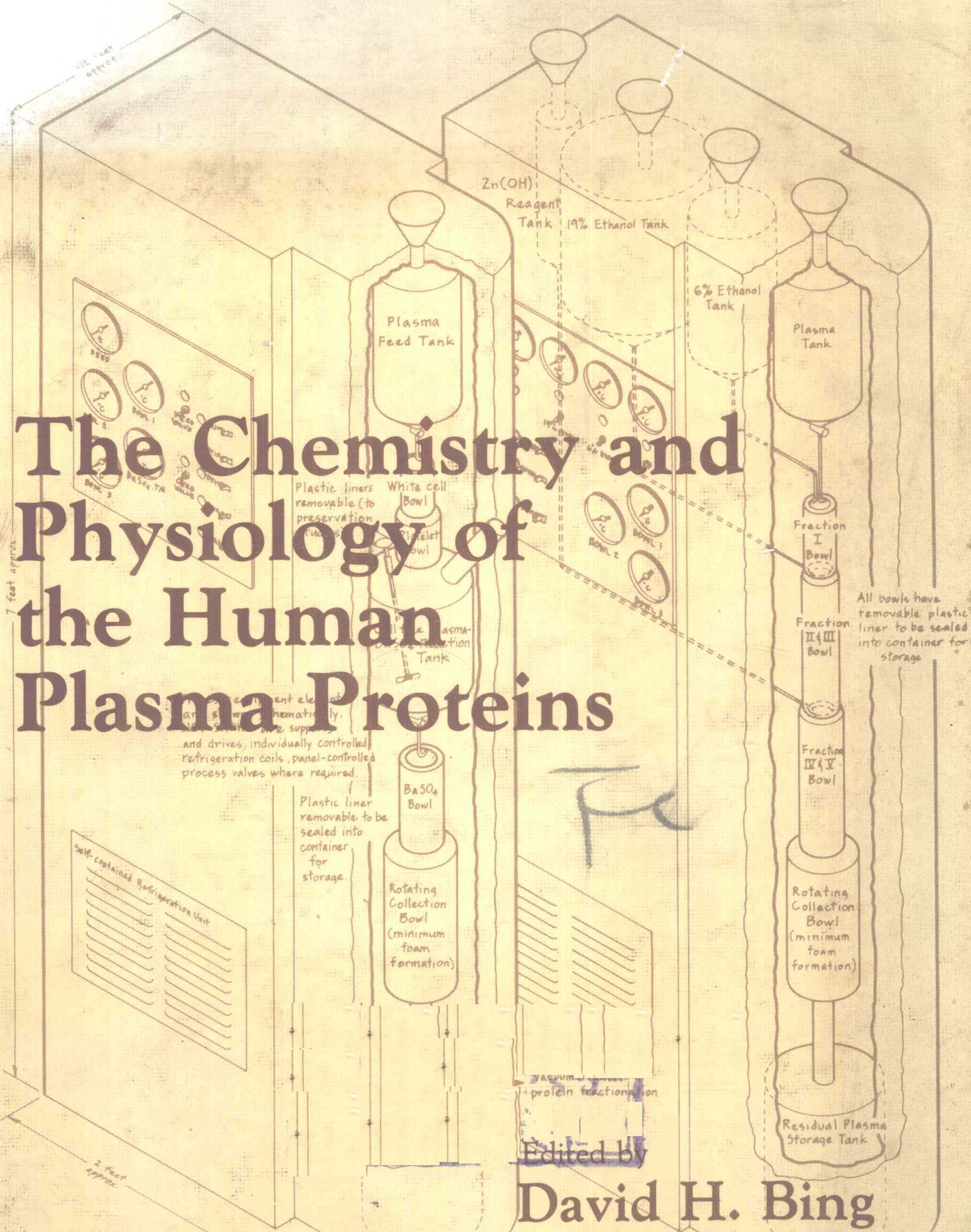
The Chemistry and Physiology of the Human Plasma Proteins

