

IMMUNIZATION FOR JAPANESE ENCEPHALITIS

Edited by

W. McD. HAMMON, M. D.

M. KITAOKA, M. D.

W. G. DOWNS, M. D.

Immunization for Japanese Encephalitis

Conducted by

The U.S. Japan Cooperative Medical Science Program
Washington, D.C.

Conference Organizing Chairmen

W.McD. HAMMON, M.D., Dr.P.H. and W.G. DOWNS, M.D. (U.S.A.)
M. KITAOKA, M.D. (Japan)

Edited by

WILLIAM McD. HAMMON, MASAMI KITAOKA
and WILBUR G. DOWNS

1971



IGAKU SHOIN LTD., TOKYO

©First Edition, July 1, 1971 by IGAKU SHOIN LTD., 5-29-11 Hongo, Bunkyo-ku, Tokyo.
All rights reserved. No part of this book may be translated or reproduced in any form by
print, photoprint, microfilm, or any other means without written permission from the
publishers.

Printed and Bound in Japan

LIST OF THOSE ATTENDING CONFERENCE

- BABA, SADAYOSHI, D. V. M., Ph. D. Technical Director of Toshiba Institute of Biological Science, Gosen, Niigata, Japan.
- BENENSON, ABRAM S., M. D. Professor and Chairman of the Department of Community Medicine, College of Medicine, University of Kentucky, Lexington, Kentucky.
- BRACKETT, ROBERT G., Ph. D. Director of Virology, Parke, Davis & Co., Detroit, Michigan.
- CHAMBERLAIN, ROY W., Sc. D. Deputy Chief, Virology Section, National Communicable Disease Center, Health Services and Mental Health Administration, US Dept. of Health, Education and Welfare, Atlanta, Georgia.
- CHAPPELL, W. ADRIAN, Ph. D. Chief of Arbovirus Reference Laboratory, National Communicable Disease Center, US Public Health Service, Atlanta, Georgia.
- COX, HERALD R., Sc. D. Director of Cancer Research, Viral Oncology Section, Roswell Park Memorial Institute, Buffalo, New York.
- DARWISH, MEDHAT A., M. D., Dr. P. H. Assistant Professor of Microbiology, Ain-Shams University, Faculty of Medicine, Cairo, Egypt.
- DAVIS, DORLAND J., M. D., Dr. P. H. Director, National Institute of Allergy & Infectious Diseases, National Institutes of Health, Bethesda, Maryland.
- DOWNS, WILBUR G., M. D., M. P. H.* Professor of Epidemiology, Yale University Medical School, New Haven, Connecticut.
- ECKERT, EDWARD A., Ph. D. Associate Professor of the Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan.
- GARD, SVEN, M. D.* Professor, Chairman of the Department of Virology, Karolinska Institutet, Sweden.
- GOTO, GORO, M. D. Chief of Communicable Diseases Control Section, Public Health Bureau, Ministry of Health & Welfare, Tokyo, Japan.
- GRAYSTON, J. THOMAS, M. D.* Professor and Chairman of the Department of Preventive Medicine, University of Washington, Seattle, Washington.
- GREIFF, DONALD, Sc. D. Professor of Pathology, Department of Pathology, Marquette School of Medicine, Inc., Milwaukee, Wisconsin.
- HABEL, KARL, M. D. Research Member, Department of Experimental Pathology, Scripps Clinic & Research Foundation, La Jolla, California.
- HAMMON, WILLIAM McD., M. D., Dr. P. H.* Professor and Head of Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.
- HERSHEY, NATHAN, LL. B. Research Professor of Health Law, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.
- HILLEMAN, MAURICE R., Ph. D. Director of Virus and Cell Biology, Division of Virus and Cell Biology Research, Merck Institute for Therapeutic Research, West Point, Pennsylvania.
- HINGSON, ROBERT A., M. D. Professor of Public Health Practice, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.
- HSU, T. C., M. Ph. M. D. Commissioner, Provincial Health Department, Taiwan Provincial Government, Taiwan, Republic of China.
- HULL, ROBERT N., Ph. D. Research Advisor, Eli Lilly Research Laboratories, Indianapolis, Indiana.
- IDA, YASUO, M. D. Technical Official, Communicable Disease Control Section, Ministry of Health and Welfare, Tokyo, Japan.
- INOUE, Y. KANDA, M. D.* Associate Professor of Virology, Institute for Virus Research, Kyoto University, Kyoto, Japan.

