

This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization.

WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES

No. 588

CEREBROSPINAL MENINGITIS CONTROL

Report of a WHO Study Group

WORLD HEALTH ORGANIZATION

GENEVA

1976

ISBN 92 4 120588 1

© World Health Organization 1976

Publications of the World Health Organization enjoy copyright protection in accordance with the provisions of Protocol 2 of the Universal Copyright Convention. For rights of reproduction or translation of WHO publications, in part or *in toto*, application should be made to the Division of Publications and Translation, World Health Organization, Geneva, Switzerland. The World Health Organization welcomes such applications.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Secretariat of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries.

The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the World Health Organization in preference to others of a similar nature that are not mentioned. Errors and omissions excepted, the names of proprietary products are distinguished by initial capital letters.

PRINTED IN SWITZERLAND

CONTENTS

	Page
1. Introduction	5
2. Causative agent	5
2.1 Identification and characterization of <i>Neisseria meningitidis</i>	6
2.2 Sensitivity to sulfonamides and antibiotics	7
3. Immunity	8
3.1 Mechanisms of antibacterial immunity	8
3.2 Protective antigens and antibodies	9
3.3 Serological tests	10
3.4 Immune response of infants and young children	11
4. Vaccines and immunization	11
4.1 Preparation and testing of vaccines	12
4.2 Combined polysaccharide and other vaccines	13
4.3 Other antigens as potential vaccines	13
4.4 Evidence of the effectiveness of vaccines	14
4.5 Immunization	18
5. Treatment and prevention with antimicrobial agents	20
5.1 Efficacy of antimicrobial agents used for treatment	20
5.2 Simple methods of treatment for rural areas	21
5.3 Principles for the use of chemoprophylaxis	21
6. Recent advances in related fields	22
6.1 <i>Neisseria</i> infections in animals	22
6.2 Studies of immunity against <i>N. gonorrhoeae</i>	23
6.3 Studies of <i>Haemophilus influenzae</i> type b vaccine	23
7. Needs for further research	25
8. Recommendations	27
Acknowledgements	29

WHO STUDY GROUP ON CEREBROSPINAL MENINGITIS CONTROL

27-31 October 1975

Members :

- Dr G. Ayme, Chief, Department of Research on Bacterial Immunology, Mérieux Institute, Charbonnières-les-Bains, France
- Dr J. Etienne, WHO Collaborating Centre for Reference and Research on Meningococci, Microbiology Research Laboratory, Institute of Tropical Medicine of the Army Health Service, Marseille-Armées, France
- Dr E. C. Gotschlich, The Rockefeller University, New York, NY, USA
- Dr M. R. Hilleman, Director, Virus and Cell Biology Research, Merck Institute for Therapeutic Research, West Point, PA, USA (*Rapporteur*)
- Dr I. Joó, Scientific Leader, "Human" Institute for Serobacteriological Production and Research, Budapest, Hungary
- Dr R. Netter, Acting Director-General, National Health Laboratory, Ministry of Health, Paris, France (*Vice-Chairman*)
- Dr J. B. Robbins, Director, Division of Bacterial Products, Bureau of Biologics, Food and Drug Administration, Bethesda, MD, USA
- Professor N. A. Vedros, Medical Microbiology and Immunology, Department of Biomedical and Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA, USA (*Chairman*)

Secretariat :

- Dr W. C. Cockburn, Director, Division of Communicable Diseases, WHO, Geneva, Switzerland
- Dr B. Cyjetanović, Chief Medical Officer, Bacterial Diseases, WHO, Geneva, Switzerland (*Secretary*).
- Dr P. H. Mäkelä, Director, Bacteriology Division, Central Public Health Laboratory (State Serum Institute), Helsinki, Finland (*Temporary Adviser*)
- Dr F. T. Perkins, Chief Medical Officer, Biological Standardization, WHO, Geneva, Switzerland
- Dr R. H. Tiesjema, National Institute of Public Health, Bilthoven, Netherlands (*Temporary Adviser*)

CEREBROSPINAL MENINGITIS CONTROL

Report of a WHO Study Group

A WHO Study Group on Cerebrospinal Meningitis Control met in Geneva from 27 to 31 October 1975. Dr W. C. Cockburn, Director of the Division of Communicable Diseases, opened the meeting on behalf of the Director-General.

1. INTRODUCTION

Meningococcal meningitis has been causing increasing concern in recent years owing to its changing patterns and the apparent rise in prevalence in several parts of the world where it was previously not considered to be a public health problem. Cerebrospinal meningitis outbreaks have continued to occur in Africa, but at the same time, the morbidity due to the disease has risen to epidemic proportions in some countries of South America, the Middle East, southern Africa, Europe, and Asia. These outbreaks are caused by meningococci of serogroups A and C, but serogroup B organisms are also prevalent in many countries. The present trend of the disease seems to indicate that another pandemic of cerebrospinal meningitis similar to those that have occurred in the past may be in sight.

The epidemiological situation in the world is disquieting and demands the urgent development of more effective tools for the control of this disease.

In the light of the above, the Group discussed various aspects of cerebrospinal meningitis and its control.

2. CAUSATIVE AGENT

The characteristics of *Neisseria meningitidis* are variable. The identification and characterization of the organism require particular attention in view of the association of particular serogroups and serotypes with clinical disease.

2.1 Identification and characterization of *Neisseria meningitidis*

2.1.1 Identification

The genus *Neisseria* consists of diplococci of Gram negative character that contain cytochrome oxidase and a catalase. Bacteria of the species *Neisseria meningitidis* are strictly aerobic organisms that grow best on enriched media in the presence of carbon dioxide at an optimal temperature of 36°C. They fail to grow at 22°C. They ferment glucose and maltose but not fructose, sucrose, mannose, or lactose, as determined in tests employing either cystine trypticase agar with 1% sugar and phenol red, or Mueller-Hinton medium containing 1% sugar and bromthymol blue indicator. Viability of the meningococcus is best preserved by storing it at -70°C or by freeze-drying.

Meningococci have been classified on the basis of their morphology and biochemical characteristics.

While identification is relatively simple, problems may, nevertheless, arise, and it is advisable in such cases that national laboratories refer the isolates to the WHO Collaborating Centre for Reference and Research on Meningococci in Marseilles, France, or to the *Neisseria* Repository, School of Public Health, Berkeley, California, USA.

The development of laboratory facilities to identify meningococci is of capital importance for meaningful surveillance which, in turn, is essential for the proper use of vaccines and antimicrobial agents.