Edited by

David H. Bing

Pergamon Press



The Chemistry and Physiology of the Human Plasma Proteins

Proceedings of a Conference
Held 19-21 November 1978 in
Boston, Massachusetts
Sponsored by the
Center for Blood Research

Edited by

David H. Bing

Pergamon Press
NEW YORK • OXFORD • TORONTO • SYDNEY • FRANKFURT • PARIS

Pergamon Press Offices:

U.S.A.	Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A.
U.K.	Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England
CANADA	Pergamon of Canada, Ltd., 150 Consumers Road, Willowdale, Ontario M2J, 1P9, Canada
AUSTRALIA	Pergamon Press (Aust) Pty. Ltd., P O Box 544, Potts Point, NSW 2011, Australia
FRANCE	Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France
FEDERAL REPUBLIC OF GERMANY	Pergamon Press GmbH, 6242 Kronberg/Taunus, Pfердstrasse 1, Federal Republic of Germany

Library of Congress Cataloging in Publication Data
Main entry under title:

The chemistry and physiology of the human plasma
proteins.

Proceedings of a meeting held November 19-21, 1978 in
Boston and sponsored by the Center for Blood Research.
Includes index.
1. Blood proteins—Congresses. I. Bing, David H.
II. Center for Blood Research. [DNLM: 1. Blood
proteins—Congresses. 2. Chemistry, Physical—Congresses.
WH400 C517 1978]
QP99.3.P7C43 612'.12 79-10742
ISBN 0-08-023860-2

Printed in the United States of America

The Chemistry and Physiology of the Human Plasma Proteins

Pergamon Titles of Related Interest

Abraham Microscopic Innervation of the Heart and Blood Vessels in Vertebrates Including Man

Birke et al Physiology and Pathophysiology of Plasma Protein Metabolism

Fareed et al Perspectives in Hemostasis

Kelemen Physiopathology and Therapy of Human Blood Disease

Maupin Blood Platelets in Man and Animals

Peeters/Wright Plasma Protein Pathology

Rosenoer et al Albumin Structure, Function and Uses

PREFACE

On November 19-21, 1978, the Center for Blood Research held a meeting in Boston, Massachusetts to commemorate fifty years of research on the proteins of human plasma carried out by the Harvard Medical School Group working under the leadership of Dr. E.J. Cohn, and by their successors in laboratories all over the world. The meeting coincided with the 25th anniversary of the founding of the Protein Foundation, the parent organization of the Center for Blood Research. This book contains the proceedings of that conference.

I would like to thank a number of people for their assistance in organizing this meeting and the subsequent publication of this book. First, I would like to express by gratitude to all the participants for their excellent presentations and contributions to this book. The Center for Blood Research is indebted to The Commonwealth Fund for its support in the publication of this book. Without the unfailing efforts of Mr. Francis G. Shaw and Mrs. Jean S. Gregory, the meeting would never have taken place. I would like to recognize the dedicated help of Mrs. Rachelle Rosenbaum, not only with the meeting, but in the typing, editing and indexing of this book. Finally, I would like to express gratitude to the individuals at Pergamon Press, and Mr. Laszlo Straka in particular, for their help in preparation of this book.

David H. Bing, Ph.D.
Center for Blood Research
Boston, Massachusetts

Robert B. Pennell
Director of Laboratories
Center for Blood Research
Boston, Massachusetts

INTRODUCTION

It is appropriate to dedicate this volume to Robert B. Pennell. In the last twenty-five years of the fifty years of research being commemorated by this congress, his energies, scholarship and administrative skills were applied totally and undividedly to the pursuit of goals and the execution of initiatives made by Doctor Edwin Cohn. His long and distinguished career has encompassed translocations, from Harvard Medical School to the Bussey Laboratories to the Center for Blood Research, and the several reorganizations, from the University Laboratory of Physical Chemistry related to Medicine and Public Health to the Protein Foundation, the Blood Research Institute and now the Center for Blood Research.

