecca for biochemists working on respiratory enzymes. Among the people who had worked at the Molteno were such well known names as Emil Smith, David Green, Bruno Straub, Britton Chance, Max Perutz, John Kendrew, E. C. Slater and many others.

I was given the problem to study the structure-function relationship of cytochrome *c* by proteolytic digestion of the protein molecule which must be an early attempt for such an approach [2-4]. In those years, we used the Keilin-Hartree heart-muscle preparation as our enzyme source. The heart-muscle mince had to be washed thoroughly and squeezed firmly through muslin several times to get rid of all the myoglobin attached to the muscle. I thought it might be easier to remove the myoglobin by washing with salt solutions, but to my surprise, not only myoglobin but also cytochrome *c* was removed. I was later able to show that cytochrome *c* can be easily extracted from heart-muscle mince by salt solutions and can be put back again under appropriate conditions. There are important qualitative as

Prof. Chen-Lu Tsou.

well as quantitative differences of cytochrome *c* in the bound and the free states [5]. I also noticed that, no matter how thoroughly I extracted the heart-muscle mince with salt solutions, there was always some pigment left which absorbed near 550 nm in the reduced state. It was cytochrome c_1 of course, as we all know now [6], however, I missed it when it was sitting right there before my eyes. Working on succinate dehydrogenase I showed that, contrary to a very popular suggestion at that time [7], it is not identical with cytochrome *b* [8].

Professor Keilin made his great contributions to science with very limited equipments. Apart from the hand spectroscope with which the professor made his discovery of the cytochromes, people at the Molteno had at their disposal only the Barcroft manometers, Thunberg tubes, and an old swing-out centrifuge. A later addition of a Beckman DU spectrophotometer was a luxury. A refrigerated centrifuge (MSE) came only a few weeks before I finished work on my Ph. D. thesis. For centrifugation in the cold, we had to put a few

ice cubes made from distilled water to each of the centrifuge tubes when dilution was no objection. The professor told us a story that once he had a rich professor from a rich country as a visitor who asked his advice on what to work on with all kinds of fancy equipments available and enough money to buy anything you can think of. I am sorry to say that this kind of thing does not happen only in rich countries. In recent years, many Chinese laboratories had generous loans from the World Bank to buy scientific instruments and I had the same experience more than once with my visitors asking not only what to do but also what to buy with all the money made available to them! In China, as anywhere else, it is not always those with the best equipment who are doing the best work.

Among those who are engaged in fundamental research in China today, we are fortunate in being generously supported by Academia Sinica and during the last few years when hard currency was indeed very hard to come by for us, we had a Grant from the National Institutes of Health of the U.S. so that we are now moderately well equipped for the work we are doing. Nevertheless, I still ask my graduate students to make their own enzymes and to synthesize whatever reagents that are good for their training; above everything I make them always remember that worthwhile contributions in fundamental research can be made from very limited resources.

I got into the bad habit of smoking when I was very young, and in England those days cigarettes were difficult to obtain: one had to know the tobacconist and get the cigarettes 'under the counter'. I therefore learnt to smoke a pipe as pipe tobacco was easier to obtain. Unfortunately, my pipe-smoking technique was not very good and someone in the Molteno once remarked that my pipe seemed to work most of the time in anaerobic conditions. It is for this reason that I kept the pilot flame of a gas burner always on so as to light my pipe whenever it went anaerobic which happened quite frequently. One day Prof. Keilin walked into my room in the corner of the third floor and saw the lighted pilot flame of the burner. He asked me to turn it off and use matches for lighting my pipe; he said that if I always left it on I might regret it if I had an accident.

It was a few days after, when I went home after work, my wife, who was then working in the Goldsmith Laboratory of Metallurgy

downstairs from the Chemistry Department at Pembroke, told me that Prof. A. R. Todd dismissed a graduate student because of an accident. The student had been doing a distillation the previous evening and forgot to turn off the cooling water when he finished the experiment and left for home. Because of the increased water pressure after working hours when everyone turned their taps off, the connection between the rubber tubing and the condenser burst and his room, which was right above Prof. Todd's office, was flooded. The water dripped through the floor and soiled some important papers. This made Prof. Todd very angry and he dismissed the student on the spot when he came to his office in the morning. After my wife told me this story, we had our dinner, talked a little and then went to bed. It happened that I could not go to sleep and sometime around midnight it suddenly occurred to me that I left the pilot flame of the gas burner in my lab on. The more I thought of it the more I was sure that I did not turn it off when I left. I hurriedly put on my mackintosh over my pajamas and rode my bicycle to the lab. I had to climb over the locked iron gate of the Dawning premises, went into my lab on the top floor of the Molteno and found to my great relief that the pilot flame was off after all.