*Session Chairman

- ISHII, KEIZO, M. D. Chief of Division of Laboratory Examination, Central Virus Diagnostic Laboratory, National Institute of Health, Murayama Division, Musashi Murayama, Tokyo, Japan.
- KAMAHORA, JUNTARO, M. D. President of Osaka University, Professor and Head of Department of Tumor Viruses, the Research Institute of Microbial Diseases, Osaka University, Osaka, Japan.
- KANAMITSU, MASATSUGU, M. D.* Professor and Head of Department of Hygiene and Epidemiology, Sapporo Medical College, Sapporo, Japan.
- KITAOKA, MASAMI, M. D. Dr. Med. Sci.* Chief of Department of Virology and Rickettsiology, WHO Regional Center for Arthropod-borne Viruses. Present Deputy Director-General, National Institute of Health, Tokyo, Japan.
- LEE, G. CHIN-YUN, M. D. Associate Professor of Pediatrics, National Taiwan University, Taipei, Taiwan. Visiting Associate Professor of Preventive Medicine (1969-1970), University of Washington, Seattle, Washington.
- LENNETTE, EDWIN H., Ph. D., M. D. Chief of Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley, California.
- MIFUNE, KUMATO, M. D. Associate Research, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan.
- MURRAY, RODERICK, M. D. Director of the Division of Biologic Standards, National Institutes of Health, Bethesda, Maryland.
- NAGANO, YASUITS, M. D.* Head of Virus Division, Kitasato Institute, Tokyo, Japan.
- NAKAMURA, HAJIME, D. M. Sc. Chief of Fourth Virus Laboratory, Research Division, Nippon Institute for Biological Science, Tachikawa, Tokyo, Japan.
- OGATA, TAKAYUKI, M. D. Associate Chief of Division, Department of Virology and Rickettsiology, National Institute of Health, Tokyo, Japan.
- OHTOMO, NOBUYA, M. D., Dr. Med. Sci. Chief Member of Research Staff, Chemo-Sero Therapeutic Research Institute, Kumamoto, Japan.
- PHIFER, KENNETH O. Geographic Medicine Branch, NIAID, National Institutes of Health, Bethesda, Maryland.
- POND, WILLIAM L., Ph. D. Associate Professor of Medicine and Microbiology, University of Miami, School of Medicine, Coral Gables, Florida.
- PRICE, WINSTON H., Ph. D. Professor of Epidemiology, Kenneth Maxcy Laboratories, Department of Epidemiology, School of Hygiene and Public Health, Johns Hopkins University, Baltimore, Maryland.
- PROVOST, PHILIP J., Ph. D. Department Head, Biological Research and Development, Lederle Laboratories, Pearl River, New York.
- ROSEN, LEON, M. D., Dr. P. H. Head, Pacific Research Section, National Institute of Allergy and Infectious Diseases, Honolulu, Hawaii.
- ROWE, WALLACE P., M. D. Chief, Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland.
- SAENZ, ARTURO C., M. D.* Medical Officer of Virus Diseases, World Health Organization, Geneva, Switzerland.
- SATHER, GLADYS E., M. P. H. Assistant Professor of Microbiology, Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.
- SCHAEFFER, MORRIS, Ph. D., M. D. Assistant Commissioner of Health and General Director, Public Health Laboratories, City of New York, New York.
- SHELOKOV, ALEXIS, M. D.* Chairman of the Department of Microbiology, University of Texas Medical School, San Antonio, Texas.
- SINGH, BALWANT, D. V. M., Ph. D. Research Associate, Department of Epidemiology and Microbiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania.