All of Doctor Pennell's formal education was taken at Michigan State University where he earned a B.S. in 1930, the M.S. in 1932 and the Ph.D. in immunology in 1937 under Doctor I.F. Huddleson. In 1939 he joined Sharp and Dohme Co. in Philadelphia as Research Associate, where, in 1950, he became Assistant Director of Immunochemical Research in the Sharpe and Dohme Division of Merck and Company. While here, he became acquainted with Doctor Cohn when sent to Boston to learn Cohn fractionation methods 6 and 9. After three years of long range collaboration with Doctor Cohn and associates, he was recruited in 1953 to join the newly created Protein Foundation. He was its first employee, assuming the title of Chief of the Chemical Division. In 1956, he became Lecturer in Immunology at Harvard School of Public Health. When the reorganization to form Blood Research Institute occurred in 1966, he was named Acting Scientific Director, a post he held for the ensuing five years.

We first met Bob Pennell by telephone from Boston to Philadelphia, having been referred to him by Doctor Cohn for help in obtaining leukocytes for the study of blood lysozyme. In his usual gracious and cooperative way, he promised buffy coat white cells from the days collection of blood. That night, when we met the plane, we were astonished to receive a gallon jug with buffy coat "cream" skimmed from <u>two thousand donors</u>! It was a supply which took years to consume. Bigness was a characteristic of Doctor Cohn's group, from brains and concepts, to engineering, technology and accomplishments. Bob Pennell was an integral member.

In the historical reminiscences made by the eminent speakers at the opening session of these meetings, one can discern many and varied ways that Bob Pennell quietly, modestly, but always competently, carried out the functions of investigator, administrator, and good friend to his colleagues. Reference was made by Doctor Surgenor of the size of the plasma fractionation industry at the present day, a descendant of the pioneering days; and Doctor Janeway traced its early trials. The World War II caused acute needs for blood and attempts to make and use bovine albumin were modestly successful, but troublesome immunologically. The military, pressured and reluctant, was persuaded artfully by Cohn to support the fractionation of human plasma, and the group was on its way. The complete reversal in attitude of the military to the acceptance of Cohn methods and the extent to which they were developed and applied is exemplified in 1970 by the gift by the

Defense Department to Blood Research Institute of 2,000 pounds (that's right, one ton!) of surplus, outdated, human albumin, condemned because of pyrogens. It was Bob Pennell's frustrating duty to collect it from laboratories and industries around the country and try to find a way to salvage the material. The Navy, too, invested heavily in a program of cryogenic red cell preservation at the Chelsea Naval Hospital under Doctors Tullis, Pyle and Pennell at a time when red cell freezing was a laboratory curiosity. Today, blood freezing has spawned a new industry and the Navy Blood Preservation Laboratory, now independent, is the foremost in the world.

Bob Pennell participated in the pioneering research on fractionation and the methods of blood collection now in standard use. The use of ion-exchange resins as anticoagulants to remove calcium ion was explored and eventually abandoned. Nevertheless, these efforts launched the career of Doctor Harry Antoniades, Senior Investigator at the Center for Blood Research, who observed that insulin activity, and later Hageman factor and serum growth factor, were removed from plasma by the resins. During this developmental period of the refinement of fractionation methodology and new applications, Doctor Pennell served for fifteen years on the Subcommittee on Plasma of the Division of Medical Sciences of the National Research Council, the last nine years of which he chaired the Committee. It was the function of this Committee to advise government agencies related to health and defense on all research relative to use of blood and its components. The many forums and symposia it sponsored were the most stimulating any young investigator could be privileged to attend. Indeed, they were in many respects like the sessions of the meeting published in this volume.