Recently, something very similar to the accident in Prof. Todd's lab happened to one of my graduate students. Although this room was not directly above my office, he did make a mess of a few laboratories. However, I had no say in what to do with him; the Security Officer of the Institute came, inspected the damage, talked to my student and asked him to compose an essay of 'self-criticism' on the accident. Depending on how well he did with this essay, he may get away with the accident, pay a fine or be dismissed as a graduate student. In case the security officer decided on a fine, as his supervisor, I should pay 80% of the fine for 'failure to teach the student to observe the security regulations'! It is only common sense that, since I have more money than the poor student, I should pay the lion's share of the fine. There you have the difference in Chinese and Western philosophies. Fortunately for him and for me, he apparently did his essay well enough so as to satisfy the Security Officer that he had learnt the lesson and further punishment was not necessary.

Early years in Shanghai

I came back to China in 1951 and, accepting an invitation from Y.-L. Wang, I joined the Shanghai Institute of Biochemistry of Academia Sinica. I was made a full professor a few years later at a relatively young age. As I also accepted the invitation to do part-time teaching at Fudan University of Shanghai, I found that my bad habit of smoking a pipe was helpful in making me look more dignified before the students.

Modern Biochemistry was introduced to China in the 1920s when H. Wu returned from the U.S. and established the first biochemical laboratory in China in the Peking Union Medical College. Biochemists of older generations might still remember the method developed by Folin and Wu for sugar determination which bore their names and appeared in many textbooks for experimental physiological chemistry. Wu was also known for his contribution to protein denaturation which was envisaged as a process involving 'change from the regular arrangement of a rigid structure (of a protein) to the irregular, diffuse arrangement of the flexible open chain' [9] and thus laid the foundation of our present concept of the unfolding of protein molecules. Apart from protein denaturation, immunochemistry and clinical analysis, Wu's group was also mainly responsible for early nutritional research in China which was the major activity of many biochemical laboratories at that time not only in medical colleges, but also in science faculties of Chinese universities.

Research activity was interrupted when the War broke out in 1937, and serious fundamental research was not possible until the founding of the People's Republic in 1949. During the period 1949–1966, the most important center for basic biochemical research in China was the Institute of Biochemistry of Academia Sinica in Shanghai, founded in 1958 with Prof. Y.-L. Wang as Director. It was originally part of the Institute of Physiology and Biochemistry which had three divisions: Physiology, Biochemistry and Chemistry of Natural Products which all later became separate Institutes. For some years, the majority of the more significant research papers came from the Institute of Biochemistry, which had five research

laboratories: proteins, enzymes, nucleic acids, metabolic studies, and radiation biochemistry. Shortly after I joined this Institute, I was made the head of the enzyme division.

My training with Prof. Keilin in Cambridge made it much easier for me to start working with a research group of my own in Shanghai. The Institute of Biochemistry of Academia Sinica had then similar equipments as the Molteno except that we had to use a model A stone mill (A for ancient, this kind of hand-operated stone mill must have been in use in China for making flour from wheat since time immemorial) instead of a mechanical mortar for grinding up heart-muscle mince with sand to make Keilin-Hartree preparations of succinic dehydrogenase-cytochrome oxidase. During our work on the purification of succinic dehydrogenase, we used the same trick of adding ice cubes made from distilled water to the centrifuge tubes to keep them cool in some of the steps where dilution did not matter.

