THE UNIVERSITY OF MANITOBA

"A STUDY OF THE EPIDEMIOLOGY OF MENINGOCOCCI IN FAMILIES IN MANITOBA"

BY

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ABSTRACT

A study was made of the epidemiology of the meningococcus in families in Manitoba. This included a determination of (1) the distribution of cases, (2) the risk of infection for individuals at various levels of association with an index case, (3) the effect of minocycline and sulfisoxazole on the carrier state, and (4) a description of antibody response following meningococcal disease as measured by the indirect hemagglutination test.

Meningococcal disease occurred with increased frequency among the native Indian population and low income families. The highest incidence of cases occurred in March, April and May in children under the age of two years.

High carriage rates were observed in the families of cases, however, no increased risk of infection was demonstrated for nursing staff or students who are associated with cases or carriers of meningococcus.

Minocycline or sulfisoxazole was given to 69 asymptomatic carriers of N. meningitidis. Nasopharyngeal cultures, taken at regular intervals following therapy, showed minocycline to be effective in eradicating the carrier state in 75 per cent of the study group. This was the same rate of reduction achieved when sulfisoxazole was used to treat carriers of sulfonamide sensitive strains of meningococcus. The presence of resistant strains in this community, further reduces the effect of sulfisoxazole.

Hemagglutinating antibody titres were determined in sera of patients with bacteriologically confirmed meningococcal disease. The indirect hemagglutination test was group specific, and sensitive enough to detect an antibody response in 75 per cent of the convalescent sera.

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I. INTRODUCTION

The first description of what was probably meningococcal disease in epidemic form was reported in Geneva, Switzerland by Vieusseux in 1805 and in North America at Medfield, Mass. in 1806. Outbreaks of disease were reported in Western Europe and United States during the next 75 years, which, on this basis of clinical and epidemiological data, may be presumed to be meningococcal infection. (Feldman, 1966)

Since Weichselbaum's isolation and description of Neisseria meningitidis in 1887, sporadic cases as well as epidemics of documented meningococcal disease have occurred throughout the temperate and tropical areas of the world.

In North America, peaks in incidence of cases have occurred in 1918, 1929, 1936 and 1945. The high rates reported in 1918 and in 1945 were extensions of pandemics of meningococcal disease that occurred during World War I and again during World War II. Since 1953, the incidence of meningococcal disease in the United States has been at a steady low level, and no major epidemics have occurred during this time. Annual incidence for the epidemiological years 1960 - 1961 through 1968 - 1969 varied from a low of 1.18 per 100,000 population to a high of 1.77 per 100,000 population. (Bennett et al, 1969).

Similar rates have been reported for Canada, During the 10 year period from 1960 to 1969, a yearly incidence of less than one

per 100,000 population was reported. (Notifiable Diseases, 1970) During those ten years the incidence of meningococcal disease in the province of Manitoba paralleled that of the rest of Canada with 3 to 11 cases reported each year. (Table I) However, in 1970 it became evidence that Manitoba was experiencing an increased incidence of meningococcal disease. Thirty-eight cases were reported representing an annual incidence almost four times greater than rates reported during the previous 10 years. (Ronald et al, 1971) The incidence for Canada for 1970 was one per 100,000 population. It appeared that the increase in cases in Manitoba was not being experienced elsewhere in Canada.

Preliminary identification of some of the strains of Neisseria meningitidis isolated in this province revealed a second disturbing fact: more than half of the isolates were identified as serogroup A. This group has not been prevalent in North America for more than 20 years and has, historically at least, been associated with all major epidemics of meningococcal disease. (Ronald et al, 1972) In view of this information, a study of the epidemiology of the meningococcus in Manitoba appeared to be warranted.

