

ADVANCES IN  
EXPERIMENTAL  
MEDICINE  
AND BIOLOGY

---

Volume 152

# **NEW VISTAS IN GLYCOLIPID RESEARCH**

Edited by Akira Makita, Shizuo Handa,  
Tamotsu Taketomi, and Yoshitaka Nagai

# NEW VISTAS IN GLYCOLIPID RESEARCH

Edited by

**Akira Makita**

Cancer Institute  
Hokkaido University School of Medicine  
Sapporo, Japan

**Shizuo Handa**

Tokyo Medical and Dental University  
Tokyo, Japan

**Tamotsu Taketomi**

Shinshu University School of Medicine  
Matsumoto, Japan

and

**Yoshitaka Nagai**

Institute of Medical Science  
University of Tokyo  
Tokyo, Japan

PLENUM PRESS • NEW YORK AND LONDON

---

Library of Congress Cataloging in Publication Data

Main entry under title:

New vistas in glycolipid research.

(Advances in experimental medicine and biology; v. 152)

"Proceedings of the Biwako Symposium on Glycolipids which was held in Otsu, Japan, September 17-19, 1981"—Pref.

Includes bibliographical references and index.

I. Glycolipids—Congresses. I. Makita, Akira, 1931- . II. Biwako Symposium on Glycolipids (1981: Otsu-shi, Japan). III. Series. [DNLM: 1. Glycolipids—Congresses. QU 85 N533 1981]

QP752.G56N48 1982

612'.01577

82-11221

ISBN 0-306-41108-3

---

Proceedings of the Biwako Symposium on Glycolipids, held  
September 17-19, 1981, in Otsu, Japan

© 1982 Plenum Press, New York  
A Division of Plenum Publishing Corporation  
233 Spring Street, New York, N.Y. 10013

All rights reserved

No part of this book may be reproduced, stored in a retrieval system, or transmitted, in any form or by any means, electronic, mechanical, photocopying, microfilming, recording, or otherwise, without written permission from the Publisher

Printed in the United States of America

## PREFACE

This book presents the proceedings of the "Biwako Symposium on Glycolipids" which was held in Otsu, Japan, September 17-19, 1981, just before the 6th International Symposium on Glycoconjugates in Tokyo. The meeting was convened by Dr. Taro Horii in commemoration of the discovery of hematoside and globoside by Dr. Tamio Yamakawa in 1951.

Glycolipid research beginning from the age of Thudichum, father of neurochemistry, developed rapidly in the third quarter of this century and is now extensive and interdisciplinary, having progressed to the isolation and chemical characterization of a great number of glycolipids, studies on catabolism and synthesis, biochemical elucidation of inherited sphingolipidoses, immunochemical characterization of various antigens, studies on membrane phenomena and cancer problems, and so on. These developments have been stimulated by both methodological advances (TLC, GLC, MS, NMR and cell cultures) and mutual interactions with many other fields of life science. Although glycolipids are relatively minor components of cells and tissues, and their functions depend essentially on their carbohydrate moiety like other glycoconjugates, they are unique in biochemistry and biophysics by way of their amphipathic properties.

The purpose of the meeting was to bring together scientists involved in a wide variety of aspects of glycolipid biochemistry, and to promote further research. The proceedings also includes the very fruitful discussions at the meeting as well as some papers which were not presented at the meeting but dedicated to Dr. T. Yamakawa on the 60th anniversary of his birth. Regretfully, we cannot communicate the pleasant atmosphere of the "get-together" on the same boat on Lake Biwako, and other informal events during the convention.

We would like to express our thanks to all participants at the meeting and contributors, especially Dr. Sen-itiroh Hakomori for communicating with the publisher.