In the opening session of these symposia, Doctor Alper began his remarks by exhibiting a bottle containing 100 gms of properdin found among the old stock of chemicals moved from the Bussey Laboratories into the new building of the Center for Blood Research, --- obviously impressed with the grand style and scope of Doctor Cohn's program. All but a few were unaware that the isolation and properties of properdin was the subject of the Ph.D. thesis many years earlier by Fred Rothstein, present in the audience, but then a graduate student in biochemistry at Harvard under Doctors Oncley and Pennell.

In the transition from the glorious times of Doctor Cohn through the hard economic period which followed after his death, Bob Pennell devoted most of his energies to keeping the Protein Foundation and later the Blood Research Institute solvent and functioning. Operating from a tiny cubicle office in the multi-partitioned and overcrowded Bussey Laboratory, his responsibilities were compounded many-fold when the happy dream, a new building on the present site, became a reality. He coordinated moves of the senior staff from various scattered laboratories, coped with architects, builders, staff, the city, and neighbors. Only a few of us fully appreciated his unstinting, tireless, and unselfish dedication to the truly Herculean task. We all tried to continue to function, with the minimum of interruption while he sacrificed his own scientific work to make us comfortable and productive. In this most difficult period (1970-1973) he was also responsible for renewing annually the Institute Program Project Grant, the main source of operational funds. Single-handedly (almost) he wrote voluminous progress reports and renewal proposals, often just from data or rough copy supplied by busy investigators. From 1961-1971, as principal investigator, he was responsible for and successfully raised these funds. With what time he could generate, he and his close friend and colleague, Jack Bergen of the Worcester Foundation, conducted research on the identification of chemical factors in plasma related to schizophrenia. After the merger with Blood Grouping Laboratory made time available to him to concentrate on research, one of his biggest frustrations ever was his inability to raise funds through grant channels to continue the project.

To dedicate a volume to Bob Pennell without commenting on his extraordinary character would be an injustice. The adjectives, kind, gentle, self-effacing, courteous, considerate, unassuming, and the cliche, gentleman's gentleman, do not exaggerate. In the opening session, remarks by Doctor Tullis, bedridden with flu and unable to attend, were read by Pennell. Doctor Tullis cited the difficult times of 1952 when he and Pennell met weekly to struggle with financial problems and decide how to meet the payroll. The tenure of the Protein Foundation "hung by a gossamer thread". For these years and many to follow Bob Pennell was the thread.

Fabian J. Lionetti
Senior Investigator
Center for Blood Research
November 27, 1978

CONTENTS

PHYSICAL CHEMISTRY AT HARVARD MEDICAL SCHOOL: THE FIRST TWENTY YEARS

John T. Edsall
Harvard University, Biological Laboratories, Cambridge, Massachusetts 02138

THE FOUNDING OF THE DEPARTMENT OF PHYSICAL CHEMISTRY

The Department of Physical Chemistry at Harvard Medical School was unique, both in name and in character, among medical school departments. To explain its origins I must note that my father, David Linn Edsall, had become Dean of Harvard Medical School in 1918. One of the early problems that faced him was that of holding Lawrence J. Henderson at Harvard. Henderson was the discoverer of buffer action, and the author of a series of papers on the physical chemistry of blood -- studies that were to culminate ten years later in his great monograph "Blood". He was also the author of an influential monograph on the biological significance of the properties of matter: "The Fitness of Environment" and of a sequel entitled "The Order of Nature". He had been teaching a pioneer course on the history of science in Harvard College for some years. Yet, in spite of his brilliance and his versatility --- partly indeed perhaps because his range of interests was so broad --- he had not yet received adequate recognition from the Harvard Administration. Johns Hopkins University was trying to lure him away with a very attractive offer, and David Edsall was determined to keep him at Harvard if possible. Suitable action became possible because the Medical School had just received a gift of two million dollars, later increased to four million, through the will of an enterprising industrialist, Captain Joseph R. DeLamar. This was the largest single gift that the Medical School had ever received up to that time, and it went to support research in several departments. David Edsall used a fraction of it to set up a new Department of Physical Chemistry with Henderson in charge. Its responsibility was to pursue research fundamental to medicine, with no specific obligations for teaching medical students. Henderson happily accepted the proposal, and decided to stay at Harvard.