Reports that have appeared while our study was in progress indicate that this trend toward an increased incidence of disease is now being experienced elsewhere in Canada. Newfoundland (Severs, 1972), Ontario (Joshua, 1972) as well as British Columbia (Notifiable Diseases, 1972) have all experienced a rise in the incidence of meningococcal

TABLE I

MENINGOCOCCAL DISEASE IN CANADA AND THE PROVINCE OF MANITOBA*

Year	Canada	Rate/100,000	Manitoba	Rate/100,000
1960	158	0.9	9	1.0
1961	120	0.7	6	0.7
1962	110	0.6	11	1.2
1963	111	0.6	2	0.2
1964	115	0.6	9	0.9
1965	88	0.4	6	0.6
1966	85	0.4	5	0.5
1967	105	0.5	3	0.3
1968	96	0.5	9	0.9
1969	153	0.7	8	0.8
1970	205	1.0	38	3.9
1971	209	1.0	61	6.3

* from Notifiable Diseases, 1970.

disease. The cumulative totals of 1972 over 1971 for Canada show more than a 200 per cent increase.

Unfortunately, serogrouping of all isolates had not been performed routinely in other provinces, making it impossible to determine the degree to which serogroup A was responsible for this increase. One identification of group A meningococcus in Ontario (Severs, 1972) suggests that it has not been confined to the province of Manitoba.

As the incidence of meningococcal disease increases, the continual problems of management of individuals exposed to a case and of eradication of the nasopharyngeal carrier state become apparent. One of the objectives of this study was to provide information to serve as a guideline in these instances. This included an assessment of the risk of infection, as well as an evaluation of the effectiveness of current prophylactic therapy.

A limitation in any study concerned with the epidemiology of the meningococcus is the lack of bacteriological confirmation of infection in many instances. A technically simple test, that is serogroup specific and sensitive enough to identify the etiology of a clinical case or detect the asymptomatic carrier of meningococcus, would be an invaluable laboratory tool. A second objective of this investigation was to evaluate the indirect hemagglutination test as an epidemiological method in the study of group A meningococcal infection in this province.

During the course of this study the areas of investigation have been:

1. a study of the distribution of meningococcal disease in Manitoba
2. a determination of the risk of infection for individuals at various levels of association with the proband
3. a comparison of the efficacy of sulfisoxazole and minocycline in eradicating the carrier state
4. an evaluation of the indirect hemagglutination test in terms of its usefulness in detecting systemic and/or nasopharyngeal infection with group A meningococcus.

II. REVIEW OF THE LITERATURE

SEROLOGICAL GROUPS OF MENINGOCOCCUS

According to Branham (1953) serological differences among meningococci were first reported by Dopter in 1969. In 1915, Gordon and Murray separated the meningococci isolated from spinal fluids into Types I, II, III, and IV. Later it became evident that there was no clear distinction between Type I and III. During 1935-7, a previously unrecognized group of meningococci became common in the United States. Branham et al (1942) designated these strains Group II alpha. The classification of meningococcus was reviewed in 1950 by a Subcommittee of the Nomenclature Committee of the International Association of Microbiologists. These serogroups are currently designated as Groups A, B, C, and D which corresponds to the previous classifications of Group I, II, II alpha, and IV respectively, which had been in common use since 1940.