2.1.2 Serogrouping and serotyping

Meningococci have been classified serologically on the basis of the capsular polysaccharides they carry on their surface. The chemical composition of the group-specific polysaccharides, according to the best present information, is tabulated below :

Chemical composition of the meningococcal group-specific polysaccharides

Group A.	<i>N</i> -acetyl-3- <i>O</i> -acetyl mannosamine phosphate (α 1-6)
Group B.	<i>N</i> -acetyl neuraminic acid (α 2-8)
Group C.	<i>N</i> -acetyl and <i>O</i> -acetyl neuraminic acid (α 2-9)
Group D.	Under investigation
Group X.	<i>N</i> -acetyl glucosamine phosphate (α 1-4)
Group Y.	<i>N</i> -acetyl neuraminic acid : glucose
Group Z.	Under investigation
Group Z'.	3-deoxy-D <i>manno</i> -octulosonic acid
Group W135.	Under investigation

It has been possible, using serological procedures, to subdivide Group C meningococci into a majority of strains called C₁+ and a

minority of strains designated C₁-. The C₁+ antigen is O-acetylated, whereas the C₁- lacks O-acetyl groups. The O-acetyl group, when present, is found on carbon 7 and 8 and the distribution between those two carbons is somewhat variable.

The serotype antigens of Group B meningococci have been partially defined, particularly for serotype 2 which commonly causes disease in man. It has been demonstrated that the antigen responsible for serotype specificity is the major outer membrane protein. Similar antigens have also been demonstrated for several other Group B serotypes. These antigens may be shared by meningococcus strains belonging to Groups B, C, and Y, but not Group A.

Group identification is usually carried out by agglutination on slides, in test tubes, or in plastic trays. The latter is the simplest and most reliable method. Grouping of meningococcal isolates is not always easy or definitive since polyagglutinable, autoagglutinable or nonagglutinable strains are sometimes found and since the findings in different laboratories may not be in complete agreement. This problem seems to be due mainly to the use of non-standardized antisera that may have been prepared using different animal species, different strains of bacteria, and different regimens of immunization.

Tests for precipitation of meningococcal antigens, whether by the Ouchterlony procedure or immunoelectrophoresis, are of little value for serotyping but have particular usefulness for studying antigenic structure. Counter immunoelectrophoresis has been tried in several laboratories and halo formation in agar gel may be of special use in research investigations.

Serotyping of meningococci within each Group is best carried out by the serum bactericidal test of Frasch,^a who has applied the test to Groups A, B, and C. This test is more difficult to perform than the agglutination assay. Typing by use of bacteriocins or bacteriophage has not yet been developed.

2.2 Sensitivity to sulfonamides and antibiotics

Examination for sensitivity to sulfonamides and antibiotics is essential to the bacteriological study of *N. meningitidis* from the clinical and epidemiological viewpoints.

^a FRASCH, C. E. & CHAPMAN, S. S. Classification of *Neisseria meningitidis* Group B into distinct serotypes. I. Serological typing by a microbacterial method. *Infect. Immun.*, 5: 98 (1972).

Sensitivity to antibiotics is usually investigated by the disc method. This method is adequate in routine practice. The choice of the antibiotics to be tested may vary from one laboratory to the next and should be standardized. In addition to the antibiotics most frequently studied (penicillin, ampicillin, streptomycin, chloramphenicol, erythromycin, rifampicin, tetracycline, minocycline, cephalosporins and their derivatives), sensitivity to spiramycin should be included on account of its excretion in the saliva.

Investigation of sensitivity to sulfonamides should not be neglected in the sensitivity tests; it should cover not only simple sulfonamides such as sulfadiazine, but also long-acting sulfonamides (sulfamethoxypyridazine and sulfadoxine) and trimethoprim-sulfamethoxazole.

The disc method is not to be recommended for tests of sensitivity to sulfonamides. Instead, the sensitivity of the strain is tested in the presence of increasing concentrations of sulfonamides incorporated in Mueller-Hinton medium. The concentrations normally used in the laboratory (1, 10, 50, and 100 mg/litre) seem adequate. The resistance threshold needs to be defined. For the long-acting sulfonamides 50 mg/litre can be considered as sufficiently accurate.

The sensitivity of strains *in vivo* is generally higher than sensitivity *in vitro* would suggest. Nevertheless, the latter parameter remains a valuable indicator of the overall sensitivity of the strains at a given time and place.

3. IMMUNITY

Immunity against cerebrospinal meningitis has been studied in numerous epidemiological and laboratory studies.

3.1 Mechanisms of antibacterial immunity

Immunity to meningococcal disease has been clearly shown to be antibody-mediated. In young children, the age-specific incidence of the disease is related to disappearance of maternally acquired antibodies to the organism. Agammaglobulinaemic children are very susceptible to meningococcal disease which is effectively prevented by passive immunization with pooled gamma globulin. In a prospective study,^a it was

^a GOLDSCHNEIDER, I. ET AL. *J. exp. Med.*, 129 : 1307 (1969).

demonstrated that individuals who developed meningitis did not have circulating bactericidal antibodies to the organisms with which they were infected. The role of cell-mediated immunity is being studied currently. Attempts have been made to define the susceptible host by serological methods. It should be kept in mind that both the human antibody response and the antigenic composition of the meningococcus are so complex that it is unrealistic to expect any single test to identify the susceptible host.

3.2 Protective antigens and antibodies

It is well established that Group A and Group C meningococcal polysaccharides can serve as protective antigens in humans of certain age groups. For other meningococcal antigens, the evidence is much less clear and more indirect. Protection afforded by the polysaccharide vaccines is strictly group-specific.

Evidence that antibodies against the serotype antigens of Group B may be protective is limited and is based on studies done in chick embryos. It has been shown, for example, that antiserum against the serotype 2 antigen protects to some extent against type 2 meningococci of Groups B and C when injected intravenously in chick embryos. The protective effect of these sera is markedly enhanced if they are administered along with antiserum against the Group B polysaccharide. Apparently, the two antibodies act synergistically. Studies are in progress to prepare the type-specific antigens in a relatively non-pyrogenic form and suitably characterized to permit tests in human beings.

Protection against meningococcal infection has also been obtained with other antigens, but, as yet, they are insufficiently characterized to allow firm conclusions as to their origin or their chemical composition. Two antigens have been isolated ^a from Group A meningococci, one of which was the group-specific polysaccharide with an associated polypeptide; the other was of greater complexity. Both antigens protected mice challenged with live meningococci in the presence of mucin. The second antigen also afforded a lesser degree of protection against challenge with a heterologous meningococcus strain. A complex protein antigen was isolated ^b by calcium chloride extraction of meningococci. This

^a CHENG, W. C. ET AL. *J. Immunol.*, **114** : 1497 (1975).

^b JENNINGS, H. J. ET AL. *Infect. Immun.*, **5** : 547 (1972).

material, when used to immunize mice, produced considerable immunity to challenge with meningococci of several serogroups.