With Prof. Keilin, I made all the enzymes that I used including the proteases for the study on the effect of proteolytic digestion of cytochrome *c*. One day I was discussing with him my work on the reactions of cyanide with cytochrome *c* and succinic dehydrogenase and he suggested that I try the effect of methyl carbylamine on the respiratory enzymes; it is after all similar to cyanide in structure. It was not available commercially and, as I had some training in organic chemistry, I tried to make it myself. On account of its smell, Prof. Keilin suggested that it would be better to work with as little material as was convenient. The Molteno was no chemical lab and a hood was not available for such experiments. I therefore worked on the roof which served not only as a cold room for Bill (E. C. Slater, in spite of his first name Edward, he has always been known as Bill), but also as a hood for me. Prof. Keilin commended me upon my dexterity for making the final fractional distillation step with 0.5 ml of material collecting fractions with a drop each and determining the boiling point of each of the fractions. However, I was an unwelcome person for some time because of the smell which was retained in my lab coat for quite a while. Joan Keilin also used this reagent to study its reactions with haems but fortunately for her, and unfortunately for the others working in the same room, she temporarily had no sense of smell precisely at the time when she was working on this problem.

In my early years in Shanghai, it was apparent that my training in organic chemistry and the habit of making myself some of the things I needed stood me in good stead. In those early days, we had to synthesize even dimercaptopropanol ourselves [10]. For our work on the resynthesis of insulin from its chains, we made sodium tetrathionate for the splitting of the insulin into the *S*-thiosulfonate derivatives of the chains.

Collaborating with Wang and Wang, I continued my interest in succinate dehydrogenase and purified this enzyme using the method of butanol extraction first suggested by R. K. Morton whom I had known in Cambridge and was deeply impressed by his success in the extraction of a number of membrane-bound enzymes with butanol which was dubbed Mortonol at that time. I was very much saddened when I heard that Morton was killed in an accident of acetone explosion while drying acetone powder in his laboratory in Adelaide.

The successful purification of this enzyme [11] depended very much on a convenient and reliable assay method. It was not known at that time that dyes commonly used for the assay of the enzyme in particulate preparations (membrane-bound) such as methylene blue or 2,6-dichlorophenolindophenol do not react directly with dehydrogenase and require another factor so as to accept the electrons from the dehydrogenase. I suspected this when working on this enzyme in Cambridge and we hit on the correct idea of using potassium ferricyanide as the artificial acceptor. Relying too much on my Cambridge experience, I suggested to include substrate and cyanide in the extraction of this enzyme, the substrate was to protect the enzyme from being inactivated and cyanide to prevent the exhaustion of the substrate during extraction; as I had shown previously it did no harm to the dehydrogenase in the presence of the substrate. Little did I realize that the added cyanide made the enzyme no longer reconstitutable with the particulate enzyme preparation of the respiratory chain then known as the Keilin–Hartree preparation. This was demonstrated by Keilin and King a little later [12].

The excitement came when it was found that this enzyme has as its prosthetic group an FAD molecule covalently linked to the protein moiety of the enzyme [13]. It was at that time very difficult for

us to attend international meetings, especially when they were held in a Western country. Y.-L. Wang was the only one of our group who went to the Third International Congress of Biochemistry in Brussels and presented our results. Coincidentally, T. P. Singer was also there to present his paper on the purification of succinate dehydrogenase. Although the purification procedures were necessarily different, the final preparations obtained by the two groups were remarkably similar [14]. The method of Wang, Tsou and Wang modified by various authors is still commonly used for the preparation of this important enzyme.

During my early years in Shanghai, I had the good fortune to get help of my young colleague, Dr. C. Y. Wu, in my studies on respiratory enzymes [15]. Unfortunately, he was to fall a victim of the 'Cultural Revolution' while still in his thirties.

Total synthesis of insulin

The year 1958 was a memorable one in Chinese history. Mao Zedong's idea of a great leap forward caught the imagination of hundreds of millions of Chinese people who, understandably, were eager to shake off the legacy of over a century of poverty and backwardness and the scientists, who can be at times very unscientific in nonscientific matters, were no exception. When everyone else was achieving wonders, what could we poor biochemists do? In the Shanghai Institute of Biochemistry, we had many heated discussions on the great problem we were going to tackle; then suddenly someone hit on the idea of synthesizing a protein. Considering that at least the amino acid sequence of one protein, insulin, was then known (the sequence of myoglobin was to come a little later) and an active fragment of ACTH 26 amino acid residues long had already been synthesized, the idea appeared logical. In fact, apart from insulin, we had no other choice. It could have been myself who first made the proposal, but during those days when many people often spoke at the same time, the original proposal could have come from a dozen different people. Anyway, the idea seemed to be the only proposal acceptable to both the authorities and the scientists and