The Editors

## CONTENTS

Glycolipids and I: Past, Present and Future . . . . .	1
T. Yamakawa	
Aspects of the Future for the Analysis of Glycosphingolipid Mixtures. . . . .	15
K.-A. Karlsson and G. Larson	
High Performance Liquid Chromatography and Structural Analysis by Field Desorption Mass Spectrometry of Underivatized Glycolipid. . . . .	23
S. Handa and Y. Kushi	
High Resolution $^1\text{H}$ Nuclear Magnetic Resonance Spectroscopy and 'Soft Ionization' Mass Spectrometry of Glyco- sphingolipids . . . . .	33
H. Egge, J. Dabrowki, P. Hanfland, A. Dell and U. Dabrowski	
Carbon-13 Nuclear Magnetic Resonance Studies of Hematoside and Globoside . . . . .	41
R.K. Yu and L.O. Sillerud	
New Strategies for Detection and Resolution of Minor Gangliosides as Applied to Brain Fucogangliosides . . . .	47
R.W. Ledeen and J.R. Sclafani	
Chemistry of Gangliosides Carrying O-Acetylated Sialic Acid. . . . .	55
S. Sonnino, R. Ghidoni, V. Chigorno and G. Tettamanti	
A Novel Pentaglycosyl Ceramide Containing Di- $\beta$ -N-Acetyl- galactosaminyl Residue (Para-Forssman Glycolipid) Isolated from Human Erythrocyte Membrane. . . . .	71
S. Ando, K. Kon, Y. Nagai, and T. Yamakawa	

Ganglioside Patterns of Frog Nervous Tissues . . . . .	83
M. Ohashi	
The Isolation and Characterization of 4-O-Methyl- glucuronic Acid-containing Glycosphingolipid from Spermatozoa of a Fresh Water Bivalve, <i>Hyriopsis schlegelii</i> . . . . .	93
T. Hori, O. Itasaka, M. Sugita, and S. Ando	
Phosphosphingoglycolipids, A New Class of Ionic Sphingoglycolipids . . . . .	103
A. Hayashi and T. Matsubara	
Glycophosphosphingolipids: "Ganglioside-like" Glyco- lipids from Plants and Fungi . . . . .	115
R.A. Laine	
Discussion . . . . .	121
A Method for the Determination of the Activity of Glycosyltransferases with Endogenous Glyco- sphingolipid (GSL) Substrates . . . . .	125
P. Stoffyn and A. Stoffyn	
Biosynthesis and Characterization of Globoside and Forssman Glycosphingolipids in Guinea Pig Tumor Cells. . . . .	131
S. Basu, M. Basu, H. Higashi, and C.H. Evans	
Glycolipid <i>N</i> -Acetylgalactosaminyltransferase Activities in Normal and Kirsten Murine Sarcoma Virus Transformed Balb/c 3T3 Cells. . . . .	139
M.W. Lockney, R.M. Soltysiak, and C.C. Sweeley	
<i>N</i> -Acetylgalactosaminyltransferases Related to Biosynthesis of Globo Series Glycolipids . . . . .	149
A. Makita, N. Taniguchi, S. Kijimoto-Ochiai, T. Ishibashi, F. Mizuno, and T. Osato	
Glycolipid Glycosyltransferase Levels in Surgical and Transplanted Human Lung Cancers. . . . .	159
S. Gasa and A. Makita	
The Antigens Ii, SSEA-1 and ABH Are an Interrelated System of Carbohydrate Differentiation Antigens Expressed on Glycosphingolipids and Glycoproteins. . . . .	167
T. Feizi	