Pili have been demonstrated to exist on meningococci but their role in the virulence of the organisms has not been defined. No studies have been performed on the immunochemical characterization of these structures and research in this area seems warranted.

3.3 Serological tests

A number of tests have been used for detecting antibodies against the various intact meningococci or their antigens. The most common are listed below :

Bactericidal assay. The antibody complement-dependent bactericidal reaction has been employed widely. It does not require purified antigen. The test measures antibodies that are known to be associated with protection against the disease but it has serious shortcomings : it is difficult to duplicate from laboratory to laboratory ; it depends in unknown ways on the media used to grow the meningococci ; and it is affected by differences in target strains and complement sources.

Haemagglutination assay. Several modifications of the haemagglutination assay have been developed. The assay is highly specific for the group-specific polysaccharide if the purified polysaccharides are used to sensitize the red blood cells. It is a sensitive test and could be modified to give even better results. The major disadvantage of the test is that it measures IgM antibodies mainly and the results of the test are only semi-quantitative.

Quantitative precipitation reaction. The antibody response to meningococcal infection in adult human subjects is often sufficient to permit measurement by the quantitative precipitation test. The technique measures antibody independently of its immunoglobulin class. The same procedure has been used to measure pneumococcal antibodies and may provide especially useful data in confirming responses to vaccines against these two groups of organisms. The method is technically difficult and insensitive, hence its chief value is as a standard of comparison.

Radioimmunoassay. Several radioimmunoassays for the detection of polysaccharide antibodies have been developed. In the tests the polysaccharide used can be intrinsically or extrinsically labelled. The latter antigen has the disadvantage of requiring modifications of the poly-

saccharide but presents an advantage in that the isotope ^{125}I can be counted in a gamma spectrometer. In all tests, the antigen-antibody complex precipitated with ammonium sulfate permits measurement of all antibodies without regard to their immunoglobulin class. The test might be modified for use with anti-immunoglobulin reagents to detect the classes of antibody present. The amount of antibody can be expressed either indirectly as the amount of antigen bound, or directly by testing for antigen-binding capacity of dilutions of an antiserum in comparative tests with a known standard antiserum. The second treatment has the advantage that it relies on stable antiserum reagents as standards, and also that it facilitates comparisons between different immunogens.

3.4 Immune response of infants and young children

It has been found that children at the age of 3 months are already able to make feeble responses to Group C antigen but have essentially no response to Group A antigen. At 7 months infants respond to both antigens and their response approaches adult levels between 2 and 6 years of age. The response to a second injection has been studied. Infants 7 or 13 months old who have already received Group C antigen at age 3 months, show a significantly lower response than infants who receive this antigen for the first time. The hyporesponsiveness to secondary injection with Group A antigen is not seen after the age of 2 years.

On the other hand, in the case of the Group A antigen, a booster effect is seen upon second injection of this antigen into infants between 7 and 18 months of age. The infants produce antibody levels akin to those seen in older children or adults. This hyperresponsiveness is less evident after the age of 2 years.

4. VACCINES AND IMMUNIZATION

The emergence of resistance to antimicrobial agents, such as sulfonamides for meningococci and ampicillin for *Haemophilus influenzae*, as well as the logistic difficulties, expense, risk, and transient benefit of chemoprophylaxis, emphasize the need for other methods for the prevention of cerebrospinal meningitis caused by these bacteria.

Advances in the field of immunochemistry have permitted the preparation of highly purified and immunogenic capsular polysaccharides

from meningococcal serogroups A and C and from *H. influenzae* type b. Their immunizing properties have been studied in controlled field trials.

4.1 Preparation and testing of vaccines

Details relating to the preparation and testing for safety and potency of the meningococcus vaccines are in preparation.^a

Briefly, meningococcus Group A and C strains of proved usefulness are propagated in media of specified composition. The polysaccharides are purified by either of two processes known as the "Sevag" method^b or the "cold-phenol process".^c In both procedures, the bacteria are removed and the polyanionic polysaccharides are precipitated from the culture fluid by cetrimonium bromide and dissolved in calcium chloride solution. The nucleic acids and bacterial debris are eliminated by precipitation with 25% ethanol and the polysaccharides are precipitated with 80% ethanol. By the Sevag method, residual protein and endotoxin are removed by extraction with chloroform and butanol and by ultracentrifugation. By the cold-phenol method, the proteins are removed by cold-phenol extraction and the endotoxins by ultracentrifugation. The cold-phenol method, applied most frequently to meningococcus A, tends to give polysaccharide of high molecular weight.

The final polysaccharides are characterized chemically and by physical measurements to assay for purity and molecular weight. The product is then formulated into the final product, dispensed into vials and dried. The product is tested for safety and potency by the procedures given in "Requirements for meningococcal polysaccharide vaccine".^a

^a Note added during editing: A proposed text for "Requirements for meningococcal polysaccharide vaccine" was prepared by the Study Group and submitted to the Twenty-seventh WHO Expert Committee on Biological Standardization for their consideration. The Group proposed the following requirements for the polysaccharide vaccines: "Group A vaccine shall contain 3.75 µg of phosphorus per 50 µg of polysaccharide and Group C vaccine shall contain 37.5 µg of *N*-acetyl neuraminic acid per 50 µg of polysaccharide. The molecular size of the polysaccharide in at least one final container from each filling lot shall be determined by Sepharose 4B gel filtration. Chromatography shall be carried out in a solvent having a concentration of 0.2 ionic strength. Molecular weight characterization shall be determined by measuring the proportion of polysaccharide that elutes with a *K*_d less than or equal to 0.40. At least 50% of the polysaccharide shall elute with a *K*_d less than or equal to 0.40." The full text of the "Requirements for meningococcal polysaccharide vaccine" as approved by the Expert Committee on Biological Standardization will be published shortly in *WHO Technical Report Series*, 1976, No. 594.

^b SEVAG, M. G. *Biochem. Z.*, **273**: 419 (1934).

^c GOTSCHLICH, E. C. ET AL. *Progr. immunobiol. Standard.*, **5**: 485-491 (1972).

4.2 Combined polysaccharide and other vaccines

The continuing development of new viral and bacterial vaccines makes it necessary to devise means whereby these vaccines can be administered as efficiently as possible and at least cost. One principal approach to the problem is to use combined vaccines in which more than one vaccine is administered in a single injection.

Combined meningococcus Groups A + C vaccine has been developed recently and offers important advantages in situations in which both serotypes are causing a significant amount of illness in a population within a limited time period. Such a situation has existed in the recent epidemic of Groups A and C meningococcal meningitis in Brazil.

Carefully conducted clinical studies have been carried out in the USA and in Central America to compare the antibody responses to the clinical reactions in persons given combined vaccine or the same individual vaccine components separately. Three age groups were included: 16-69 years, 2-8 years, and 8-24 months. In the studies, there was no significant difference in antibody response between the bivalent and monovalent vaccines. Further, there was no increased local or systemic reactogenicity in recipients of the combined vaccine.