- STOKES, JOSEPH JR., M. D., Sc. D. Emeritus Professor of Pediatrics, School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania.
- SWEET, BENJAMIN H., Ph. D. Director of Medical Microbiology and Cell Biology Division, Gulf South Research Institute, Lakefront Laboratories, New Orleans, Louisiana.
- TAGAYA, ISAMU, M. D. Chief of Department of Enteroviruses, National Institute of Health, Tokyo, Japan.
- TAKAHASHI, KATSUMI, M. D. Director of Nagasaki Prefectural Institute of Public Health, Nagasaki, Japan.
- TAKAKU, KEISUKE, M. D. Head Investigator, Department of Vaccine and Serum Production, Kan-onji Institute, the Research Foundation for Microbial Diseases of Osaka University, Kan-onji, Kagawa, Japan.
- TIGERTT, WILLIAM D., M. D. Professor of Experimental Medicine, University of Maryland, School of Medicine, Baltimore, Maryland.
- TINT, HOWARD, Ph. D., Director of Development, Wyeth Laboratories, Philadelphia, Pennsylvania.
- WAGNER, JOHN C., Sc. D. Assistant Director, Division of Biologic Standards, National Institutes of Health, Bethesda, Maryland.
- WARREN, JOEL, Ph. D. Director, Life Science Center, Nova University, Fort Lauderdale, Florida.
- WATANABE, MORIMATSU, D. V. M., Ph. D. Chief of the Second Research Division (Virus), National Institute of Animal Health, Kodaira, Tokyo, Japan.
- WORK, TELFORD H., M. D., M. P. H., D. T. M. & H. Professor of Infectious and Tropical Diseases, School of Public Health, University of California at Los Angeles, California.
- YAMADA, AKIRA, D. V. M. Chief of Japanese Encephalitis Vaccine, Institute for Chemo and Serotherapy, Kumamoto, Japan.
- YAMAMOTO, SHIGEO, M. D. Chief of Department of Biological Products, Takeda Chemical Industries Ltd., Hikari Plant, Yamaguchi, Japan.
- YAMASHITA, KOSHI, M. D. Lecturer of Department of Pathology, Yamaguchi University School of Medicine, Ube, Japan.
- YOSHINO, KAMESABURO, M. D. Professor and Head of Department of Virology, Institute of Medical Science, University of Tokyo, Tokyo, Japan.
- YOSHIOKA, ISAO, M. D., Dr. Med. Sci. Virology Division, Kitasato Institute, Tokyo, Japan.

TABLE OF CONTENTS

I.	Foreword	1
II.	Introduction	2
III.	Basis for selection of virus vaccine strain.....	5
	A. General introduction	5
	B. Comparison of strains by cross protection and common sero- logical tests	12
	1. Cross protection tests with Japanese encephalitis vaccines in mice and monkeys.....	12
	2. A study on immunogenic differences between strains of Japa- nese encephalitis virus.....	15
	3. Comparative studies of strains of Japanese and St. Louis en- cephalitis viruses	23
	4. Host-dependent antigenic variation of Japanese encephalitis virus	26
	5. Antigenic differences of Japanese encephalitis virus strains.....	27
	C. Comparison of strains by gel precipitin tests.....	34
	1. Strain variation of Japanese encephalitis virus on the basis of antigenic structure by agar gel precipitation test	34
	2. Gel precipitin study of Japanese encephalitis virus antigen and antibody	41
IV.	Inactivated viral vaccine	48
	A. General introduction.....	48
	B. Problems common to all vaccine tissue sources.....	52
	1. Preparation, inactivation and control procedures.....	52
	a. Inactivated viral vaccines: Preparation, inactivation and control procedures	52
	b. The method of preparing the alcohol-protamine purified, inactivated Japanese encephalitis vaccine (AP vaccine).....	55
	c. Inactivation of Japanese encephalitis viruses for use as vaccines: Summary of studies.....	57
	d. Inactivated vaccine from Japanese encephalitis infected mouse brains (Ultracentrifuge-purified vaccine).....	59
	2. Methods for testing potency in animals and their significance....	65
	a. General introduction: The potency testing of Japanese en- cephalitis vaccine.....	65
	b. Vaccine potency testing methods and their significance.....	72
	c. Mouse challenge tests for vaccine potency.....	76
	d. Neutralizing antibody response to vaccine.....	79
	e. Antigenic potency test of Japanese encephalitis virus vaccine..	80
	f. Relationship between immunizing potencies in man and animals of Japanese encephalitis vaccine.....	83
	g. Monkey intranasal challenge against Japanese encephalitis vaccine	87