Glycosphingolipid Biosynthesis in Rat Bone Marrow Cells . . . . .	179
T. Taki, R. Kamada, and M. Matsumoto	
The Sulfoglycolipid, Highly Acidic Amphiphiles of Mammalian Renal Tubules . . . . .	195
I. Ishizuka and K. Tadano	
Discussion. . . . .	207
Substrate Specificity of Viral, Bacterial and Mammalian Sialidases with Regard to Different N,O-Acetylated Sialic Acids and GMI. . . . .	215
M. Sander-Wewer, R. Schauer, and A.P. Corfield	
Protein Activators for the Catabolism of Glycosphingolipids. . . . .	223
Y.-T. Li and S.-C. Li	
Degradation of Glycolipids by Water-Soluble Lysosomal Glycosidases. . . . .	227
E. Conzelmann, J. Burg, G. Stephan, and K. Sandhoff	
Proteins That Transfer Sphingoglycolipids . . . . .	235
N.S. Radin and R.J. Metz	
Galactosialidosis ( $\beta$ -Galactosidase-Neuraminidase Deficiency): Clinical and Biochemical Studies on 13 Patients. . . . .	241
Y. Suzuki, K. Fukuoka, H. Sakuraba, K. Hayashi, and Y.-M. Ko	
The Twitcher Mouse: Fate of Exogenously Administered [ $^3\text{H}$ ]Galactosylsphingosine . . . . .	253
T. Kobayashi and K. Suzuki	
Studies on Sialidoses: $\text{GM}_3$ Ganglioside Sialidase in Human Leucocytes. . . . .	261
T. Yamada, Y. Tabata, T. Ariga, S. Tsuji, and T. Miyatake	
Membrane Glycosphingolipids in Chicken Muscular Dystrophy . . . . .	273
E.L. Hogan, R.D. Happel, and J.-L. Chien	
Gangliosides and Neutral Glycosphingolipids in Human Brain Tumors: Specificity and Their Significance. . . .	279
Y. Eto and S. Shinoda	

Abnormalities in Cerebral Lipids and Hepatic Cholesterol Glucuronide of a Patient with GM <sub>1</sub> -Gangliosidosis Type 2 . . . . .	291
T. Taketomi, A. Hara, and T. Kasama	
Discussion . . . . .	301
Possible Function of Brain Gangliosides in Survival in the Cold . . . . .	307
H. Rahmann	
Glycolipid Composition of Fish Brains and Its Bearing on the Phylogeny . . . . .	315
Y. Tamai, H. Kojima, K. Abe, and S. Saito	
Biosynthesis of Glycoconjugates in Pure Cultures of Neurons: Presence of Ectoglycosyltransferases. . . . .	325
H. Dreyfus, B. Hoflack, J.C. Louis, S. Harth, and R. Massarelli	
Gangliosides of Human Intestinal Mucosa: Detection of a Possible Fetal Antigen . . . . .	333
L. Svennerholm, P. Fredman, J.E. Månsson, O. Nilsson, and J. Holmgren	
Studies on the Cell Association of Exogenous Glycolipids . . .	343
H. Wiegandt, S. Kanda, K. Inoue, K. Utsumi, and Sh. Nojima	
Reactivity of Sphingoglycolipid Haptens in Glycero- glycolipid Liposomes as Well as in Glycero- phospholipid Liposomes . . . . .	353
T. Endo, S. Nojima, and K. Inoue	
Complexity of Glycolipids in Erythrocyte Membranes Is Promoted by $\beta$ -Galactoside $\alpha 1 \rightarrow 2$ Fucosyltransferase. . . .	365
E. Zdebska, A. Magnuska, H. Miller-Podraza, B. Lenkiewicz, and J. Kościelak	
Distinctive Characteristics of Ganglioside-Profiles in Human Leukemia-Lymphoma Cell Lines . . . . .	369
M. Saito, H. Nojiri, F. Takaku, and J. Minowada	
Discussion . . . . .	385
Myxovirus-Induced Membrane Fusion Mediated by Phospho- lipids and Neutral Glycolipids . . . . .	393
R.T.C. Huang	

# CONTENTS

xiii

A New Type of Glycolipid Change Associated with Oncogenesis: A Fucoganglioside Accumulation in Premalignant Hepatic Lesion and in Hepatoma Induced by N-2- Acetylaminofluorene . . . . .	401
S.-I. Hakomori, E.H. Holmes, and T. Nakayama	
Localization of Asialo GM <sub>1</sub> and Forssman Antigen in the Small Intestine of Mouse. . . . .	415
A. Suzuki, Y. Umesaki, and T. Yamakawa	
Cytoskeleton-Associated Glycolipid (CAG) and Its Cell Biological Implication. . . . .	425
Y. Nagai and K. Sakakibara	
Using Gangliosides to Study Normal and Abnormal CNS Functions . . . . .	435
M.M. Rapport	
Sulfatide: Localization by Immunohistochemical Techniques: Relevance to Metabolism . . . . .	439
B. Zalc and N. Baumann	
Expression of Hanganutziu and Deicher Type Heterophile Antigen on the Cell Surface of Marek's Disease Lymphoma. . . . .	445
M. Naiki, Y. Fujii, K. Ikuta, H. Higashi, and S. Kato	
Antiglycolipid Antibodies in Human Sera . . . . .	457
T. Yasuda, J. Ueno, Y. Naito, and T. Tsumita	
Discussion. . . . .	467
Remarks for the Biwako Symposium D.M. Marcus. . . . .	475
List of Participants. . . . .	477
Subject Index . . . . .	485