At present, there are no further vaccines in which the current meningococcus A and C vaccines might be combined. This is primarily because the meningococcus vaccines are not highly effective in infants in whom such vaccines as DPT, poliomyelitis, etc. are routinely given. The meningococcus vaccines might be profitably combined with *H. influenzae* type b vaccine or a polyvalent pneumococcal vaccine at some future time when the last named vaccines have been developed. However, it should be recorded that a candidate combined vaccine including live measles virus vaccine, meningococcus A vaccine, and tetanus vaccine is at present being tested in children in Africa.

4.3 Other antigens as potential vaccines

The antibody response to *N. meningitidis* is directed primarily against the cell surface components such as the group-specific capsular polysaccharide and the less well defined type-specific and cross-reacting antigenic structures. Sufficient evidence exists in the literature to indicate that serogroup-specific polysaccharides from Groups A and C are deficient in certain respects for effective control of cerebrospinal meningitis. Thus, the serogroup C polysaccharide does not appear to be effective in very young children; the production, stabilization, and

biological assay of the polysaccharides are complex and costly ; and the polysaccharides are very group-specific. In addition, there is as yet no satisfactory polysaccharide vaccine from the serogroup B meningococcus.

Bacterial cell fractions other than group-specific purified polysaccharides have been examined. A protein-polysaccharide fraction from serogroup A has been isolated that appears to induce protection in mice to challenge by the homologous Group A and Groups B and C. Other studies have been concerned with tests for immunogenicity of cell-wall components and of cell membrane and somatic antigens.

It is important that a search be made to find means for enhancing the immunogenicity of the current polysaccharide by the classical method of attachment to carrier proteins. It has been suggested that methylated protein might be a good choice. Other suggested studies include the use of adjuvants such as emulsified vegetable oil, and the use of cross-reacting non-pathogenic bacteria, live attenuated strains for intranasal immunization, and *Neisseria* species other than *N. meningitidis* as vaccines. Since the Neisseriae share many major antigens, studies should be conducted on those antigens involved in meningococcal virulence that may be present in other human *Neisseria* species.

The inherent antigenic variability of the Neisseriae as evidenced by the multiplicity of serogroups implies that some bacterial component(s) other than the group-specific polysaccharide should be isolated and studied that may be highly immunogenic and might induce broad serogroup protection.

4.4 Evidence of the effectiveness of vaccines

Data are available for eight field trials of meningococcus polysaccharide vaccines in which an acceptable control group was included. These are presented in Table 1 and referred to by the numbers 1 to 8. The results of these controlled field trials have to be interpreted with caution in view of the fact that only small numbers of cases have been observed in the control and vaccinated groups.

In trials on army recruits (No. 1 and 6) the vaccinated groups were selected on a volunteer basis but the vaccinated and control groups were otherwise comparable. All the other trials were double-blind trials employing an antigenically unrelated vaccine for control purposes (tetanus toxoid, *H. influenzae* type b polysaccharide).

Vaccines from four producers and produced using either the Sevag or the cold-phenol extraction method have been used with no differences in efficacy attributable to the purification method. The molecular weight

of the polysaccharide (indicated by the Kd value ^a) is closely related to the efficacy of the vaccine. The failure of the vaccine in trial No. 2 was most probably the result of degradation of the Group A polysaccharide caused by extended exposure of the vaccine to high temperatures in the field. Since that time it has been recommended to store Group A vaccine at -20°C until used. This requirement increases the costs of vaccine administration.

Trials No. 1, 3, 4, 5, and 6 involved a total of 500 000 people in whom more than 100 cases of meningitis occurred. They demonstrated the clinical efficacy of both Group C and Group A vaccines in adults and children above 6 years of age. The protective effectiveness of both vaccines was about 90%.

The duration of immunity could be followed for only 8 weeks in the case of Group C vaccine (trial No. 1). Immunity after Group A vaccine may be long lasting; it has been shown to persist for 3 years in Egypt in trial No. 3. In trial No. 5, also with Group A vaccine in Egypt, there appeared to be a loss of protection after 1 year.

The efficacy of the vaccines for infants and young children has not been definitely determined. In trial No. 8, Group C vaccine gave no protection in infants between 6 and 23 months of age but the vaccine appeared to be effective in children aged 2-6 years.

Trial No. 7 involved Group A vaccine and the age range was 3 months to 5 years. Those under 18 months of age received a booster dose of vaccine. There were no cases in the vaccinated group but 5 cases occurred in the control group during the first 9 months of observation suggesting protection at all ages. The numbers were too small, however, to establish this point.

The findings in several studies suggest that vaccination of only part of a population may decrease the transmission of infection in the population as a whole. Thus, in trial No. 6, the epidemic in the army stopped when approximately 40% of the men had been vaccinated. In trial No. 7, approximately 40% of children in the age groups in question received the Group A vaccine, and the rate of Group A disease decreased appreciably in all children of these ages in the vaccination area without a corresponding decrease in disease (both meningitis and bacteraemia) caused by other meningococcal groups or of meningococcus Group A disease in other age groups or other areas of the country.

^a Partition coefficient of the main polysaccharide component upon Sepharose 4B gel filtration.

TABLE 1. CEREBROSPINAL MENINGITIS POLYSACCHARIDE VACCINES TESTED IN CONTROLLED FIELD TRIALS

VACCINES		FIELD TRIALS												
Trial No.	Type	Lot no.	Producer	Kd ^a	Country (reference)	Year of observation	Age	Population		Homologous cases	Duration of immunity in the field	Temperature in the field		
								Total no. Vaccinated	Control					
1	C	C-6 ^a	Walter Reed	0.28	USA (7)	1968/69	army recruits	68 072	54 309	1	38	2 months	15-20°C	
		C-7 ^a	Squibb	0.26 0.34	USA (7)	1969/70	army recruits	74 644	60 172	1	35	2 months	15-20°C	
2	A	V-5 ^b	Mérieux	0.27 0.60 ^a	Nigeria (2)	1969	school children	14 426	7 239	8	5	0	35-40°C	
3	A	V-6 ^b	Mérieux	0.27	Egypt (3, 5)	1972/75	school children	124 349	62 054	0	15 ^c	3 years	15-20°C	
4	A	V-7 ^b	Mérieux	0.39 0.46 ^a	Sudan (4)	1973/75	all ages	21 640	10 881	10 759	0	7 ^d	4 months	35-45°C
5	A	V-7 ^b	Mérieux	0.39	Egypt (5)	1973/75	school children	176 646	88 263	88 383	4	7 ^e	1 year	15-20°C
6	A	453 ^a 553 ^b	Merck, Sharp & Dohme Merck, Sharp & Dohme	0.41	Finland (6) Finland (6)	1974/75 1974/75	army recruits army recruits	13 023	5 158	7 865	1	8	9 months	0-20°C
								24 183	11 300	12 883	0	3	9 months	0-20°C

7	A	572 ^b 573 ^b	Merck, Sharp & Dohme	0.41	Finland (7)	1975	children 3 months to 5 years	100 000	50 000	50 000	0	5	9 months (being continued)	0°C
8	C		Merck, Sharp & Dohme		Brazil (8)	1972/75	6-23 months 24-35 months	79 225	39 674	39 551	22	25		
								55 216	27 580	27 636	9	20		

^a Prepared by the Sevag method.