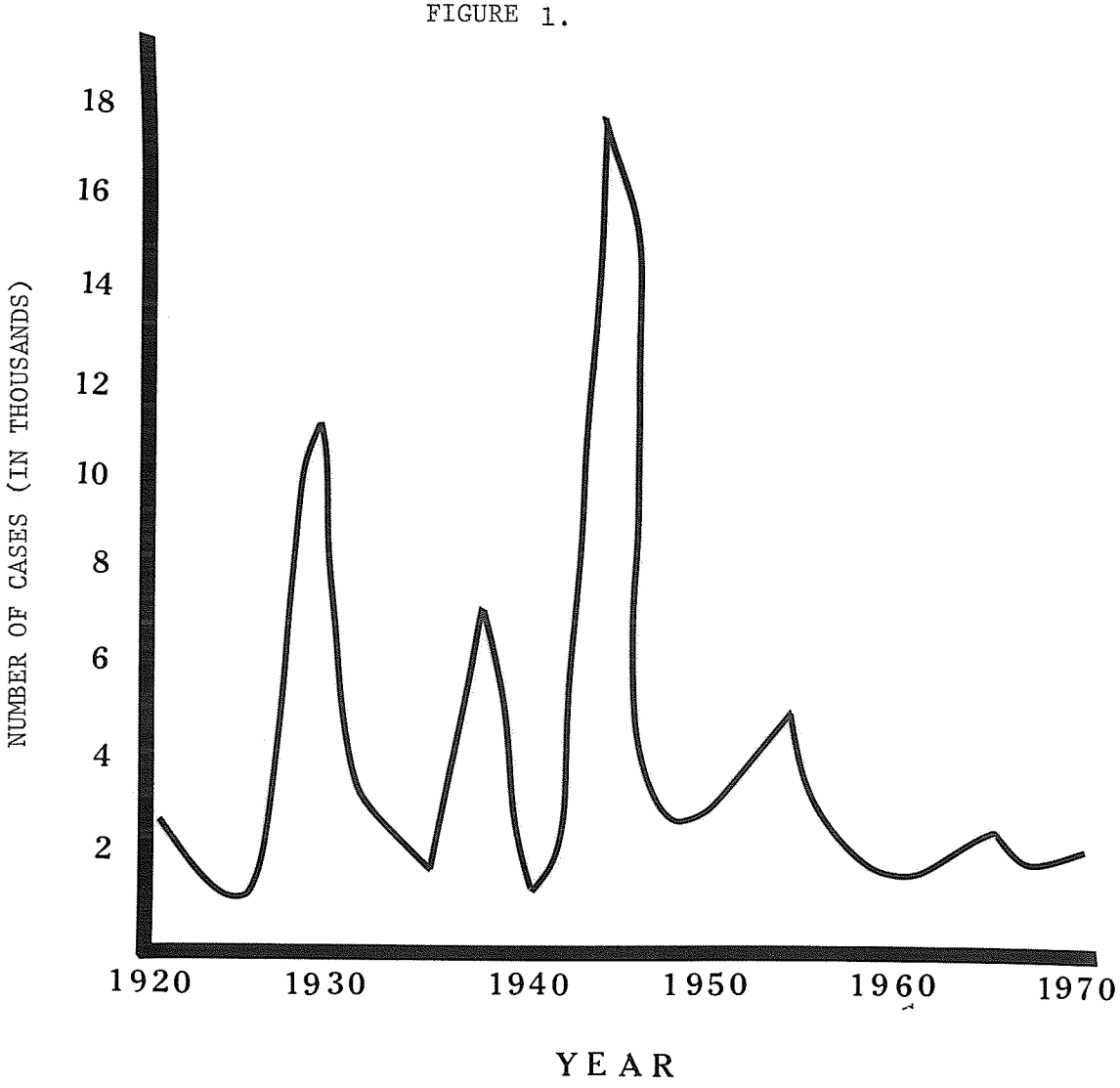
These four groups (Groups A, B, C, and D) historically, have caused the majority of meningococcal disease. Several additional groups have been described by Slaterus (1961) and named X, Y, and Z. Evans et al (1967) recently described three new groups isolated from patients with systemic infection; these groups were designated B_o, 29E, and 135. Of the 843 meningococcal isolates studied by these authors. 4.3 per cent were attributed to one of these strains. B_o strain was shown to be similar if not identical to the Y strain of Slaterus.

Of these sero-variants, Group A has been associated primarily with major outbreaks of meningitis. With the mobilization of the U.S. forces in 1918, the disease appeared in epidemic proportions. During the First World War 2,466 military personnel were admitted to hospital in the United States with meningococcal disease. (Daniels, 1950). Branham et al (1937), in a study of the strains of meningococci recovered during this period, reported that Type I and III (Group A) represented 81.6 per cent of the strains studied.

Figure I shows the number of cases of meningococcal disease (in thousands) which occurred in the United States from 1920 to 1970, demonstrating peaks in incidence occurring in 1929, 1936, and 1942. The peak shown in 1929 reflects the massive epidemics beginning in 1928 and ending in 1931, that involved civilian populations particularly in the Detroit and Indianapolis areas. Branham et al (1942) reported on the sero-grouping of strains recovered from spinal fluid of patients during this epidemic as well as those recovered during the epidemic year of 1936. Eighty-one per cent of the strains studied in 1929 and 89 per cent of those studied in 1936 were Type I and III (now designated Group A).