# GLYCOLIPIDS AND I: PAST, PRESENT AND FUTURE

Tamio Yamakawa

Department of Biochemistry, Faculty of Medicine,  
University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113

It was in 1950 that I found a peculiar sphingoglycolipid in a large amount of horse erythrocyte stroma (1). It gave a beautiful purple color when heated with Bial's orcinol reagent due to the presence of neuraminic acid. But I named it hematoside, because of some discrepancy with the reported properties of ganglioside (Fig. 1).

In those days, the situation was very poor both economically and as regards scientific conditions. I was only a beginner at biochemical research and did not have enough experience in the chemistry of glycolipids from natural sources.

I wrote a letter to Prof. Klenk of the University of Cologne and asked his opinion on my finding of hematoside. He was the main authority at the time and had already discovered ganglioside before the 2nd World War. A few months later, Prof. Klenk wrote a letter of reply and mentioned some doubt about the identity between hematoside and ganglioside. In the same letter, he also informed me that he had recently isolated a glycolipid from human erythrocytes which had galactosamine but no neuraminic acid (2).

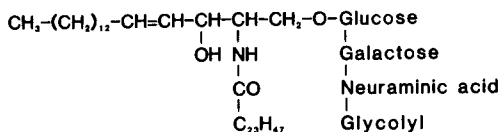


Fig. 1: Structure of hematoside as the major glycolipid of equine erythrocytes

I reexamined his work and separated a glycolipid from human erythrocytes which had the same chemical composition as the one he had given me in his letter (3). I named it Globoside, but the first discoverer of Globoside was Prof. Klenk. And he reconfirmed my work on hematoside immediately (4) (Fig. 2). This was the start of my research work on glycolipids.

In those days, the purification of glycolipids was carried out on the basis of the solubility difference of glycolipids in various organic solvents. After column chromatography using silicic acid as an adsorbent became available, glycolipids were purified more and more by this technique (5). More recently DEAE-Sepharose together with the application of thin-layer chromatography has been a powerful method of purification of individual glycolipids. For example, we now have excellent separation with the ganglioside mapping technique of Nagai and co-workers (6). Thus far, we have applied high performance liquid chromatography to separation of individual glycolipids from erythrocytes by detecting UV-absorption after a p-nitrobenzoyl group is introduced to the acylamide nitrogen of peracetylated glycolipids (7) (Fig. 3).

Recently, Otsuka in my laboratory applied DCC or droplet counter-current chromatography to the separation of glycolipids. The DCC apparatus was devised by Tanimura and himself and they have applied it to the separation of natural products including alkaloids, phenolics, peptides and saponins. The DCC apparatus is equipped with 50 to 500 thin glass columns, connected to each other by Teflon tubings in series and the separation is carried out by passing fine droplets of a mobile phase successively through the columns of the stationary liquid phase in an ascending or descending manner. Thus, in this system, no mechanical shaking is involved and there is no solid support which may reduce the recovery of polar substances by irreversible adsorption or chemical modification.

As much as 1 - 2 grams of crude glycolipid mixture can be applied to DCC. Several examples of separation profiles are presented here (8) (Fig. 4).