^b Prepared by the cold-phenol method.

^c Partition coefficient of the main polysaccharide component upon Sepharose 4B gel filtration.

^d Material returned from the field.

^e Vaccinated/control: 0/8 after 1 year; 0/4 after 2 years; 0/3 after 3 years.

^f Vaccinated/control: 0/7 after 1 year; 0/0 after 2 years.

^g Vaccinated/control: 1/5 after 1 year; 3/2 after 2 years.

REFERENCES

1. GOLD, R. ET AL. *Bull. World Health Organ.*, **45** : 279 (1971).
2. SANBORN, W. R. ET AL. *Progr. Immunobiol. Stand.*, **5** : 497 (1972).
3. WAHDAN, M. H. ET AL. *Bull. World Health Organ.*, **48** : 667 (1973).
4. ERWA, H. ET AL. *Bull. World Health Organ.*, **49** : 301 (1973).
5. WAHDAN, M. H. Unpublished results.
6. MÄKELÄ, P. H. ET AL. *Lancet*, **2** : 883 (1975).
7. MÄKELÄ, P. H. ET AL. Results not yet published.
8. TAUNAY, A. DE E. ET AL. *Ped. Res.*, **8** : 429/155 (1974).

In addition, field studies (which did not include a control group) carried out in several countries provided further information indicating the efficacy of the vaccines.

In most of the trials, these vaccines were reported as causing no severe side effects. An exception is the experience in Finland, in which Group A vaccine caused minor systemic reactions in 40% of the vaccinated infants and young children (trial No. 7) and quite high fever reactions (38.5–41°C) in 1.8% of these persons. These reactions were probably caused by endotoxin in the vaccine. Young children appear to be especially sensitive to the pyrogenic effect since the same vaccine did not cause fever in army recruits.

The effect of the vaccines on the nasopharyngeal carriage of meningococci has been studied in some trials. The vaccine did not appear to have any effect on the rate in established carriers. However, Group C vaccine did reduce new acquisitions of Group C strains in various camps of military recruits by a factor of 2 to 10. Group A vaccine (trial No. 5) had a slight effect on new acquisitions of Group A strains (21 persons in the vaccine group compared with 32 persons in the control group) and decreased somewhat the duration of the carrier state.

In the controlled field trials, efficacy in preventing the disease of the order of 90% has been demonstrated for Groups A and C vaccine in adults and children above 6 years of age. Group A vaccine seems to protect down to 3 months of age, while Group C vaccine does not appear efficacious below 2 years. The effect of the vaccines on nasopharyngeal carriage is less marked than on clinical disease.

4.5 Immunization

In several controlled field trials meningococcal Groups A and C vaccines have been shown to be highly effective in preventing cerebrospinal meningitis although they influence to a lesser degree the acquisition of nasopharyngeal carriage. Thus they provide a safe means of producing, after a single dose, immunity for a certain period against two of the three major serotypes of meningococci causing the disease. These highly purified capsular polysaccharides do not cause serious adverse reactions.

There are indications that the serogroup C vaccine does not protect children under 2 years of age, while Group A vaccine seems to protect children over 3 months of age. These antigens produce or boost antibodies in persons of certain age groups.

Ongoing studies carried out in Finland and USA with the currently available *H. influenzae* type b polysaccharide showed little or no pro-

protective effect against meningitis and/or epiglottitis caused by this organism. An antibody response was, however, observed in children, although it was slight in most of those below 2 years of age—the age groups with the highest attack rates.

The available information justifies the use of the Group A and Group C meningococcal polysaccharide vaccines in population groups with high attack rates in areas experiencing epidemics of cerebrospinal meningitis due to these serogroups. Booster inoculations with serogroup A vaccine may be considered in the face of a continuing epidemic for children under 18 months of age, while Group C vaccine should not be given to infants under 6 months of age, because their antibody response after a subsequent booster dose is poorer than that of children receiving their first dose after that age. Available data indicate that the antibody response is equally good when the Group A and Group C vaccines are given together. The costs of combined vaccine may argue in favour of the use of monovalent vaccines, in particular when only one serogroup is active.

The justification for routine immunization of children with the currently available vaccines remains to be established.

A need exists for the combination of meningococcal polysaccharide antigen with other commonly used vaccines. Present evidence suggests that simultaneous injection of *H. influenzae* type b polysaccharide in infants and pneumococcal polysaccharide in mice with whole-cell pertussis vaccines depresses the response to the polysaccharide vaccines. Thus the latter may have to be given alone and this could necessitate an additional visit to a medical facility.

Susceptibility to cerebrospinal meningitis has been correlated with the absence of detectable serum bactericidal antibodies. This assay is expensive and laborious to perform and hence extensive surveillance has not been possible. Therefore, immunization programmes will have to be based on information concerning the age-specific attack rates of the disease in the given community. Epidemiological surveillance, coupled with the isolation and typing of meningococci, is necessary to ensure that the appropriate polysaccharide vaccine(s) is used.

In view of the cost of the vaccine and the limited resources in developing countries, an effort should be made to utilize the available vaccine in the most economical and judicious way in order to derive maximum benefit from an immunization campaign. This is achieved by using vaccine primarily for the protection of well defined high-risk groups.

5. TREATMENT AND PREVENTION WITH ANTIMICROBIAL AGENTS

In view of the limitations and constraints of the presently available vaccines in the control of cerebrospinal meningitis, namely the lack of an effective antigen against Group B meningococci and the lack of combined vaccines, and in view of the poor immune response in young, high-risk age groups, the logistic difficulties, and the high costs, other control measures should be taken into consideration.

Among other control measures that could be considered for field application, specific chemo- and antibiotherapy and prophylaxis^a have been used most in public health practice.

Immunization and the use of antimicrobial agents are control measures that are complementary and not mutually exclusive. The possibility of using various combinations of these methods should therefore be considered.

The merits and drawbacks of immunization on the one hand, and of chemo- and antibioprophyllaxis and therapy on the other, should be considered in the light of local conditions, taking into account the cost-benefit aspects of these approaches in the control of the disease.