Pandemics of meningococcal disease occurred during the war years 1939 to 1945. In the U.S., this epidemic created serious medical problems for both the civilian and military personnel. Kuhns et al (1943), in a study of the sero-group of 800 isolates from blood or spinal fluid, reported that 95 per cent were Group A. A particularly severe epidemic was experienced in Chile during this pandemic (1941-1942) that was attributed to Group A meningococcus. This epidemic

FIGURE 1.



CASES OF MENINGOCOCCAL INFECTIONS REPORTED IN THE UNITED STATES 1920 - 1970

was concentrated in the provinces of Santiago and Valparaiso. Attack rates of 188.1 per 100,000 population in Valparaiso and 261.6 per 100,000 population in Santiago were reported in 1942. The strains isolated in both cities were Group I and III. Groups II and IV were not found. (Pizzi, 1944).

In contrast to Group A, Group B and C are usually the predominant strains isolated during interepidemic periods. Branham et al (1937), observed that the relative incidence of Type II (Group B) was greater during the endemic years of 1932 - 1935. Additional studies of sporadic cases (Silverthorn et al, 1939; Cragg et al, 1959) and of smaller spidemics (Reed et al, 1966; Bristow et al, 1945; Gould et al, 1965) have indicated a preponderance of Groups B and C.

Since 1953 the incidence of meningococcal disease in North America has been at a steady low level and no major epidemics have occurred during this time. This is coincidental with a low level of Group A isolations. In the United States, Group A meningococcus accounted for less than 1 per cent of the total isolates recorded by the National Communicable Disease Centre in Atlanta, Georgia from 1966 - 1969 (Bennett et al, 1969). In other parts of the world, however, Group A strains have continued to be responsible for epidemics of meningococcal disease; this is particularly evident in Africa (Lefèvre et al, 1969).

The majority of the cases reported in the United States since 1950 have been due to Group B or Group C meningococcus. From 1950

to 1965, approximately 90 per cent of the strains isolated were Group B. However, Group C has now become the predominant strain with 90 per cent of the cases in 1969 being attributed to Group C strains. Although there has been a change in the predominant sero-group, there has been no recorded change in the incidence of meningococcal disease (Bennett et al, 1969).

An increased incidence of meningococcal disease in Manitoba combined with the fact that more than half of the strains identified have been sero-group A, is very disturbing in view of the past history of Group A meningococcus.

NASOPHARYNGEAL CARRIAGE OF THE MENINGOCOCCUS

In addition to the serological types, the epidemiology of this organism is further complicated by the fact that it infects many, but produces disease in relatively few. Artenstein and Gold (1970) have suggested that for sero-groups B and C, the ratio of cases to carriers may be as low as one case for every 1000 aquisions of meningococcus. Greenfield et al (1971), in a study carried out in the Syracuse area found the point prevalence of carriers to range from 4.9 per cent to 10.6 per cent; the median duration of carriage was determined to be 9.6 months. This data was obtained from a population of "normal" families, and during the study period there were no cases of meningococcal disease in any of the study homes. Rake et al (1934) studied a group of individuals working at the Rockerfeller Institute in New York City. The 10 carriers discovered in this group were

described as falling into three categories: chronic, intermittent, and transient carriers. Half of these carriers carried the same sero-group for more than 2 years.

A number of investigations have been carried out on civilian populations when cases of Group B disease were present. Silverthorn et al (1939) reported that 31 per cent of the immediate family of a case carried meningococcus in the nasopharynx. The majority of strains isolated in this study from cases as well as contact carriers were identified as Type II (Group B). A similar study by Cragg et al, (1959), concluded that meningococcal meningitis in children is associated with a high carrier rate among contacts. Seventeen of the 18 cases in this study were identified as being Group II (Group B).

Studies of meningococcal carriage among military personnel have been extensive for both epidemic and non epidemic periods (Aycock et al 1950; Reed et al, 1966; Bristow et al, 1945; Millar et al, 1963; Farrell et al, 1966). Military populations however, differ significantly from the civilian population in terms of distribution of sex, age, and in the type of housing they occupy. Consequently, results obtained from these investigations will not necessarily apply to civilian populations.