h.	Immune response of Japanese encephalitis virus in dd strain of mouse colony from different areas.....	92
3.	Measures of serological responses in man.....	96
a.	Antibody response to inactivated Japanese encephalitis vaccine of man living in a nonendemic area.....	96
b.	Antibody response in man to cell culture Japanese encephalitis vaccine (Preliminary report)	103
c.	Response in children to an experimental inactivated virus vaccine grown in hamster diploid cell culture.....	104
d.	Response in adults to an inactivated cell culture vaccine employing a highly attenuated strain, OCT-541-35C-24CP1.4-5..	109
e.	Study on the method of inoculation of Japanese encephalitis virus vaccine.....	114
f.	Response to formalinized mouse brain vaccine in adults previously infected with dengue viruses.....	119
4.	Use of adjuvants including poly I:C.....	121
a.	Adjuvant vaccine and interferon approaches to control of arbovirus infections	121
b.	Effect of poly I:C on Japanese encephalitis virus infection in mice.....	128
c.	Hemagglutination inhibition antibody response and protection against challenge in pigs vaccinated with adjuvant vaccine	133
5.	Final form of product and additives.....	138
a.	Freeze drying of viruses.....	138
b.	Final form of the alcohol-protamine vaccine.....	142
c.	Final form of the ultracentrifuged vaccine.....	144
C.	Mouse brain virus source.....	147
1.	Purification procedures.....	147
a.	Purification by chemical means.....	147
1)	Application of alcohol-protamine method of partial purification of Japanese encephalitis virus to production of a partially purified, inactivated vaccine.....	147
2)	Chemical purification of Japanese encephalitis viruses for use as vaccines: Summary of studies.....	149
3)	A new purification procedure for biological vaccines (Adsorption on magnetic iron oxides).....	152
b.	Purification by ultracentrifugation and chemical means.....	155
c.	Purification by exclusion chromatography.....	158
2.	Tests for purity of vaccine.....	160
a.	Tests for protein content and absence of encephalitogenic properties of vaccine.....	160
b.	Tests for purity and virus protein concentration in ultracentrifuge-purified vaccine	162
3.	Safety tests to detect indigenous mouse viruses or other contaminating viruses in preinactivation and postinactivation tests	169

a.	The problem of murine virus contamination of a mouse-grown vaccine.....	169
b.	Detection of contaminants in seed virus.....	172
4.	Safety tests for the inactivation of the virus, inoculated and replicated in vaccine production.....	173
D.	Cell culture virus source.....	175
1.	Introduction	175
2.	Discussion	178
3.	Preparation, stability and immunologic response in animals....	180
a.	Development of an inactivated vaccine from an attenuated strain of OCT-541 produced in primary hamster kidney cells	180
b.	Development of an inactivated vaccine for Japanese encephalitis from virus produced in primary cynomolgus kidney cells	185
c.	Development of an inactivated vaccine grown in embryonic hamster diploid cell culture.....	192
d.	Development of alum precipitated inactivated Japanese encephalitis vaccine produced in primary porcine kidney cells, and primary hamster kidney cells, alum precipitated....	198
e.	Development of inactivated Japanese encephalitis vaccine produced in porcine kidney cells.....	199
4.	Safety tests peculiar to cell culture produced vaccines.....	200
V.	Live attenuated cell culture vaccines.....	202
A.	General introduction	202
B.	Development of a live attenuated Japanese encephalitis vaccine of the M strain.....	204
C.	Development of a live attenuated Japanese encephalitis virus in chick embryo tissue culture.....	205
D.	Development of a live attenuated AT ₃₁ strain of Japanese encephalitis virus by hamster kidney cell passage.....	212
E.	Antigenic changes occurring during attenuation of a virus.....	225
F.	Significance of attenuation criteria in animal tests.....	227
G.	Laboratory safety testing of live attenuated virus vaccines.....	230
H.	Possible effects of mosquito infection and transmission on virulence of live attenuated cell culture vaccines.....	231
I.	Stability of Japanese encephalitis attenuated virus strains in <i>Culex tritaeniorhynchus</i>	233
J.	Results of human volunteer studies with live, attenuated strain of OCT-541.....	237
VI.	Noninfectious, purified, fractionated viral antigens.....	239
A.	Purified fractionated viral antigens: Introduction and application to influenza virus	239
B.	Vaccines of purified, fractionated viral antigens: Discussion and application to measles virus (Abstract)	246
VII.	The jet inoculator in the control of epidemics through mass immunization program.....	247