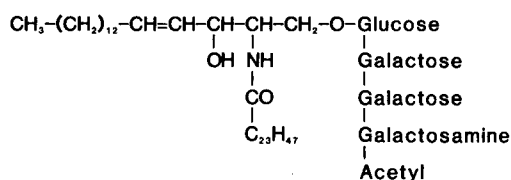


Fig. 2: Structure of Globoside as the major glycolipid of human erythrocytes

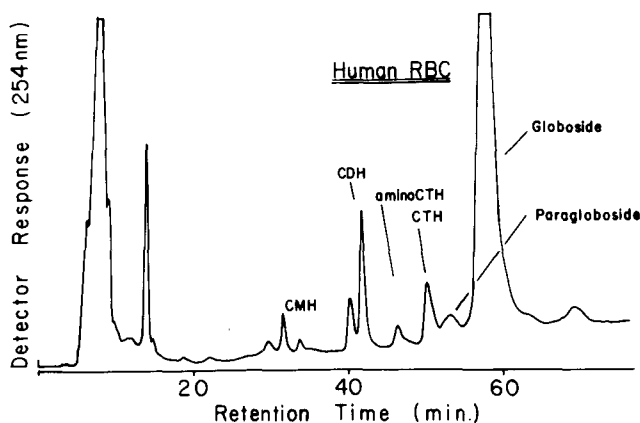


Fig. 3: The analysis of neutral glycolipid composition of human erythrocytes by high performance liquid chromatography using O-acetyl-N-p-nitrobenzoyl derivatives

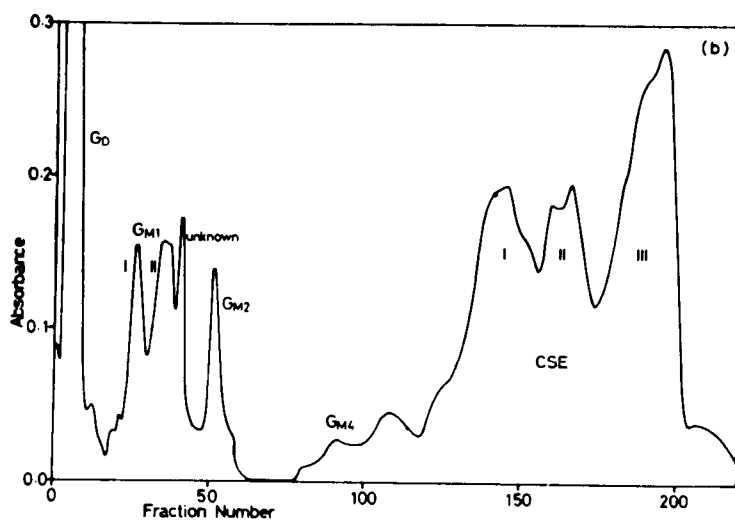
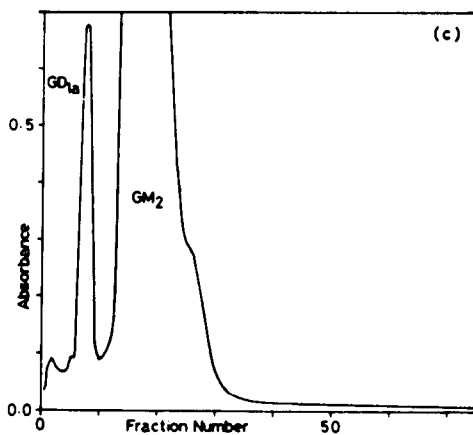
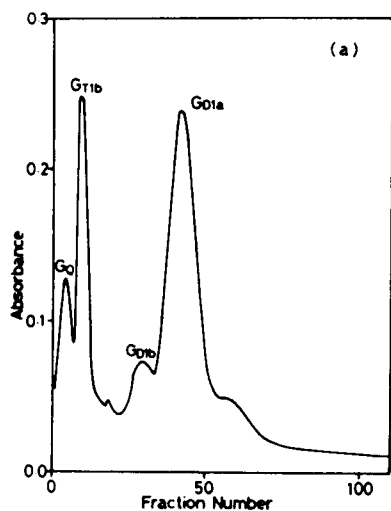
The efficiency of separation by DCC is not so high, but it is a useful technique for the preparation of rather large amounts of glycolipids, and I think it can be applied to a variety of glycolipids by improving the selection of solvents and modification of the mixing proportions.

In 1953, we noticed the presence of ABO blood group activity in the crude globoside preparation, it was the first evidence of biological activity of glycolipid (9).