There have been no reports of studies on civilian populations during an interepidemic period when cases of Group A were prevalent.

PROPHYLAXIS

From the late 1930's, when they were first introduced, to 1963, sulfadiazine remained the drug of choice for the treatment of meningococcal disease. Even after the discovery of penicillin, sulfadiazine remained the drug of choice for the eradication of the carrier state and for use prophylactically. During World War II, Kuhns et al (1943) demonstrated that sulfadiazine, when administered as a mass prophylactic during an epidemic, dramatically reduced the carrier rates as well as the appearance of new cases. However, in 1963, Millar et al (1963) reported on a concentration of cases due to sulfadiazine resistant organisms. This outbreak occurred at the U.S. Naval Training Centre in San Diego, and was attributed to sero-group B sulfadiazine resistant strains. Since that time there have been an increasing number of reports of sulfadiazine resistance.

In the past four years in the United States, roughly half of all Group B strains isolated at the National Communicable Disease Centre at Atlanta, Georgia have required concentrations in excess of 1.0 mg./100 ml. of sulfadiazine to inhibit growth. Group B isolates are now outnumbered by strains belonging to group C. The proportion of sulfonamide resistant group C organisms has increased from 15 per cent in 1966 to approximately 90 per cent in 1969. (Bennett et al, 1969).

Epidemics of sulfonamide resistant Group A infections have occurred in Africa in recent years. In a meningitis epidemic in

Greece in 1968 (Vassiliadis et al, 1969), 90 per cent of the isolates were Group A, only 3 per cent of which were sensitive to 1 mg./100 ml. of sulfadiazine. An epidemic in Meknes, Morocco, which involved several thousand cases, was attributed to Group A meningococcus. (Alexander et al, 1968). Ninety per cent of the strains were resistant to 1 mg./100 ml. of sulfadiazine. Isolates of sero-group A in the United States, with one exception (Clark et al, 1971), have never been found to be resistant to sulfonamides.

These reports of sulfonamide resistance have prompted a search for an alternate form of treatment. Although penicillin is highly successful in the treatment of disease, it has proven to be ineffective in the management of contacts of cases. Decelopment of disease following several days of oral penicillin has been reported (Tobin 1956). Neither penicillin or oxytetracycline were effective in aborting the epidemic in 1963 at the naval station in San Diego (Millar et al, 1963). Dowd et al (1966) evaluated four antibiotics (penicillin, erythromycin, ampicillin and oxytetracycline) for their effectiveness in eradicating sulfadiazine resistant meningococci from the nasopharynx. These authors found none of the four antibiotics to be completely successful. All showed some degree of suppression but failed to eradicate the organism from the nasopharynx.

Recently, a new antibiotic, rifampin (3-(4-methylpiperazinyl minomethyl) rifamycin SV), has been considered as superior to other currently available drugs in the eradication of meningococcus from the

nasopharynx. Deal et al (1969) administered 600 mg. of rifampin daily to 15 unknown carriers of meningococcus. During the study period, rifampin reduced the carrier rate by 93.3 per cent. Devine (1970) and Guttler (1971), using larger study groups, reported an 84 per cent reduction in carriage rate during therapy. However, the high frequency of rapidly emerging rifampin resistant strains following therapy (Guttler et al, 1971) has dampened the enthusiasm for mass prophylaxis with this drug.

Minocycline, a semisynthetic derivative of tetracycline, was reported to reduce the carrier rate by 84 per cent in two separate studies (Devine et al, 1970; Guttler et al, 1971). There were no minocycline resistant strains encountered by these authors following prophylaxis with this drug.

Indications are that minocycline may prove to be a useful prophylactic agent. Further studies are necessary to determine the duration of its effect as well as its effect on a wider range of meningococcal sero-groups. Studies of minocycline carried out by Guttler and Devine used populations in which Group Y and non-groupable meningococci were the predominant strain carried. Neither of these studies included carriers of sero-group A.

IMMUNOLOGY OF THE MENINGOCOCCUS

Rake and Scherp (1933) described a number of antigenic determinants associated with the meningococcus. These included three fractions; a carbohydrate which these authors designated as "C" substance found to be common to all meningococci, a protein present

in all meningococci as well as a number of other organisms, and a third fraction found to be a group-specific polysaccharide.

