Letter to Editor

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Hsien Wu and his major contributions to the chemical era of immunology

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Abstract: One of the first biological fields to achieve a formal chemical wing is believed to be immunology, and in the early twentieth century, many investigators have studied this field in terms of chemical reactions. Hsien Wu was one of China's outstanding biochemist leaders during the first half of the twentieth century, an influential immunology scientist. By introducing his academic and scientific background, I elaborate on Hsien Wu's achievements in the immunochemical period. Wu has solved one of the most serious issues on the accurate perception of immunology, and he made quick and universal attention in this regard. Hsien's most fundamental contribution to this field is his concentration on quantitation, chemical approaches, and physicochemical, opening a new window for other scientists to find out more about the chemical approaches and theories.

Keywords: Hsien Wu, immunochemistry, immunology, physicochemical approaches

1 Introduction

Hsien Wu was more than a great biochemist and immunochemist and blood specialist. He is a pioneer of Chinese biochemistry and one of China's skillful and truly original chemist leaders. The first Chinese director of Peking Union Medical College (PUMC) was Wu [1]. Even though all biochemistry candidates are familiar with Folin–Wu and their blood assessment method, Hsien's life story and his key role in immunology are still unknown. He came from an educated family and was born on 24 November 1893, in Foochow, China. Hsien's interest in science and his passion for learning pushed him toward taking an exam to pursue his education in the USA [2]. Among the 160 scholarship winners, he achieved scholarship and went to the USA in 1911 and started studying at the Massachusetts Institute of Technology (MIT) [3].

His bachelor's thesis was spermaceti derivatives and after graduation in 1916 [2]. He graduated in organic chemistry at MIT for a year and, at the same time, worked as an assistant [4]. For the next year, he started his Ph.D. at Harvard University under Dr. Otto Folin's supervision [4], and published a paper entitled "Blood System Analysis" in Biological Chemistry journal in 1919, which was his first published article among the 159 papers in American and Chinese scientific journals [2]. Dr. Wu and the late Dr. Otto Folin worked out to trace sugar termination in the blood system [3]. He designed a method by which major blood components could be quantified by 10 cc of blood samples; therefore, one drop of blood or urine was enough to measure the amount of sugar by this method. Many scientists approved this discovery's effectiveness and mentioned that insulin invention would not be plausible without this discovery. Later, the method has been known as Folin–Wu method [1]. Blood sugar measurements made up a friendly competition between the Folin and Stanley Benedict groups. Best and Banting discovered insulin after Hsien discovered blood glucose measurements [4].

Wu started working with an outstanding biochemist called Donald Van Slyke at Rockefeller Institute in New York in early 1922 [5]. Donald Van Slyke was working on chemical mechanisms that contributed to the respiratory cycle, which involved exchanging oxygen, carbon dioxide, and other blood materials during the respiratory cycle. Wu trained Chinese technicians to perform some research regarding Van Slyke's design after moving to PUMC, and the results of the research were published in 1923 [6]; this paper elaborate on exchanging between red cells and electrolytes in plasma and water under varying pressure of oxygen and carbon dioxide by the forth mathematical formula. This paper stands as a landmark in our understanding of chemical basis for respiration and demonstrates the first application of the

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Gibbs–Donnan law of heterogeneous equilibrium, a deduction from thermodynamics, to the study of physiology [7].

After Hsien received his Ph.D. at Harvard, he returned to China and started his professional life at recently established university, Peking Union Medical College [8]. His experiments there were mostly based on solving denaturation as he had rich experience on blood protein analysis. Hsien's and Yen studies on electrolytes and water changes in the blood led to changes in the Duboscq colorimeter [4]. From 1924 to 1940, 16 articles were published under the main title of "Studies on Denaturation of Protein." Hsien published an article in 1931 named "Studies on denaturation of proteins. XIII. A theory of denaturation" has opened up a new course of understanding protein, more than half a century and scientists have worked hard on this road. As the famous protein chemist Felix Haurowitz commented in 1950, this was "the first reasonable theory on protein denaturation," and he believed that this theory was published at least 5 years earlier than others [1] (Figure 1).

Hsien and his team have published 163 academic papers and three books. He was chosen as an honorary member of the Deutsche Akademie der Naturforscher Leopoldina and selected as an American Society of Biological Chemists [1]. He was interested in research in the following areas [9]: blood chemistry; gas and electrolyte equilibria; nutrition, immunochemistry; proteins; metabolism of amino-acids labeled with nitrogen-15 as a tracer; molecular weights and osmotic pressure of hemoglobin of experimental animals.

N excretion pattern, followed by the consumption of labeled amino acids, is known as his most recent research [2]. In 1953, after breaking the Crohn's thrombosis, Wu was retired and passed away on 10 August 1959 at Philips. At that time, he was 65 years old and was living in Boston [1].

Figure 1: Hsien Wu [1].

