PROTEINS OF IRON STORAGE AND TRANSPORT

Edited by G. SPIK, J. MONTREUIL,
R. R. CRICHTON and J. MAZURIER

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Proceedings of the 7th International Conference on Proteins of Iron Metabolism held in Villeneuve d'Ascq (France) on 30 June-5 July, 1985

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1985

ELSEVIER SCIENCE PUBLISHERS
AMSTERDAM · NEW YORK · OXFORD

ISBN 0 444 80722 5

Published by:
Elsevier Science Publishers B.V. (Biomedical Division)
P.O. Box 211
1000 AE Amsterdam
The Netherlands

Sole distributors for the USA and Canada:
Elsevier Science Publishing Company Inc.
52 Vanderbilt Avenue
New York, NY 10017
USA

Library of Congress Cataloging-in-Publication Data

International Conference on Proteins of Iron Metabolism
(7th : 1985 : Villeneuve-d'Ascq, France)
Proteins of iron storage and transport.

Bibliography: p.
Includes index.
1. Transferrin--Congresses. 2. Ferritin--Congresses.
3. Iron proteins--Congresses. I. Spik, G. (Geneviève)
II. Title.
QP552.T7I567 1985 612',3924 85-20603
ISBN 0-444-80722-5 (U.S.)

Printed in The Netherlands

PREFACE

Since the First International Conference on the Proteins of Iron Storage and Transport, organised in 1973 by Drs Phil Aisen, Pauline Harrison and Ernie Huehns at the University College in London, the number of participants has more than doubled and the field of iron metabolism has undergone a considerable expansion due to major advances in structure determination, gene cloning and cell receptors.

All of the six previous meetings held in London, Louvain-La-Neuve, New-York, Davos, San Diego and Sapporo marked an important step in the development of the research on Proteins of Iron Storage and Transport, due to the enthousiasm of the participants to share their new results and to exchange ideas.

The VII th International Conference on Proteins of Iron Metabolism which was held from the 30th of June to the 5th of July 1985 in France, on the Campus of the Université des Sciences et Techniques de Lille at Villeneuve d'Ascq, has not failed in this tradition.

206 participants from 22 countries have presented exciting new results in 18 plenary lectures and more than 150 poster communications. Since it is impossible to publish all of the results in this book, we have chosen to give an overview. In particular, the eighteen plenary lectures are included as well as a number of oral communications which have been selected by the chairmen who had, in addition, the heavy responsability during the round table discussions to summarize the most relevant results presented in the poster sessions. I would like to thank them very warmly.

One of the most pleasant aspects of scientific meetings is to offer an opportunity to honour personalities who have contributed, by their discoveries, to enrich our knowledge of Nature and Life. During the VIIth International Conference on Proteins of Iron Metabolism we have chosen to honour two pioneers in the field of transferrins : Professor Arthur Schade for his work on conalbumin and siderophilin and Professor Jean Montreuil for his discovery, 25 years ago, of lactotransferrin from human milk. The meeting was dedicated to both of them.

The Conference and satellite events have been achieved thanks to the financial support which has been offered by the Ministère de la Recherche et de la Technologie, the Conseil Régional du Nord/Pas-de-Calais, the Centre National de la Recherche Scientifique, the Institut National de la Recherche Médicale, the Université des Sciences et Techniques de Lille, the City of Lille and by several french and foreign industrial firms. I express my gratitude to all of them for their help.

Financial support is not enough to be able to organized a congress and if we have succeeded, it is thanks to the aid of devoted men and women who have made the stay of the participants as pleasant as possible. With enthusiasm, everyone of the "Laboratoire de Chimie Biologique" contributed to this, and I thank them with all my heart !

May I express my particular gratitude to the Members of the Organizing Committee for their constant and efficient help : Jean Montreuil, Robert R. Crichton, Joël Mazurier and André Verbert.

The meeting was placed under the shadow of the belfry of the North of France and under the sign of iron. I hope that the forged friendships which are the basis to link scientific connections will be solid and durable as iron itself!