From 1943 to 1963, it appeared that meningococcal infections were managed well with sulfonamides and investigators showed little interest in the immunology of this organism. However, in 1963, the emergence of sulfonamide resistant strains stressed the need for further information in this area.

Goldschneider et al (1969_a) proved conclusively that meningococcal disease was related to a lack of humoral antibody to the pathogenic strains of meningococcus. Goldschneider et al (1969_b) also described the role of asymptomatic nasopharyngeal infection in the development of immunity to disease. Carriers of meningococci were shown to develop bactericidal activity against the sero-group of meningococci carried in the nasopharynx and, in addition, these individuals were shown to develop appreciable bactericidal activity against heterologous strains. Another significant event in the development of the understanding of the immunology of the meningococcus was the extraction and purification of specific Group A and Group C high molecular weight polysaccharides (Gotschlich et al, 1969). This method used the cationic detergent Cetavlon to precipitate polysaccharide from overnight cultures of meningococci.

The extraction of the group specific polysaccharides, combined with the indirect hemagglutination test as described by Edwards and Driscoll (1967), formed the basis for sensitive and highly specific measurement of meningococcal antibody. Artenstein et al (1971), using

the indirect hemagglutination test and purified meningococcal polysaccharides as antigens, was able to detect group specific antibodies following systemic infection. This investigation included five patients with meningococcal disease in which Group C organisms were isolated from the spinal fluid and one patient whose illness was caused by a Group Y strain. The patients with Group C disease all showed a significant rise in hemagglutinating antibody titre. The patient with Group Y disease, showed no antibody change using the A, B, and C antigens. Eickoff (1971),also reported a correlation between recent infection and a rise in homologous antibody. This was demonstrated to be specific in the detection of antibody following Group C associated systemic disease and asymptomatic carriers of Group Y meningococci.

There have been no reports of the sensitivity or specificity of the indirect hemagglutination test in detecting homologous antibody following natural infection with Group A organisms.

III. MATERIALS AND METHODS

COLLECTION OF CLINICAL MATERIAL

Posterior nasopharyngeal specimens were obtained with a commercially prepared calcium alginate-tipped swabs bent to a 45^{o} angle. Swabs which were contaminated with oral secretions or which did not reach the nasopharynx were discarded. Swabs were inoculated directly onto Thayer-Martin plates. The plates were kept warm until they were transferred to a CO_2 incubator at $35^{o}C.$, generally within two hours.

Blood specimens were obtained at intervals from patients with systemic infection and from asymptomatic nasopharyngeal carriers. Samples were drawn, allowed to clot at room temperature for one hour, then refrigerated overnight. Sera was removed the following day and stored at $-20^{o}C$ until the serological tests were performed.

LABORATORY METHODS

Nasopharyngeal specimens were plated onto Thayer-Martin medium. (Martin et al, 1967).

Method of Preparation of Media

1. Double strength base was prepared by suspending 7.2 grams of GC Medium Base in 100 ml of distilled water. This was boiled for one minute to assure solution of ingredients.
2. Two grams of Bacto Haemoglobin (Difco) were suspended in 100 ml. of distilled water and thoroughly mixed using a magnetic mixer.
3. GC Medium Base and hemoglobin suspension were sterilized by autoclaving at $121^{o}C$ for 15 minutes, then cooled to $50^{o}C$.

4. Haemoglobin, two ml. of Isovitalex Enrichment (Difco) and two ml. of V-C-N antibiotic combination, were aseptically added to the sterile base. The antibiotic combination consisted of 300 mcg. Vancomycin, 750 mcg. colistin and 1250 mcg. nystatin /100 ml. of GC base.
5. These were thoroughly mixed and dispensed into petri dishes.

After incubation for 18 to 24 hours at 35°C and 6 per cent CO_2, colonies were selected and subcultured onto blood agar. Organisms that were morphologically consistent with meningococcus were tested for the ability to ferment glucose, maltose, sucrose and lactose. Fermentation tests were carried out using CTA medium (BBL) with 1 per cent carbohydrate and incubation at 35°C.