2 The role of Hsien Wu on the evolution of immunology

Systematic physicochemical research, extracted from laboratory studies in the early 1920s, made up our present antibody structure information. In 1930s, immunochemists attempts introduced the globular nature of antibodies, their continuous polypeptide chain nature, bi- and multi-specificity within the unique molecule, and the early notions of how antigen-binding occurred. The most significant knowledge that Wu brought is about the basic underlying mechanisms of the chemistry of life [10].

To evaluate the small amount of hemoglobin deposition by anti-hemoglobin, Wu and his colleagues used the micro-Kjeldahl method. However, they failed to achieve a reliable result in specifying fixed composition with various percentages of antigens and antibodies [11]. The immunochemical methods have become a useful tool in characterizing proteins and polysaccharides [12]. One of the most outstanding issues to understanding immunology has been removed by Wu and opened the new windows to further investigations. In the middle of the 1920s, it has been revealed that an organism's immunity against infection proceeds by a reaction between an invading antigen and a defensive antibody to produce a precipitate, which is finally removed from the bloodstream. Because of no measurement ways of precipitate composition, there is less understanding of the nature of such reactions [13,14]. In 1928, Wu had another contribution to such research, again drives from his study of blood. Years earlier, Wu had developed a method to determine a small amount of hemoglobin. Now using a measured quantity of hemoglobin as the antigen, the amount of antibody can be achieved as the variance among the total protein precipitate and the hemoglobin using this method. After understanding certain limitations of using hemoglobin, Wu used color tracer, iodoalbumin, for his investigations. This method's effectiveness was quickly identified, and additional quantitative surveys of immunological studies started [12–15].

Years later, Michael Heidelberger et al. performed more extensive data in this field and used nitrogen-free carbohydrate antigens. According to Lawry or by ultraviolet absorption, the amount of antibody was determined by the Kjeldahl method and later by calorimetry at approximately 280 nm, and gravimetry with micro balances is used in order to specify the total weight of the sediment.

Wu proposed the main discussion on the protein denaturation theory in the American Physiological Society in 1929: The protein molecule is regarded as a compact structure. It is worth mentioning that joining amino acids and different kinds of linkage make protein unique. The chain may be considered to fold frequently at short intervals forming a three-dimensional network slightly similar to a crystal lattice in which amino acid molecules substitute the atoms. Denaturation includes breaking many of the labile linkages, or bonds, within a protein molecule responsible for the highly ordered structure of the protein in its natural state. The protein molecule now converts a "diffuse" structure. The surface and the interior of the protein molecule are changed and exposed. It led to decreased solubility and increased acid and base binding power and altered immunological specificity known to accompany denaturation [10].

The whole data about denaturation were collected by Hsien and also mentioned some incisive and controversial ideas that are briefly explained below.

The necessary part of the same process is supposed to be denaturation and coagulation. A chain has the potential to be folded repeatedly by molecular attraction if it is so long. There are some doubts on peptide chain structure; however, "the peptide linkage remains the best supported theory regarding the constitution of the protein;" denaturation involves no change of molecular weight; denaturation changes antigen properties; an increase in viscosity accompanies denaturation; long chain or linear structures for native proteins are incompatible with observed molecular weights and the associated molecular dimensions of an extended structure; the probability of random linear chains being able to crystallize in regular arrays is "… so small as to be negligible;" protein molecules are not an open chain, flexible molecules but have a compact structure [16,17].

Wu understood the association between the native and denatured protein for the first time. This theory elucidates the interaction between the polar groups in the chain and the polar side chains, which causes to form a native protein molecule is in a highly compact and well-ordered structure [8].

Mirsky and Pauling published their famous paper entitled "On the structure of native, denatured, and coagulated proteins although puzzlingly at this stage without any reference to the work of Wu" without mentioning the name of Wu. Their paper was similar to the Wu paper with some small changes on describing the hydrogen bond as the non-covalent "glue" that holds folded chains together. They focused on the thermodynamically more rigorous treatment of what Wu describes as "labile linkages" [18].

Hsien and his colleagues investigated the denaturation mechanism. In this study, they used crystalline ovalbumin from egg whites and examined its denaturation and dialysis. After making a series of corrections to the enzymes' nitrogen, they calculated the total digested nitrogen percentage. They achieved the optimal pH and the maximal digestion of the different proteins, indicating the digestion rates of natural and different forms of denatured proteins were the same except for a few cases in the peptic series. This result intimates that the significant alteration underlying denaturation does not affect the albumin's linkages, which are hydrolyzed in peptic digestion, and hence declared that the denaturing changes probably had the same nature as tryptic digestion. In addition, Hsien and his colleagues displayed that denatured and natural proteins differ in optimal pH digestion, representing the isoelectric point, and the maximal dissociation of the albumin is shifted toward the neutral point by denaturation [19].

Hemoglobin is determined based on the benzidine reaction with low content of 0.02 mg and this amount is also sufficient to determine the antigen-precipitin composition introduced by Hsien Wu, Lan Hua Cheng, and Cheen-Pien Li. The benzidine reaction also can be determined directly with antigen-precipitin amount when hemoglobin is used as an antigen. The difference between total protein and hemoglobin determined the amount of precipitin in the precipitate. We should also mention in this study that hemoglobin meant the general oxyhemoglobin and its immediate derivatives. They tried various techniques to immunize rabbits, but none of them were perfectly adequate because of hemoglobin's antigenic properties and also declared that the antigen-precipitin deposition was a stable mixture and reaction between antigen and precipitin was chemical [20].