VIII.	Use of human volunteer in vaccine tests.....	250
A.	The contribution of volunteers to immunization procedures.....	250
B.	Legal and ethical aspects of field trials to determine effectiveness of agents in man.....	254
IX.	Controlled field tests of vaccine in man.....	258
A.	A completed field trial for an evaluation of the effectiveness of mouse-brain Japanese encephalitis vaccine.....	258
B.	Supplementary report. Effectiveness of Japanese encephalitis vaccine: Study in the second year following vaccination.....	266
C.	Planning and organizing of a Japanese encephalitis vaccine field trial in Korea	268
D.	Evaluation and analysis of a Japanese encephalitis vaccine field trial in Korea	271
X.	Follow-up on use of vaccine in children in Japan.....	275
A.	Host reactions following vaccination against Japanese encephalitis with special reference to neurological complications.....	275
B.	Evaluation of the effectiveness of vaccination in Japan: Clinical and laboratory follow-up.....	278
C.	Shift of age distribution of cases of Japanese encephalitis in Japan during the period 1950 to 1967.....	287
XI.	Use of vaccine in pigs.....	292
A.	Effects of immunization of swine upon the ecological cycle of Japanese encephalitis virus.....	292
B.	Formalinized vaccine, antibodies and swine fetus resistance to Japanese encephalitis virus infection.....	304
C.	Field trials of Japanese encephalitis vaccine for prevention of viral stillbirth among sows.....	305
D.	Trial of living virus vaccine of Japanese encephalitis in pigs.....	313
XII.	Predictions and plans for future in new arbovirus vaccines.....	319
A.	A sequential immunization procedure against Group B arboviruses employing four live attenuated Group B arboviruses.....	319
B.	Predictions and plans for the future of arbovirus vaccines with emphasis on advantages of live attenuated virus.....	323
C.	The implications of virus mutation for vaccine production.....	327
D.	Predictions and plans for new arbovirus vaccines in the future with emphasis on a subunit product.....	329
XIII.	Summary	331
	Index of authors	333

I

FOREWORD

DORLAND J. DAVIS

It is a pleasure to welcome you to the U.S.-Japan Cooperative Medical Science Program Conference on Japanese Encephalitis Virus Vaccine and it is a particular pleasure to welcome our Japanese colleagues to our Nation's Capital.

The first conference sponsored by the Joint Panel on Virus Diseases was held in December 1967 in Honolulu on smallpox. The second was in Tokyo in December 1968 on the myxoviruses and this is the third conference. We are privileged to hold this conference in the Pan American Health Organization Building which is an appropriate location for this meeting with scientists from Japan, United States and other countries.

The U.S.-Japan Cooperative Medical Science Program, now under the aegis of the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, has completed 4 years of activity and one can readily see its influence. For instance, in 1965 the NIAID supported 8 research grants dealing with rabies, encephalitis and smallpox, subjects of primary interest to the Program. This year there are 16 grants, twice as many, and one contract. I am also aware that the Japanese scientists have increased substantially the amount of research on these diseases. There is every reason to believe that continuing interchange between scientists of our 2 countries will accelerate our knowledge of these virus diseases which are of such special importance to Southeast Asia.

II

INTRODUCTION

WILLIAM McD. HAMMON

At the combined meeting of the Panels on Virus Diseases, of the United States-Japan Cooperative Medical Science Program, held in Kyoto on December 5, 1968, the topic for the annual working conference of 1969 was selected. The last, on Myxoviruses, had just been held on December 2, 3 and 4, 1968 in Tokyo. The title tentatively decided upon for that of 1969 was "Working Conference on Viral Vaccines with Special Emphasis on Japanese Encephalitis." The meeting, on the basis of the customary rotation would be in the United States. It was felt that since so many aspects of so many virus diseases had been discussed at the just completed conference, it had not been possible to deal in adequate detail with a lot of important applied aspects, and that an attempt should be made in the future one to deal in adequate depth with a more limited subject, one aspect, control, of one disease.

Immunization against Japanese encephalitis was a topic of great, immediate importance for much of Asia, and many of the Pacific Islands. Two partially purified mouse brain vaccines had gradually come into rather extensive use in Japan and one of these to a lesser degree in Taiwan. Elsewhere, none was used or available from a local source. Research on vaccine was in progress in many laboratories in Japan, Taiwan and the United States. These efforts involved live, attenuated vaccines and inactivated tissue culture vaccines, both types claimed to have potential advantages over those in use. Field trials were being planned in Korea for one of those already in use in Japan, and for an American developed tissue culture vaccine. Only one controlled field trial of a currently available or approved vaccine had ever been conducted and results of this were not accepted by all, the data never having been published. The time appeared appropriate to bring together as many of the scientists as possible who were working on such problems to share their knowledge and obtain additional guidance from other distinguished virologists, experienced manufacturers of other viral vaccines, epidemiologists, and those responsible for licensing and approving new vaccines.