Application of column chromatography made it possible to separate the active portion from the major Globoside I. The activity was further divided into two fractions: Globoside II and Globoside III (10) (Fig. 5). Further purification of these active glycolipids was carried out and their structures were proposed by Hakomori and by Hanfland.

In those days, chemical structure of blood group antigens was investigated by using materials from mucous secretions and the carbohydrate structures of the antigenic determinants were elucidated. You will see that the carbohydrate determinant groups attached to the ceramide moiety are of the same chemical structure as the blood group active glycoproteins isolated from body fluids or secretions which are attached to peptide by O-glycosidic linkages (Fig. 6).

Since then, I have had the idea that the antigenic materials at the surface of erythrocytes are glycolipid in nature and the blood group active material in the secretion is glycoprotein in



nature. Our findings were not accepted immediately, but now everybody accepts the existence of ABO active glycolipid.

However, in recent years, ABO blood group activity has still been reported to be present also in glycoprotein from human erythrocyte membrane (11). Several other people isolated a highly polymerized glycolipid called megaloglycolipid or macroglycolipid from human erythrocytes and mentioned that the major activity resides in such high molecular glycolipids (12).

The separation of water-soluble high molecular glycolipids from glycoprotein is difficult and the activity detected in the glycoprotein fraction might be due to contamination by such a high molecular glycolipid.

I think it is not so simple to determine which is the real active group material, because the procedure to separate and purify such a high molecular substance is rather complicated as compared to the methods used for relatively low molecular glycolipids such as ceramide hexa- or octa-saccharide.

A recent examination by Handa, my co-worker, showed the distribution of ABO blood group activity in human erythrocytes (Table 1). He showed that the activity is found mostly in the low molecular glycolipid and only 1% of the activity was found in the megaloglycolipid and no activity was detected in the glycoprotein fraction.

Several workers in Finland stated that the ABO activity was found in the glycoprotein fraction called poly(glycosyl)peptide of erythrocytes (13). However, their assay method for blood group activity was not the hemagglutination inhibition test using iso-antibody, they used the binding activity with a lectin, *Bandeiraea simplicifolia*, as a measure of blood group activity. My opinion

---

Fig. 4: Separation of glycolipids by droplet counter-current chromatography (DCC). (a) Mouse brain gangliosides (75 mg of crude glycolipids prepared by acetone precipitation and an alkaline treatment) were separated with 500 columns using solvent system of chloroform - methanol - 0.5% NaCl in water - *n*-propanol (50:60:40:6, by volume). (b) Crude glycolipids (150 mg) from whale brain were separated by using chloroform - methanol - water - *n*-propanol (50:60:40:2).  $GM_1$  was separated into two peaks and CSE into three. (c) Separation of crude glycolipid obtained from the brain of a patient with Tay Sachs' disease. Crude mixture (700 mg) was separated with chloroform - methanol - water - *n*-propanol (50:60:40:5, by vol.) Sugar was determined by phenol-sulfuric acid method.

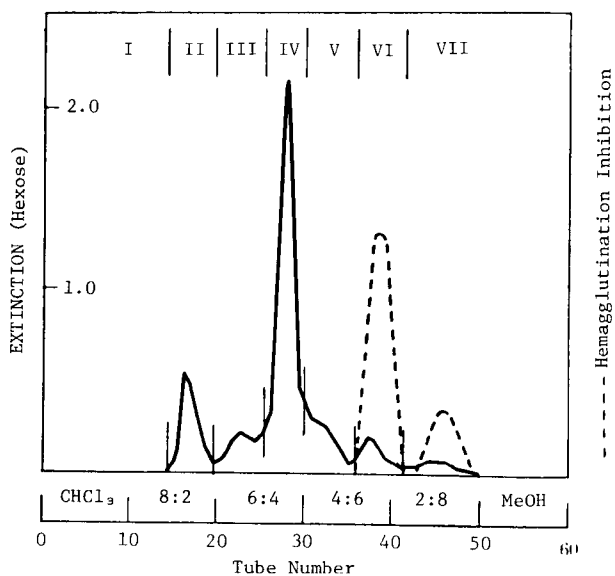


Fig. 5: Silicic acid chromatography of the glycolipids obtained from human erythrocytes with blood group A. Fraction IV is Globoside I and the activity of hemagglutination inhibition was detected with Globoside II in fraction VI and Globoside III in fraction VII. For the inhibition assay anti-A isoagglutinin and blood group A erythrocytes were used.