5.1 Efficacy of antimicrobial agents used for treatment

5.1.1 Sulfonamides

Sulfadiazine is not generally used for treating meningococcal meningitis, in contrast to the long-acting sulfonamides that are widely used in certain countries because of their properties, their ease of application, and their low cost.

However, an increasing *in vitro* resistance to these agents has become apparent. Thus, in France, the percentage of strains resistant to sulfamethoxypyridazine increased from 19.8% in 1972 to 49% in October 1975, and for sulfadoxine from 23.5% to 53% during the same period. The difference is only slight (2% to 5%) for trimethoprim-sulfamethoxazole.

It should be noted that this phenomenon varies according to serogroup: for example, 30% of strains of serogroup A were at one time

^a Chemoprophylaxis and prophylaxis by the use of antimicrobial agents refers, in this context, to mass medication of a population among whom there are carriers rather than to the use of such agents by healthy persons to prevent possible infection.

sensitive to sulfamethoxyipyridazine but by 1975 this percentage had fallen to 18.5%.

5.1.2 *Antibiotics*

The overall sensitivity of strains seems to be decreasing. Since 1972, it has declined from 97.1% to 84%.

This has occurred principally with the streptomycin-resistant strains (16.4% for B, 3.7% for A). Rare strains have been found resistant to chloramphenicol (less than 1%).

5.2 Simple methods of treatment for rural areas

Any therapeutic agent that is to be used for treating patients in rural areas in developing countries must be highly efficacious, easily administered and inexpensive.

The long-acting sulfonamides (sulfamethoxyipyridazine and sulfadoxine) are still widely used with success in a single injection of 2.5 g in the adult, the dose decreasing with age. There have not been any accidents in treatment with this method.

An identical method has been proposed that employs chloramphenicol in an oil suspension in a single dose. This method gives excellent results and can be applied along with long-acting sulfonamides.

Early application of drugs, immediately the first signs of illness appear, is of great importance for success in treatment. Nevertheless, case fatality rates range between 5% and 10% in treated persons and are probably related to the toxic effects of the organism.

5.3 Principles for the use of chemoprophylaxis

The necessity for chemoprophylaxis has declined since the development of specific immunization. Protection disappears immediately after the drug has been eliminated from the body. Serious untoward effects may result from uncontrolled and indiscriminate use of sulfonamides and antibiotics. However, chemoprophylaxis may be justified during an epidemic due to Group B organisms when its use should be limited to certain special situations, such as a closed community that can be kept under strict medical supervision. The risk-benefit ratio of chemoprophylaxis should be carefully considered before a decision is taken to use it. Whatever product is chosen—sulfonamide or antibiotic—it is essential that the treatment be applied only once and at a therapeutic dose level.

6. RECENT ADVANCES IN RELATED FIELDS

Research on the biochemical and biological properties of *N. meningitidis* and related aspects of immunity and disease control can possibly benefit from current studies on other *Neisseria* and similar organisms causing meningitis. Examples are studies on capsular polysaccharide vaccines against meningitis caused by *H. influenzae* type b and studies on the antigenic structure of *N. gonorrhoea*.

6.1 *Neisseria* infections in animals

The meningococcus and the gonococcus are pathogenic only for man. There have been many attempts during the last 75 years to infect animals with the *Neisseriae* in order to develop an *in vivo* model for the study of host-parasite relationships. Studies are currently in progress on the mouse-mucin, chick embryo, and rabbit uvea models, as described below.

The mouse-mucin test. Meningococci, but not gonococci, are virulent for mice when injected intraperitoneally in a mucin suspension. The strain of mice, source of hog gastric mucin, and growth phase of the bacteria are critical parameters. Mice injected with either Group A or Group C polysaccharide vaccine were resistant to challenge by homologous but not heterologous serogroups. Serum from vaccinated humans did not passively protect mice but typing sera produced in rabbits showed high protective capacity.

Chick embryo. The chick embryo at various ages has been found to be susceptible to meningococcal infection. The most satisfactory results in regard to serum protection have been obtained in 12-day embryonated eggs injected intravenously. Because of the highly technical and demanding requirements and the large numbers of eggs required per dilution point for statistically valid results, the model has certain disadvantages.

Rabbit uvea. The uvea of rabbits has been used to study the cellular response to meningococci, gonococci, and certain cellular fractions of these bacteria. The cellular response observed was typical of that observed with delayed hypersensitivity and was not related to humoral antibodies.

In conclusion, animal models are currently being used to study the host-parasite relationship of gonococci and meningococci. These animal

models are not intended to simulate infections in humans but rather to answer specific questions such as how the *Neisseria* attach to and penetrate mammalian cells and what roles humoral and cellular immunity play in these biological interactions.

6.2 Studies of immunity against *N. gonorrhoeae*

Gonococci and meningococci share many antigens. Extensive research is needed on the antigenic components of the gonococci that will permit effective serological detection of asymptomatic gonococcal infections. Antibodies induced by meningococcal infection may be responsible for the high numbers of false positive reactions currently observed in gonococcal serological surveys.

The virulence of gonococci has been correlated with colonial morphology and the presence of pili. It is assumed that, because of constant flushing by secretions and urine, gonococci need to attach to the epithelium lining the urogenital tract. The pili are believed to play a major role in this attachment. In addition, as yet undefined surface proteins also serve to attach gonococci to cells. Pili have been chemically characterized and there is antigenic diversity among pili of different gonococcal strains. Further study is needed to evaluate the human response to pili in relation to natural and specific acquired immunity. A parallel situation may exist with the acquisition of meningococci in the nasopharynx. Knowledge of how meningococci may be prevented from attaching to oral membranes may be applied to gonococcal infections and vice-versa.

The serological diversity of gonococci demonstrated by pili studies is further confirmed by analysis of the major outer protein membranes of this organism. Many serotypes have been identified and the effect of this antigenic variability on reinfection will play a major role in our understanding of the epidemiology of gonorrhoea. Suitable animal models are lacking for gonorrhoea as well as meningococcal meningitis. Further studies are needed in this area for an understanding of the important biological factors involved in attachment of the gonococci and penetration of epithelial cells, and of the role of local immunity.

6.3 Studies of *Haemophilus influenzae* type b vaccine

H. influenzae type b is the most common cause of bacterial meningitis in infants and children and is an important cause of epiglottitis. The incidence of *H. influenzae* type b meningitis has increased over the last

20 years in many parts of the world. Because of this, and because of the tendency of *H. influenzae* type b meningitis to cause neurological sequelae, there is a definite need for prophylactic measures.