Henderson had long ceased to be a laboratory worker, and he needed someone to take charge of the laboratory in the new Department. For this he turned to his former student, Edwin Joseph Cohn, who had done his Ph.D. thesis partly with Henderson, and partly with Frank Lillie at Chicago and Woods Hole. After completing his Ph.D. thesis, Cohn made the decision to devote his scientific life to the study of proteins. He began by going to the laboratory of the foremost protein chemist in the country --- Thomas Burr Osborne in New Haven. Our involvement in World War I soon brought him back to Harvard, to take part with Henderson in a project for making bread from potatoes and other sources; what flour was in short supply, during the war, and making bread from other sources was an important practical problem.

With the war over, Cohn received one of the first of the National Research Council Fellowships, and went in 1919 to Copenhagen to work with the great Danish protein chemist S.P.L. Sørensen. Although he spent only about six months there, this experience was crucial for Cohn's subsequent career, and greatly influenced his choice of research problems in the next few years. He returned to Harvard Medical School in 1920, to take up the work of the Department of Physical Chemistry. From the beginning Henderson gave him a very free hand, and within a relatively short time he was for practical purposes the head of the laboratory. Henderson turned his main energies to research on blood and his associates at the Massachusetts General Hospital, and later at the Fatigue Laboratory in the Harvard Business School.

In those early years the Department occupied only part of the fourth floor of Building C1 at the Medical School; later it gradually expanded. On the floor below were the Laboratories of Physiology, under the direction of Walter B. Cannon. Professor Cannon had an operating room for this animal experiments on the fourth floor, adjoining Cohn's Department. At the farther end of the hall, in what later became Edwin Cohn's office, was Professor William T. Porter, another physiologist. When Porter retired in 1928, Cohn took over that section of the fourth floor also. With Cohn in the laboratory were Ronald M. Ferry, later to become Master of John Winthrop House at Harvard, and Selig Hecht, who was in the early stages of his distinguished career in the physiology of vision, before he moved permanently to Columbia. Jessie L. Hendry was the first expert laboratory assistant, followed not long after by Ruth E.L. Berggren and Adela M. Prentiss.

Fig. 1 Edwin J. Cohn as a young man. From M. Florkin "History of Biochemistry" in M. Florkin and E.H. Stotz (editors) Comprehensive Biochemistry, Vol. 30. Elsevier Publishing Co., Amsterdam and New York 1972.

EARLY RESEARCH ON PROTEINS

From the beginning, Cohn (of whom an early photograph is shown in Fig. 1) concentrated on the factors determining the solubility of proteins, and on their behavior as acids and bases --- problems that had already been intensively studied in Sørensen's laboratory. From the beginning Cohn assumed, like his mentors Osborne and Sørensen, that the general laws of physical chemistry applied to proteins, provided that one made due allowance for their large sizes and multiple electric charges. In his titration studies he did his best to correlate the capacity of proteins to take up or release protons with their content of acidic and basic amino acid residues. The correlation was often imperfect, owing largely to the great difficulties that existed in those days in doing amino acid analyses.

Cohn did not do such analyses himself, but relied on the best available data in the literature, and sought advice from his friend H.B. Vickery in New Haven. The titration curves were done on the hydrogen electrode; glass electrodes did not come into use in the laboratory until the middle 1930s. From the start Cohn rejected the notion, then still fashionable among many of the colloid chemists, that proteins might bind hydrogen or hydroxyl ions by some rather vaguely defined process of adsorption. He held strictly to the view that combination occurred by chemical binding to acid or basic amino acid side chains. At that time he tacitly assumed that all the acid and basic side chains were freely titratable in the native protein. The idea that proteins could unfold reversibly, in acid or alkaline solution, so as to make groups titratable which had been inaccessible in the native protein, was not to emerge until much later, and Cohn was by no means the first to perceive it.