The recommended subject was heartily approved by the 2 panels and planning began immediately. Two chairmen from the American Panel and one from the Japanese were appointed to plan the conference, together with the assistance of the Panel Chairmen. This planning had to be accomplished largely by mail.

The Chairmen agreed to try a rather novel conference procedure. Instead of assigning broad major topics to a few selected speakers, a detailed outline of the subject matter was

prepared as a unified, systematically organized coverage of the total subject, as it might be prepared for a monograph to be written by a single person. The next step involved selection of appropriate persons to present data on each main topic and the many subtopics in the outline, through several descending orders. For a number of main topics one or more scientists were readily recognized as having outstanding qualifications, but these were not all residents of Japan or the United States. United States as the host country invited most of these as their guests. The World Health Organization was kind enough to provide for one of their invited staff members. Acceptances were received from Switzerland, Sweden, the United Arab Republic and the Republic of China. Ten others were selected in the same manner from Japan and the United States. Each of these was requested to present a 10 minute, formal, written paper to introduce, or in a few instances to cover completely the topic assigned. Their manuscripts were to be available in advance to the interpreters, so accurate and effective simultaneous translation would be available for all participants.

The topical outline of the total conference was mailed, following an invitation, to a specially selected list of all American and Japanese scientists whom the chairmen thought might have significant contributions to make, either because of known involvement with Japanese encephalitis immunization vaccine development, or other related experience. Certain limits had to be placed on the number of these invitations, particularly for the Japanese, because of the great travel distance and limited government funds available for such. It was felt that the total number of participants should not exceed 75. Each was requested to study the outline and indicate to what topic or topics they could contribute significant data, and would be willing to present them, and to submit to their respective country's program chairman a brief abstract of their contribution for advance evaluation. The response was very rewarding and the chairmen selected one or several volunteer discussants for each topic outline and encouraged a few to broaden their coverage. Each of these was allotted 5 minutes for each topic, with a few exceptions where it was obvious that more time was needed. Many had contributions to make on a number of specific topics. These participants were requested to prepare an informative abstract with tables and charts similar to those to be used for projection, or complete manuscripts, if preferred, for distribution at the meeting to all attendees and to interpreters. All participants were assured that no publication of the proceedings was intended.

Invited participants were also informed that time was planned, at intervals during the program, for free discussion from the floor and those who did not choose to be formally listed for topics on the program were urged to take part in spontaneous question asking or discussing. Some chose to restrict their participation in this manner.

The conference was held in Washington, D. C. at the Pan American Health Organization's (PAHO) Building, under the auspices of the Geographic Medicine-Branch of the National Institute for Allergy and Infectious Diseases, National Institutes of Health, U.S. Public Health Service on August 4, 5 and 6, 1969.

The list of the 62 attending participants and their affiliations may be found on pages vii-ix. With the excellent projection and simultaneous translation facilities made available by or through PAHO, the staff work of the 2 panel secretariats, the excellent preparation and cooperation of the many participants and the expert handling of sessions by the 12 selected session chairmen, the meeting appeared to have succeeded well in what it attempted to accomplish. As a result, Dr. Yasuiti Nagano, Chairman of

the Japanese panel made a formal request, before the close of the final session, that the proceedings be published, with Dr. William Hammon as chief editor, assisted by Dr. Masaki Kitaoka and Dr. Wilbur Downs.

Dr. Hammon then explained to the participants the obligation each of the many who had prepared only discussion abstracts would have in preparing complete papers, indicated that many papers already submitted would be more useful if accompanied by references and pointed out that a number of the tables and charts projected would require modification for publication. He agreed to accept the proposed responsibility placed on him, provided no participant of a major topic objected to have his contribution published and a great majority of those who had submitted abstracts would provide full manuscripts. The feeling was so prevalent that the data that had been presented during the 3 days was available from no other source and had been so well brought together and was of such great usefulness that one could not afford to do otherwise than to make it permanently available, not only to the participants but to all interested scientists, students and libraries.

Dr. Nagano suggested that it be published as a monograph in English, either in the United States or Japan. If it was desired to have it done in Japan he had already made inquiry and had an interested publisher. This latter suggestion was formally and enthusiastically approved. An early publication date in 1970 was urged.