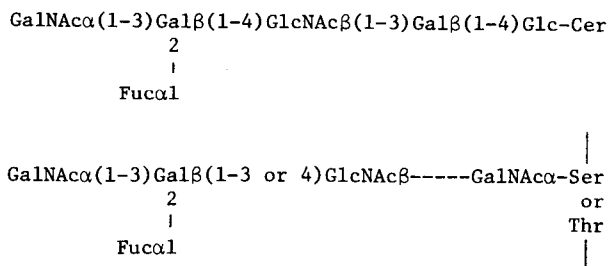


Fig. 6: The carbohydrate structure of a glycolipid and glycoprotein with blood group A activity.

Table 1. Distribution of blood group A activity in the various fractions of human erythrocytes

Fractions	Yield mg/l liter RBC A	Minimum Amount to Inhibit Agglutination $\mu$ g B	Total Active Amount A/B
Low-molecular glycolipid			
C-M-W Extract	510	25	26,100
BuOH Extract	8.6	1.5	
Megalogycolipid	5.3	20	265
Glycoprotein	38.5	>100	-

is that the binding activity with lectin has broader specificity than the immunological antigen-antibody reaction and the 'group activity' revealed by such a poly(glycosyl)peptide does not represent the true antigenic activity of the ABO blood group.

Now, I will mention our recent experience with the molecular difference of erythrocyte glycolipids. As to the hematoside-type of glycolipids, that of horse has 1 mole of N-glycolylneuraminic acid, and that of cat 2 moles of N-glycolylneuraminic acid. Previously, we reported that dog red cell glycolipid consisted of 27% N-glycolylneuraminic acid and 73% N-acetylneuraminic acid (14). On the other hand, when Klenk and Heuer proposed the structure of dog erythrocyte glycolipid in 1960, they had found that the neuraminic acid was exclusively N-acetylated (15). This apparent discrepancy had us puzzled for a long time. Recently, reexamination of this problem revealed that red cell glycolipids from some dogs have N-glycolylneuraminic acid, while others have N-acetylneuraminic acid; no mixed type exists (16).

As our previous study was carried out with pooled blood from several dogs, so the sialic acids found were not structurally uniform.

The dogs examined in these studies were mongrels, we tried to determine which breeds of dog have the N-acetyl and which the N-glycolyl type of hematoside. It was revealed that in the blood of all the European dog breeds examined N-acetylneuraminic acid existed but no hematoside of the N-glycolyl type (Table 2).

On further investigation, we found N-glycolylhematoside or the G-type in some oriental dogs. They are Shiba-dog, Kai-dog,

Table 2. Type of hematoside in dog erythrocytes (European dogs)

Breeds	NeuAc	NeuGc
Afganhound	4	0
Am. cocker spaniel	2	0
Beagle	30	0
Bulldog	1	0
Cairn terrier	2	0
Chihuahua	2	0
Collie	1	0
Dachshund	1	0
Dalmatian	1	0
Fox terrier	1	0
French poodle	1	0
Great dane	1	0
Laika	1	0
Maltese	26	0
Miniature poodle	1	0
Pomeranian	9	0
Poodle	10	0
Setter	3	0
Shephard	6	0
Shetland sheep dog	7	0
Siberian husky	5	0
St. Bernard	2	0
Yorkshire terrier	21	0
Westhighland	1	0
Total	139	0

Kishu-dog, Chin and Pekinese. However, Akita-dogs and Hokkaido-dogs were found to have N-acetylhematoside or the A-type, even though they also live in Japan. Thus, we found that there are two types of dogs in Japan, namely the A-type in northern districts and the G-type in southern districts (Table 3). Shiba-dogs are rather small in size and generally up to 9 kg in weight. The population of Shiba-dogs is decreasing in Japan, so the Society of Preservation of Shiba-dogs has been established to preserve this species as a pure breed.