The studies on solubility involved both the "salting in" of euglobulins by dilute salt solutions, and their salting out in concentrated salt. Both phenomena, of course, had long been known; and Hofmeister in Strassburg, about 1890, had described the series of salts (or rather of anions and cations) that bears his name, in terms of their capacity to precipitate proteins at high salt concentration. Cohn's first notable contribution to the interpretation of salting out was his plot of the logarithm of the protein solubility (S) against the salt concentration (C_S) or the ionic strength, which have a straight line at high salt concentrations, according to the equation:

$$\text{Log } S = \beta - K_S C_S \qquad (1)$$

Here K_S is the salting out coefficient, and β is a constant.

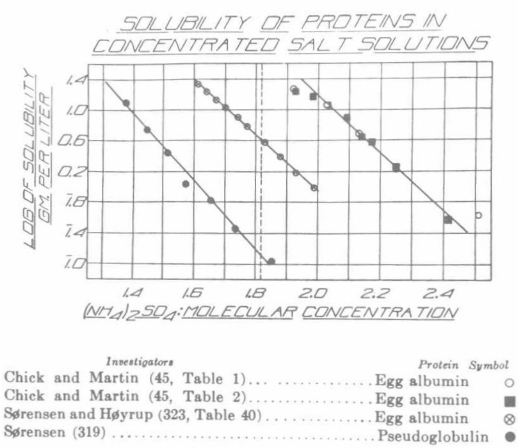


Fig. 2 The linear logarithmic relation for the salting out of proteins. From E.J. Cohn (1925) Physiol. Reviews 5, 349.

The equation is illustrated in Fig. 2, taken from Cohn's original plot in his comprehensive review of 1925. The data are not his; he took them from the published papers of Chick and Martin and of Sørensen, who certainly had had no thought of such a logarithmic relation. The fit to the linear relation is impressive, and it is interesting to note that it is just as good for Sørensen's pseudoglobulin, which was certainly a heterogeneous mixture of components, as for the highly purified ovalbumin.

Cohn's thinking about salting out, and many other matters, was certainly greatly influenced by his friend George Scatchard of M.I.T. Cohn and Scatchard had known each other as undergraduates at Amherst, and they were not then particularly congenial; but, as Scatchard was later to write in his Cohn Memorial Lecture: "Soon after I came to M.I.T. in 1923, Cohn and I attended a reception of the Science Club of Amherst College for Niels Bohr. We stayed with a mutual friend and drive back to Cambridge together. I was surprised to find that he thought more like me than anybody I had ever met. His thoughts were more developed and polished than mine, but there was little other difference. From that day we became close friends and frequent collaborators although we never published a paper together." Scatchard was much more deeply grounded than Cohn in fundamental physical chemistry and mathematics. Cohn was the more dynamic of the two, eith driving force as an organizer, and a remarkable capacity for bringing diverse people together in cooperative research. Their abilities were in many ways complementary. I well remember the important part that George Scatchard played in the departmental seminars, from the very beginning of my work in the laboratory in 1926. It was very rare, indeed almost unheard of, in those days for a "pure" physical chemist like Scatchard to come and listen to biochemists, and even to clinical investigators, and discuss their problems with them, patiently and at length. His scientific publications were generally hard reading, with rigorous thinking and highly condensed presentation. Students in his undergraduate course were reported to have a hard time understanding him. Some indeed referred to the time of his lectures as "the mystery hour". Nevertheless in personal discussion he could be almost endlessly patient in going over difficult points, with others less deeply versed in physical chemistry, until his interlocuter gradually began to take it all in. I am glad to express my own enduring gratitude to him for generout help and advice over many years. There are others in this gathering who can say the same.