Systemic *H. influenzae* serotype b infections are almost always caused by encapsulated bacteria. The capsular polysaccharide is a polyribosylribitol phosphate polymer that can be extracted in an immunogenic form. Anticapsular antibodies in hyperimmune animal sera are protective, and natural immunity to systemic *H. influenzae* type b infections in man develops parallel to the appearance of anti-capsular antibodies. This capsular antigen is currently being used in two field trials.

The vaccine is prepared from liquid cultures of the organism by precipitation with cetrimonium bromide and is purified further by cold-phenol extraction, as has been described for meningococcal polysaccharide vaccines. The final product is a high molecular weight (Kd from 0.18 to 0.28) polysaccharide and is without significant contamination with protein, nucleic acid, or pyrogenic substances. The vaccine has produced only minor side effects in field trials.

A passive haemagglutination assay, a bactericidal assay and a radio-immune assay have been used for serological tests. The radio-immune assay is necessary to test for antibody in young infants because of their low levels of antibody. Most adults have antibody against the polysaccharide and respond to vaccine (dose of 50 µg) with a more than 30-fold increase in antibody. The antibody response increases with age. Ninety percent of children over 18-24 months of age respond to an optimal dose of 3-20 µg of vaccine, while only 10-40% of infants under 12 months respond to one or two doses of vaccine and the antibody levels attained are low.

Approximately 57 000 children below 6 years of age were vaccinated with the polysaccharide vaccine in field trials carried out in 1974-1975 in Finland and in the USA. The control groups in those studies received meningococcal vaccine of Group A or C. So far, there have been 9 cases of *H. influenzae* type b disease in the *H. influenzae* vaccinated groups and 14 cases in the control groups. Thus, the overall efficacy has been very poor. However, protection was apparent when only the children who were over 18 months of age at the time of vaccination were included in the analysis. In this age group, one case of the disease occurred in the vaccinated group and 7 cases occurred in the control group. The lack of protection in infants below 18 months of age is unfortunate in view of the fact that the disease is most common in this very young age group.

7. NEEDS FOR FURTHER RESEARCH

In the course of the discussions the need for further research in several areas became evident.

The serogrouping of meningococci still presents a certain number of difficulties. Disagreements between laboratories and the existence of polyagglutinable strains are the major problems. It was suggested that antisera be prepared against the purified polysaccharides complexed with methylated bovine serum albumin in the hope that cross-reactions would be eliminated. Very encouraging results were reported by several investigators using the antiserum agar technique and further refinement of this technique would be welcome.

Problem areas of research that deserve high priority are investigations concerning the factors related to meningococcal virulence. A clear understanding of virulence attributes might permit the identification of strains capable of causing epidemics, and the prediction of impending epidemics. In addition, a clear understanding of virulence factors is a prerequisite for the initiation of studies employing putative non-pathogenic meningococci to take advantage of the particular benefits of immunization via the portal of entry.

The possibilities of developing other types of immunizing agent and possibly live attenuated vaccines should be explored.

The need to test vaccines for their endotoxin content has proved a vexing problem. The shortcomings of the biological test and the *Limulus* coagulation assay necessitate research to substitute better analyses for the presence of endotoxic lipopolysaccharides. Two answers may be suggested: the *Limulus* assay could be improved if the proteins responsible for the clotting were isolated and their action understood and if the reagents were available in a purified form amenable to standardization; the other path would appear to be to adapt some extraordinarily sensitive chemical technology, i.e., GC-mass spectrometry, to the quantitative analysis of a specific component of neisserial endotoxin.

Although the field trials concluded to date have provided a considerable body of information, there is still a lack of definitive data on the efficacy of Group C vaccines in infants and young children. The tentative conclusions drawn from previous experience indicating no protection under the age of 2 years and 75% protection between the ages of 2 and 6 years should be re-examined, especially if the epidemiological opportunity arises.

It is also essential that the poor immune response of children be investigated more deeply. To this end, genetic studies of infants who

respond poorly and their families are indicated. One lead in this direction is the reported association between the responsiveness to Group C antigen and the Gm immunoglobulin allotype, and the association of *H. influenzae* type b epiglottitis with high responsiveness to type b antigen. Cellular immune studies concerning the mitogenicity of the antigen and the enumeration of antibody secreting cells are indicated to better understand the dynamics of the maturation of the immune response to these antigens.

There is a great need for better meningococcal vaccines suitable for young children. The research needs may be divided into two broad categories: the exploration of the potential of antigens unrelated to the group-specific polysaccharides and the elucidation of possibilities of enhancing the immune response to the polysaccharide beyond what is possible with the present vaccines. Exactly the same considerations apply to the problem of producing an effective Group B meningococcal vaccine and an *H. influenzae* type b vaccine.

Under the first category may be included the development of vaccines specific to the serotype antigens, with serotype 2 deserving highest priority. Furthermore, more extensive analysis of the antigenic structure of meningococci and *H. influenzae* is warranted with the hope of finding a species-specific surface antigen that would provide broad immunization to these organisms.

With regard to improving the response to the polysaccharide antigen several tactics need to be explored. It needs to be established whether colonization with cross-reactive organisms will be sufficient to immunize children or perhaps serve to prime infants so that they will produce an adequate response to polysaccharide injected subsequently. In addition, further research is necessary to indicate to what extent the immune response can be improved simply by increasing the molecular size of the antigens as a result of improved isolation technology, or as a result of chemical polymerization. An understanding of the conformational structure of the various polysaccharides would be essential to pursue this goal in a rational manner.

A further tactic to improve the antipolysaccharide response revolves around the production of antigens that would evoke T and B cell cooperative interaction. There is a need to explore the effects on the immune response of experimental animals of protein-coupled polysaccharides, of vegetable oil adjuvant, and of soluble peptidoglycan-derived adjuvant. Such exploratory studies should be carried out with materials to which no *a priori* objections exist for their use in infants.

Once better vaccines have been prepared it will be very important

to test their compatibility with vaccines against other infections, both current and future, to facilitate the logistics of the vaccination of children.

There are insufficient data available on the antibodies to meningococcal antigens contained in commercial globulin preparations from different countries. This information is necessary to evaluate the possibilities of the prophylactic and therapeutic use of immunoglobulin. The effectiveness of pooled immunoglobulin in treating hypogammaglobulinaemic patients supports the validity of this approach as a prophylactic measure. Immunoglobulin for this purpose may be prepared from donors immunized with meningococcal vaccine.

In a study in Africa, a correlation between the incidence of cerebrospinal meningitis, crowding, and the degree of air pollution with airborne bacteria has been demonstrated. There is a need to study further the role of environmental factors with a view to the possible development of environmental control methods.

8. RECOMMENDATIONS

The Group made the following recommendations :

Vaccines

1. Studies of the mechanism, kinetics, and possible means of preventing the degradation of the meningococcal polysaccharides should be encouraged.