Within the limited period of time to collect the manuscripts, associated with travel and other commitments by many of the participants, a very few contributions to one or more topics could not be included, or have been included only as abstracts. As a whole, however, this compilation is more complete and useful than that presented originally, since translation from Japanese into English has been more carefully and accurately accomplished, the tables, charts and graphs can now be studied better and correlated more effectively with the text than during the conference, and the selected references add greatly to the coverage of a number of topics, particularly making up for the many scientists who for various reasons were unable to participate, and to summarize their valuable contributions. Unfortunately, there was no way to include the many discussions and questions presented from the floor, since there had been no recording.

Now that the compilation is complete and ready for the publisher, the editor wishes to thank all the contributors and co-editors for their enthusiastic and effective cooperation. This is an excellent example of Japanese-American cooperation in the field of medical sciences—the objective of those who envisioned and brought this organization into being, i.e., the Prime Minister of Japan and the President of the United States.

III

BASIS FOR SELECTION OF VIRUS VACCINE STRAIN

A. GENERAL INTRODUCTION

MASAMI KITAOKA

The Japanese encephalitis (JE) strain for preparation of the vaccine should, needless to say, be selected in general from among many strains kept in the laboratory and isolated in nature on the basis of the following criteria; (1) antigenic characteristics and immunizing potential as much to produce both detectable humoral and tissue immunities as to protect the vaccinees against disease with current, wild strains of JE virus, (2) virus yield from the host animal or tissue culture cells suitable for the mass-production of the vaccine, (3) stability and preservation of the liquid vaccine for at least one year, and much longer in its freeze-dried state, and (4) a safe vaccine without including any component in its preparation which might produce an adverse reaction, local or general, following a course of vaccination, especially after repeated boosters.

During the period 1935 to 1945 a formalin-killed vaccine, mouse-brain type, was prepared by Japanese investigators using the Kalinina strain and tested for its immune response in man¹ and animals,² and its protective effect had been established by direct challenge of mice³ and horses.^{4,5} The Kalinina strain was used because it was first isolated by several Japanese investigators from a human brain in 1935,⁶ and JE strains were thought to be almost the same in antigenicity, even though a little antigenic difference was found by Mitamura⁷ in cross-mouse neutralization tests. After the war the Nakayama strain was obtained again from the U.S.A. and mouse-brain type vaccine was prepared mainly using this strain, for man⁸ in small field trials and for horses⁹⁻¹¹ especially used country-widely in horses¹² in 1948 and 1949. The fact that this vaccine prepared with Nakayama-NIH was rejected for animal use by the national assay potency test in immunized mice challenged with the Nakayama-Yakken strain,¹³ a strain even originating from the same Nakayama strain, focused our attention again on the variation of antigenic characteristics and immunizing potential of various JE strains. Thus, it has been made clear that the antigenic characteristics and immunizing potential of many strains so far tested are not all the same *in vitro*, by cross HI,¹⁴ CF,^{14,15} NT,^{14,16} and other tests^{17,18} with homologous and heterologous immune sera, and also by the cross-challenge test^{13,14,19} *in vivo*, in im-

munized mice against the homologous and heterologous strains. However, the differences found were not as great as seen among influenza viruses, A, A1, A2 and C. Dr. Watanabe²⁰ pointed out the variation of JE strains due to selective host passage and Okuno¹⁷ of our study group found additional, definitely detectable differences in antigenic characteristics and immunizing potential between both G1 early and G1 late strains due to the mouse passage level. There can be no doubt but that 3 sublines, NIH, Yakken and RFVL, originating from the Nakayama strain isolated by Kasahara²¹ in 1935 have been found by laboratory testing to differ from each other, to some extent, in antigenic characteristics and immunizing potential due to extended passage in 3 different laboratories. On the other hand there are some strain differences in antigenic characteristics and immunizing potential found in viruses in nature, as pointed out first by Hale.¹⁵ From the foregoing it can be seen that the antigenicity and biological characteristics of JE virus are, to some extent, changeable by adaptation and selection by passage in the host animals in nature and laboratory, but not as markedly as occurs with some other viruses. At present, JE strains so far isolated can be divided roughly into the Nakayama-NIH type, and the JaGAR type as mentioned by Drs. Yoshioka, Nakamura, Watanabe, Takaku and our group. The former type is classical and a laboratory strain, while the latter is current and a wild strain. Almost all current isolates are classified as the latter type, though minor antigenic difference can be observed among them. By the way it is pointed out that the antigenic variation in 3 strains described by Yaoi²³ is not so conclusive from data obtained by i.p. challenge and a host-dependent antigenic variation was not so easily confirmed by Murakami¹³ and our group as described by Watanabe.²⁰

Table I presents a summary obtained by cross-neutralizing antibody and cross-protection tests against direct challenge of mice immunized with the classical Nakayama-NIH strain and a current one, JaGAR-01.