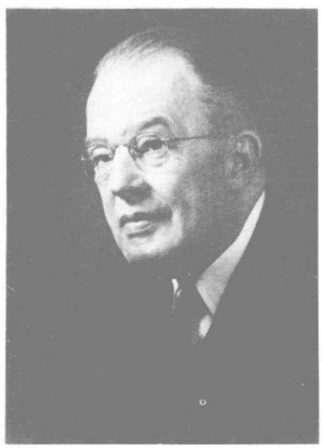


Fig. 3 George Scatchard in 1954.

A masterly experimenter in the solubility work was Dr. Arda Alden Green, who did an outstanding series of studies on horse carboxyhemoglobin. She determined both salting in and salting out curves, for a variety of salts, as well as studying the influence of pH on solubility. The solubility of course was at a minimum, at or near the isoelectric point of the protein, and it increased on going either to lower or to higher pH. She found the salting out coefficient, K_S, for any given salt, to be independent of temperature and pH; change of either of these variables displaced the linear portion of the salting out curve up or down, parallel to itself, but did not change the slope. Figure 4 gives an example of her solubility data for several salts over a wide range of ionic strength.

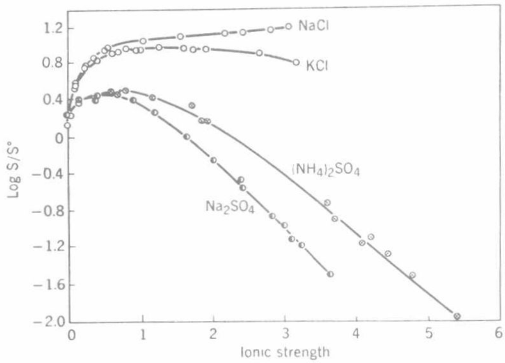


Fig. 4 The salting-in and salting-out of horse carboxy-hemoglobin by various salts. From the work of Arda A. Green [J. Biol. Chem. 93, 495-516, 517-542 (1931); 95, 47-66 (1932)] as plotted by E.J. Cohn and J.T. Edsall (1943, p. 608).

Beginning about 1926 Cohn, while actively continuing his protein studies, devoted much effort to an attempt to purify the anti-pernicious anemia factor in liver. George R. Minot's work had demonstrated the curative properties of liver in this disease, and a search for the active principle was obviously called for. The work of Cohn and his associates was a practical triumph, for they prepared a concentrated liver extract that saved patients from the previous necessity of eating about 200 g of liver a day in order to stay well. Yet for Cohn the outcome was a bitter disappointment, for he never got the active principle in pure form; indeed his extracts never even approached purity. Attempts to find an assay for the active component in animals proved fruitless. It was not until much later, in 1948, that a successful bacterial assay was developed, and pure vitamin B-12 was at last obtained, but not in Cohn's laboratory.

When I joined the laboratory in 1926, working in my limited free time while I was a third year medical student, Cohn set me to work on a globulin from muscle which had already been studied before me by W.T. Salter. It was messy stuff to handle, and gave extremely viscous solutions. I sometimes came close to despair in working on it. The arrival of Alexander von Muralt from Switzerland in 1928 opened entirely new vistas, when he showed us that a flowing solution of the globulin showed strong double refraction, which disappeared when the flow stopped. This indicated that the protein particles were long and asymmetrical. That simple observation led us to two years of joint research, in which Alex's superb experimental resourcefulness, and his knowledge of physics in relation to biology, enabled us to learn much about what was evidently an important component of the structural basis of muscular contraction. Following earlier workers, we called the protein myosin. Today it is clear, from the work of Albert Szent-Gyorgyi and others, that it was a form of what would now be termed actomyosin.

THE SECOND DECADE: THE STUDY OF AMINO ACIDS AND PEPTIDES AS PROTEIN MODELS

Beginning about 1930, and continuing for almost exactly a decade, the Laboratory undertook a far-reaching study of the physical chemistry of amino acids and