3 Conclusion

Hsien Wu (1893–1959) was a biochemist and nutritionist pioneer who made a significant contribution to medicine's progress. He was perhaps the well-known Chinese scientist and the most outstanding chemist in the first half of the twentieth century and the Biochemistry and Nutrition founder in China [1]. He is a pioneer in clinical chemistry, protein chemistry, immunochemistry and nutrition, with important creations and famous discourses. Hsien Wu was one of the chemically oriented researchers who began studying antigens and antibodies. In the meantime, Hsien and his colleagues, using the science of immunochemistry, established valuable chemical methods and quantitative chemical methods of analysis to improve immunology further. This research's significance was immediately recognized, and further quantitative study of immunological reaction followed [4–10]. Antibodies isolation has been done for the first time by Wu in the pure form ref. [21], and it could present a significant advance in the light it shed on the nature of immunity besides treating many bacterial severe diseases as well [22]. His commitments were the basis for massive advances in medical science for humankind's benefit in the twentieth century. A bright mind, driven by intellectual curiosity, is significant enough to make scientific breakthroughs, even in meager circumstances, devoid of luxuries. He was passionate about the development of his homeland, willing to serve with China's scientific development.

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References

[1] Zheng S. Hsien Wu, the founder of Chinese biochemistry and nutriology. Protein Cell. 2012;3(5):323–4.

[2] Hsien Wu. Nutr Rev. 1974;32(10):319, https://doi.org/10.1111/j.1753-4887.1974.tb07322.x.

[3] Dr Hsien Wu. Blood specialist; biochemist, co-developer of analysis method, is dead. Taught at U. of Alabama, New York Times, vol: 65; 1959. p. 27. Retrieved from https://www.nytimes.com/1959/08/10/archives/d-r-hsien-wu-65-blood-specialist-biochemist-codeveloper-of-analysis.html

[4] Bishop C. Hsien Wu (1893–1959): a biographical sketch. Clin Chem. 1982;28(2):378–80.

[5] Wu DY. Hsien Wu 1893–1959, in loving memory. Boston, 1959–1960.

[6] Reardon-Anderson J. The study of change: chemistry in China, 1840–1949. Cambridge: Cambridge University Press; 2002.

[7] Van Slyke DD, Wu H, McLean FC. Studies of gas and electrolyte equilibria in the blood. J Biol Chem. 1923;56:765–849.

[8] Edsall J. Hsien Wu and the first theory of protein denaturation (1931). Adv Protein Chem. 1995;46:1–5.

[9] Carmichael E. Dr. Hsien Wu. Nature. 1960;185(4716):809–10.

[10] Rees A. The antibody molecule. Oxford: Oxford University Press; 2015.

[11] Heidelberger M, Kendall F. The beginnings of quantitative immunochemistry. Trends Biochem Sci. 1979;4(7):168.

[12] Kabat EA, Mayer MM. Experimental immunochemistry. 1st edn. USA: Charles Thomas Publisher; 1948.

[13] Wu H. Studies of immunological reaction. PSEBM. 1928;25:341–5; Wu H. Studies of immunological reaction. PSEBM. 1929;26:737–8.

[14] Heidelberger M, Kendall FE. A quantitative study of the precipitin reaction between type III pneumococcus polysaccharide and purified homologous antibody. J Exp Med. 1929 Dec;50(6):809–23.

[15] Boyd WC, Hooker SB. The influence of the molecular weight antigen on the proportion of antibody to antigen in precipitates. J Gen Physiol. 1934 Jan;20(3):341–8.

[16] Wu HA. Studies on denaturation of proteins. XIII. A theory of denaturation. Chin J Physiol. 1931;5(4):321–44.

[17] Wu HA. Studies in denaturation of proteins. XIII. A theory of denaturation. Adv Protein Chem. 1995;46:6–25 (Reprinted in English from Reference 2).

[18] Mirsky AE, Pauling L. On the structure of native, denatured and coagulated proteins. Proc Natl Acad Sci. 1936;22:439–47.

[19] Lin K, Wu H, Chen T. Effect of denaturation on digestibility of ovalbumin by pepsin and trypsin. Exp Biol Med. 1927;25(3):199–200.

[20] Wu H, Cheng L, Li C. Composition of antigen-precipitin precipitate. Exp Biol Med. 1928;25(9):853–5.

[21] Bacon Chow. E Biochemist, dies: professor helped to isolate first pure antibody, New York Times (1923-current file); 1973 Sep 28;63. ProQuest Historical Newspapers: The New York Times with Index. p. 36.

[22] Antibody isolated by Chinese doctors. New York Times (1923-current file); 1936 Oct 2. ProQuest Historical Newspapers: The New York Times with Index. p. 27.