2. It is not advisable that the currently available polysaccharides be stored as reference standards for meningococcal Group A and C vaccines. A constant rate of degradation observed in both meningococcal vaccines, especially Group A, renders them unreliable as reference preparations. Accordingly, it is recommended that the molecular size of the polysaccharide(s) be measured by gel filtration on a standard lot of Sepharose 4B. *N*-acetylneuraminic acid, *N*-acetyl mannosamine and phosphorus reference preparations available from WHO collaborating laboratories serve as references for colorimetric assays.

3. Improved vaccines for the prevention of meningococcal and *H. influenzae* type b meningitis should be developed particularly for use in infants and children in whom the available vaccines are not completely effective.

4. Vaccines for the prevention of meningococcal Group B meningitis should be developed.

5. The need for vaccines effective against Groups Y and W135 should be verified by epidemiological studies.

6. Since the occurrence of reactions, especially fever in young infants, is related to the endotoxin content of meningococcal vaccines, efforts should be made to remove these toxic components. Methods for the specific detection of endotoxin, such as chemical measurement of its unique components, should be developed.

7. The possibility of inducing "natural" immunization by colonization of infants with cross-reacting non-pathogenic enteric and/or nasopharyngeal bacteria should be studied.

8. Studies in animal models in order to understand better the meningococcal virulence factors should be developed, as well as studies on the effect of avirulent variants upon the acquisition of immunity.

9. The Group approved the proposed text for the "Requirements for meningococcal polysaccharide vaccine" and recommended that it be adopted.^a However, in order to further improve the vaccine, it would be useful to make arrangements for the workers actively engaged in research and in the production and testing of vaccines to meet and discuss the results of further studies at an appropriate time and place in 1976.

Serogrouping and serotyping

10. More reliable serogroup antisera must be made available. Efforts should be made to adapt uniform methods for immunization of animals. For the major serogroups, the prototype strains are recommended. In a number of laboratories, the following strains have been used for vaccine preparation and for polysaccharide characterization: Group A—M1027, Group B—B11, Group C—C11.

Further study is needed to identify suitable reference prototype strains for all groups. It is recommended that reference antisera for serogrouping be made available by the WHO Collaborating Centre for Reference and Research on Meningococci.

^a Note added during editing: The "Requirements for meningococcal polysaccharide vaccine" have since been considered by the Twenty-seventh WHO Expert Committee on Biological Standardization and the approved text will be published shortly in *WHO Technical Report Series*, 1976, No. 594.

11. The value of antisera produced against the purified group-specific polysaccharides for serogrouping should be explored.

12. Collaborative studies should be conducted to evaluate the reliability of the antiserum agar technique by comparison with the available agglutination techniques for serogrouping.

13. More extensive use should be made of the serotyping techniques recently developed to better characterize meningococcal isolates from patients and asymptomatic carriers and to define the geographical distribution of serotypes.

Sensitivity to antimicrobial agents

14. The test for sulfonamide and antibiotic sensitivity of *N. meningitidis* and *H. influenzae* type b should be standardized. For clinical purposes 50 mg/litre (50 µg/ml) should be considered as the threshold of sulfonamide resistance. The origin and patterns of sulfonamide resistance should also be investigated.

Surveillance

15. Efforts should be intensified to improve laboratory facilities and to strengthen epidemiological surveillance in order to monitor and rapidly report trends in the epidemiology of cerebrospinal meningitis and, accordingly, plan control measures. To this end, studies to define, evaluate and standardize serological tests and tests for determination of the sensitivity of *N. meningitidis* to antimicrobial agents are indicated.

ACKNOWLEDGEMENTS

The Group acknowledges the special contributions made to its deliberations by the following: Dr J. Bond, WHO Regional Office for the Americas/Pan American Sanitary Bureau, Washington, DC, USA; Dr B. Bytchenko, Bacterial Diseases, WHO, Geneva, Switzerland; Dr G. Causse, Venereal Diseases and Treponematoses, WHO, Geneva, Switzerland; Dr L. Lapeyssonnie, WHO Regional Office for the Eastern Mediterranean, Alexandria, Egypt; Dr M. Radovanović, WHO Regional Office for Europe, Copenhagen, Denmark; and Professor A. B. Sabin, Medical University of South Carolina, Charleston, SC, USA.

**WORLD HEALTH ORGANIZATION
TECHNICAL REPORT SERIES**

Recent reports :

No.		Sw. fr.
545	(1974) Pesticide Residues in Food Report of the 1973 Joint FAO/WHO Meeting (42 pages)	5.—
546	(1974) Assessment of the Carcinogenicity and Mutagenicity of Chemicals Report of a WHO Scientific Group (19 pages)	4.—
547	(1974) The Planning of Medical Education Programmes Report of a WHO Expert Committee (25 pages)	4.—
548	(1974) Planning and Organization of Geriatric Services Report of a WHO Expert Committee (46 pages)	5.—
549	(1974) WHO Expert Committee on Malaria Eradication Sixteenth Report (89 pages)	7.—
550	(1974) Fish and Shellfish Hygiene Report of a WHO Expert Committee convened in cooperation with FAO (62 pages)	6.—
551	(1974) WHO Expert Committee on Drug Dependence Twentieth Report (89 pages)	7.—
552	(1974) WHO Expert Committee on Tuberculosis Ninth Report (40 pages)	5.—
553	(1974) Ecology and Control of Rodents of Public Health Importance Report of a WHO Scientific Group (42 pages)	5.—
554	(1974) Health Aspects of Environmental Pollution Control: Planning and Implementation of National Programmes Report of a WHO Expert Committee (57 pages)	6.—
555	(1974) The Use of Mercury and Alternative Compounds as Seed Dressings Report of a Joint FAO/WHO Meeting (29 pages)	5.—
556	(1974) Detection of Dependence-Producing Drugs in Body Fluids Report of a WHO Meeting of Investigators (50 pages)	5.—
557	(1974) Evaluation of Certain Food Additives Eighteenth Report of the Joint FAO/WHO Expert Committee on Food Additives (37 pages)	5.—
558	(1974) Community Health Nursing Report of a WHO Expert Committee (28 pages)	4.—
559	(1974) New Approaches in Health Statistics Report of the Second International Conference of National Committees on Vital and Health Statistics (40 pages)	5.—
560	(1975) Chemical and Biochemical Methodology for the Assessment of Hazards of Pesticides for Man Report of a WHO Scientific Group (26 pages)	6.—
561	(1975) Ecology and Control of Vectors in Public Health Twenty-first Report of the WHO Expert Committee on Insecticides (35 pages)	6.—
562	(1975) Services for Cardiovascular Emergencies Report of a WHO Expert Committee (129 pages)	10.—
563	(1975) Guidelines for Evaluation of Drugs for Use in Man Report of a WHO Scientific Group (59 pages)	7.—