Organisms that fermented glucose and maltose, but not sucrose or lactose were sero-grouped using A, B, C, D, X, Y, and Z antisera.

Antisera were obtained from Difco Laboratories and from the National Communicable Disease Centre, Atlanta, Georgia.

Gram negative diplococci, with colony morphology resembling Neisseria meningitidis and that fermented glucose and maltose, but not sucrose or lactose and that failed to grow at 22°C, were considered to be meningococci independent of their agglutination in specific antisera.

ANTIBIOTIC SENSITIVITY TESTING

The minimum inhibitory concentration of sulfisoxazole, rifampin, minocycline, and penicillin G was determined for 81 strains of meningococcus using a modification of the agar dilution method described

by Bennett (1966).

Materials

Rifampin (Dow Chemical of Canada) was dissolved in methanol to give a concentration of 10,000 mcg. of rifampin activity per ml. This stock solution was serially diluted with sterile phosphate buffer (Sorensen's) pH 7.2 - 7.4. Final concentration used in test plates were two-fold dilutions ranging from 1.0 to 0.002 mcg per ml.

Sulfisoxazole was serially diluted to yield final concentrations of 0.1, 0.5, 1.0, 2.0, 5.0, 10, 20 mg per ml. per test plate.

Penicillin G and minocycline were tested in two-fold dilutions ranging from 1.0 to 0.002 mcg. per ml.

Method

Mueller-Hinton broth was inoculated with organisms from an overnight culture grown on a blood agar plate. The broth was incubated in a shaking waterbath at 35°C for 18 hours. This suspension was diluted, if necessary, with sterile distilled water to a density visually equivalent to that of a standard prepared by adding 0.5 ml. of 1 per cent $BaCl_2$ to 99.9 ml of 1 per cent H_2SO_4.

Using a Steers-Foltz replicator, the diluted cultures were applied to the surface of a series of plates containing various concentrations of antibiotics. Control plates containing Mueller-Hinton Agar without drug were included with each series of antibiotics.

Plates were incubated in 6 per cent CO_2 at 35°C for 24 hours. The minimum inhibitory concentration was the lowest concentration of drug which produced an abrupt change from uninhibited growth to slight or no growth.

PASSIVE HI TEST FOR NEISSERIA MENINGITIDIS

Materials

The organisms used to prepare the crude antigen were Group A and Group C meningococcus isolated from the CSF of two patients admitted to the Winnipeg Children's Hospital with meningococcal disease while this study was in progress.

Diluent	Phosphate Buffered Saline (PBS) 0.01 M pH 7.2	
$NaH_2PO_4.HO$	0.233	g/l
$NaHPO_4$	1.165	g/l
NaCl	8.1	g/l

Sheep cells collected in Alsever's solution were obtained from BBL and stored at $6^{o}C$.

Method

1. Preparation of meningococcal polysaccharide crude antigen 4.5 ml. of Mueller-Hinton broth was inoculated with 0.5 ml. of fresh inoculum (culture check for purity), and incubate overnight. Next morning 50 ml. of Mueller-Hinton broth is inoculated with 5 ml. of the overnight culture, incubated in a shaking waterbath at $37^{o}C$ for 6 hours. Flasks were then culture checked for purity. 0.73 ml. of Formaldehyde was added to the flasks; the flasks were then incubated in the shaking waterbath for an additional 15 minutes. The flasks were then culture checked for killing of organisms. The broth was then centrifuged at 2000 rpm for 15 minutes, the supernatant (the antigen) was removed and stored at $4^{o}C$.

2. Treatment of Sera

All sera (including normal rabbit serum and antiserum) was inactivated at 56°C for 30 minutes. All sera was absorbed with 0.2 ml. of washed packed sheep cells per ml. of serum, for 1 hour at room temperature, then centrifuged.

3. Sensitization of Cells

To 1.0 ml. of polysaccharide antigen at the correct dilution was added 0.5 ml. of a 2 per cent suspecsion of saline washed sheep cells. The mixture was incubated in the 37°C waterbath with occasional shaking. Sensitized cells were sedimented by centrifugation, washed three times in saline and reconstituted in 2.5 ml. of 1:200 normal absorbed rabbit serum in 0.01 M phosphate buffered saline.