A good immune response and high level of protection against the homologous virus, and a low immune response and poor protection against the heterologous can be observed

TABLE I
Comparison of Cross-neutralizing Antibody Response and Cross-protection
Value against Direct Challenge in Mice Immunized with
Classical Nakayama-NIH and Current JaGAR-01 Strains

		Challenge virus	
Host	Strain of vaccine	Nakayama-NIH classical strain	JaGAR-01 current strain
Cross-neutralizing antibody response			
Mice	Nakayama-NIH	High	Low
Man	JaGAR-01	Low	High
Rabbits	Nakayama-NIH	High	Low
Guinea- pigs	JaGAR-01	Low	High
		Relative high at hyper immunization	High at hyper immunization
Cross-protection value			
Mice	Nakayama-NIH	High	Low
Mice	JaGAR-01	Low	Relative high
Cross-kinetic neutralization test			
Rabbit hyper immune serum	Nakayama-NIH	almost the same kinetic curve and dose response curve but not on anti-JaGAR-01 serum	
	JaGAR-01		

by both cross-NT tests and cross-protection tests. Also, the immunizing potential of the latter strain was found to be less than that of the former, even though as given in Figures 1 and 2, the kinetic curve and dose response curve of neutralization of both strains on anti-Nakayama-NIH serum are apparently similar to each other but not on anti-JaGAr-01 serum (Figures 3 and 4). Table II indicates that the EID_{50} obtained by cross-challenge

TABLE II
 EID_{50} of Japanese Encephalitis Vaccines Prepared with 3 Nakayama Strains, NIH, Yakken and RFVL

Vaccine		Challenge virus dose	
		NIH 13 LD_{50}	Yakken 10 LD_{50}
NIH	107 mg/dl*	1.75**	0.43
Yakken	110 mg/dl	0.78	1.67
RFVL	93 mg/dl	1.09	0.88

*Each virus used for vaccine was 3 times cloned and N-content of each vaccine was adjusted to roughly 100 mg/dl.

** $EID_{50} = \text{Log } 4$.

tests in mice immunized with each of the 3 sublines of Nakayama is not high against the heterologous one, except for RFVL. To select a better strain for vaccine than Nakayama-NIH, a comparative study continues on antigenic characteristics and immunizing potential of strains selected at random from old and new isolates from man and mosquitoes in various areas, such as Thailand, Peking, and Japan. Table III indicates the EID_{50} obtained by cross-challenge tests in mice immunized with each vaccine prepared from suckling mouse brain suspension, in which the virus titer was adjusted to $10^{8.5}LD_{50}$, infected with Nakayama-NIH, JaGAr-01, Chieng-Mai and Q-180. As a result the Q-180 strain might be a candidate for the vaccine strain because its antigenicity seems to be similar to that

TABLE III
 Comparison of Cross-protection Values of 4 Vaccines against Direct Intracerebral Challenge with 4 Japanese Encephalitis Virus Strains

Challenge virus	Vaccines used			
	Nakayama-NIH	JaGAr-01	Chieng-Mai	Q-180
Nakayama-NIH human strain Japan 1935	7.1	5.3	5.4	7.1
JaGAr-01 mosquito strain Japan 1958	6.0	6.4	6.4	6.7
Chieng-Mai human strain Thailand 1964	6.8	6.3	7.0	>7.0
Q-180 mosquito strain Thailand 1957	>7.0	5.5	5.8	>7.0

Each virus titer before preparation of vaccine was roughly $10^{8.0}$ — $10^{8.5} LD_{50}$.

Each vaccine was suckling mouse brain type.

Vaccine used: 1:10 dilution of each vaccine.

Challenge dose: 10^5 , 10^6 and $10^7 LD_{50}$.