Geneviève SPIK

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Conalbumin and Siderophilin as Iron-binding Proteins : A Review of Their Discovery

Arthur L. Schade

Appropriate to the opening of this "Conference on the Proteins of Iron Metabolism", an historical review of the original investigations leading to the elucidation of what biochemically constitutes the iron-binding factors of hen's egg white and of human plasma has been requested by interested well-wishers. I am pleased to comply. References are appended (1-30). To introduce this tale of scientific adventure, I offer you an extract of a letter that Horace Walpole of England wrote to his friend Horace Mann on January 28, 1754 :

> "I once read a silly fairy tale, called "The Three Princes of Serendip" : as their highnesses travelled, they were always making discoveries by accidents and sagacity, of things they were not in quest of : for instance, one of them discovered that a mule blind of the right eye had travelled the same road lately, because the grass was eaten only on the left side, where it was worse than on the right -- now do you understand "serendipity" ? One of the most remarkable instances of this "accidental sagacity" (for you must observe that "no" discovery of a thing you "are" looking for, comes under this description) was that my Lord Shafsbury, etc." (1).

From this letter, "serendipity" and its derivatives became accepted, if at times misused, words of the English vocabulary. In what follows, I trust that Walpole would approve the use of "serendipity" to describe our discovery of a protein whose property of iron chelation under physiological conditions has stimulated many significant biological and biochemical studies.

During the past World War we were engaged in an effort, among others, to serve the Medical Corps of the U.S. Army in the production of a polyvalent bacteriophage preparation effective against Shigella

dysenteria as well as against a variety of paradysentery strains (2, 3, 4). Individual bacteriophage lysates were evaluated for potency by the recording of effective lysis of a succession of ten-fold dilutions of a test lysate in a standard culture of its specific bacterial host grown in nutrient broth for 24 to 48 hours incubation at 37°C. When we had succeeded in the production of an effective polyvalent combined lysate, the Army suggested that it be made available in a dry form suitable for pill administration as a prophylactic to military personnel in dysentery-threatening areas. Lyophilization of the individual and combined lysates resulted in some losses of viral activity of the lysates, but the loss was particularly severe in the lysates active against Sh. Dysenteriae. To protect the latter phages from the effects of dessiccation, a great variety of additives were tested, including hen's egg white (5). For such tests, a control series of original lysate dilutions without egg white or other additives were run against the water-reconstituted lyophilized "protected" lysates. With egg white as additive and Sh. dysenteriae as test bacterium, we noted, after 48 hours incubation, that the customary secondary growth of the organism following initial lysis as seen in the control series did not appear in the first tubes of the duplicated dilution series where the concentration of egg white was the highest. Further investigation showed that a comparable amount of egg white, raw or lyophilized, added to nutrient broth inhibited the primary growth of this bacterium. Titrations run in the absence of phage with various concentrations of egg white proved that the inhibition of growth depended upon the amount of egg white added to the inoculated medium and not to the number of active bacterial viruses.

The problem presented by the fiven results simply stated was : Why did 0.02 ml of fresh egg white when added to 1 ml of 1 % meat broth plus 0.5 % peptone at pH 7.2 inhibit the growth of 2-20 X 10^5 Sh.

dysenteriae organisms for 24 hours at 37°C ? Serial dilutions of transfers from inhibited cultures to egg-white-free broth showed that failure to grow was not due to death of the cells. This result made lysozyme an unlikely culprit for the observed bacteriostasis. Avidin, a known constituent of egg white and complexer of biotin, was ruled out as the inhibitory factor when additions of twice the egg white concentration of avidin to the growth medium failed to prevent normal bacterial development. Conversely, additions of biotin in amounts double that estimated to be bound by the avidin in egg white failed to overcome the growth inhibition. We observed that the inhibitory effect of egg white resisted dialysis ; was active following incubation at 60°C for one hour, but was destroyed after one hour at 70°C ; and was salt-precipitable. The immediate indications were that the active agent was a protein.

Attempts to reverse the growth inhibition of Sh. dysenteriae by egg white were successful by additions to the nutrient broth of yeast extract, corn steep liquor, or meat extract in relatively large amounts. Ten recognized growth factors in yeast extract were tested singly and in combination. None were effective in abolishing the bacteriostasis. When yeast extract was ashed and the ash dissolved in hydrochloric acid, its addition in graded amounts following neutralization resulted in good bacterial growth in the inoculated egg white-nutrient broth. Of 31 elements tested, iron alone, both ferrous and ferric, overcame the growth inhibition. By the use of all-or-none growth measurements, we determined that 1 ml of egg white could make 15-20 micrograms unavailable to Sh. dysenteriae. Investigating the effect of pH on the dializability of iron from egg white-iron saline mixtures, we observed a close relationship between increasing acidity on the lability of the iron from the egg white-iron complex and the failing inhi-