4. Microtiter

0.025 ml. of 1:200 normal absorbed rabbit sera in 0.01 M phosphate buffered saline was added to each well of a Microtitre "U" plate (Cooke Engineering Co.) 0.025 ml. of serum was serially diluted by two-fold dilutions. 0.025 ml. of diluent was added to each well. 0.025 ml. of sensitized sheep cells were added to each well. Plates were rotated gently, covered and allowed to stand at room temperature until cell controls (containing 0.05 ml. of 1:200 normal absorbed rabbit sera in 0.01 M phosphate buffered saline and 0.025 ml of sensitized sheep cells) formed buttons (approximately 2 hours). The antibody titre was considered to be the reciprocal of the

highest dilution showing definite agglutination.

5. Titration of Crude Antigen

In order to determine the optimum sheep rbc sensitizing concentration of the antigen preparation, varying concentrations of the antigen ranging from undiluted to 1:200 were used to sensitize sheep cells. Grid titrations were set up in Micro "U" plates with homologous meningococcus antisera (Difco). Maximum dilution yielding maximum sensitization of sheep cells was found to be 1:20 for both A and C antigens. To assure adequate sensitization of cells 1:10 dilution of antigen was used in the test.

6. Controls

Controls used for all tests included unsensitized red cells with positive sera, sensitized red cells with normal and immune sera, and a cell control which consisted of sensitized sheep cells and diluent.

RADIOACTIVE ANTIGEN BINDING TEST

Quantitative determinations of antibody concentration were made using the radioactive antigen binding test. These tests were performed by Dr. E. Gotschlich at the Rockefeller University in New York, using group A polysaccharide antigen and the method of Gotschlich et al (1972).

IV. DISTRIBUTION OF CASES

The increased incidence of meningococcal disease, combined with the recent appearance of a number of clinical cases of group A meningitis in this province, has suggested the need for epidemiological deliniation of meningococcal disease in Manitoba. This portion of the study was undertaken to determine the risk of disease in terms of the attack rate for various geographical areas of the province as well as a determination of the effects of age, sex, ethnic origin, and socio-economic level on the incidence of meningococcal disease.

The area under study was the province of Manitoba. This province has a population of 1,018,535, approximately half of which live in the city of Winnipeg. Most of the remaining population is concentrated in the southern region of the province, with the large northern areas being very sparcely inhabited.

Epidemiological data for this study included all cases of meningococcal disease occurring in the province of Manitoba and having onset of illness in the period January 1, 1970 to May 31, 1972. Information on these cases was acquired from a number of sources;

1. Department of Health and Social Development, Preventive Medical Services
2. Medical record departments of hospitals in this province
3. Clinical Microbiology Research Laboratory, Winnipeg General Hospital

From these sources, 135 clinical cases of meningococcal disease were recorded. A review of hospital charts and laboratory records provided information on the bacteriological confirmation of the

etiology of these infections. The Cadham Laboratory, which provides much of the bacteriological service for the rural areas, supplied information on their isolates, as well as provided us with all strains that were submitted to them. In 1970, the Clinical Microbiology Research Department of the Winnipeg General Hospital requested that isolates of meningococcus in this province be sent to their laboratory where identification was confirmed and serogrouping was carried out. The majority of the strains submitted to the Winnipeg General Hospital during 1970 and 1971 were also sent to Dr. Harry Feldman, State University of New York, Syracuse, New York. The data on these isolates includes the results of these three laboratories in addition to our own bacteriological work with these strains that extended from Oct. 1, 1971 to May 31, 1972.

Of the 135 cases clinically diagnosed as meningococcal disease, 68 had bacteriological confirmation. (Table II) Paired sera was available for 19 of the 67 cases for which there was no bacteriological confirmation of etiology. Of these 19 cases, 13 had serological evidence of meningococcal infection as indicated by the indirect hemagglutination test (4-fold or greater rise in titre). It must be pointed out that sera was tested only for antibody to group A and group C. Group B associated infections were not identified with this method.

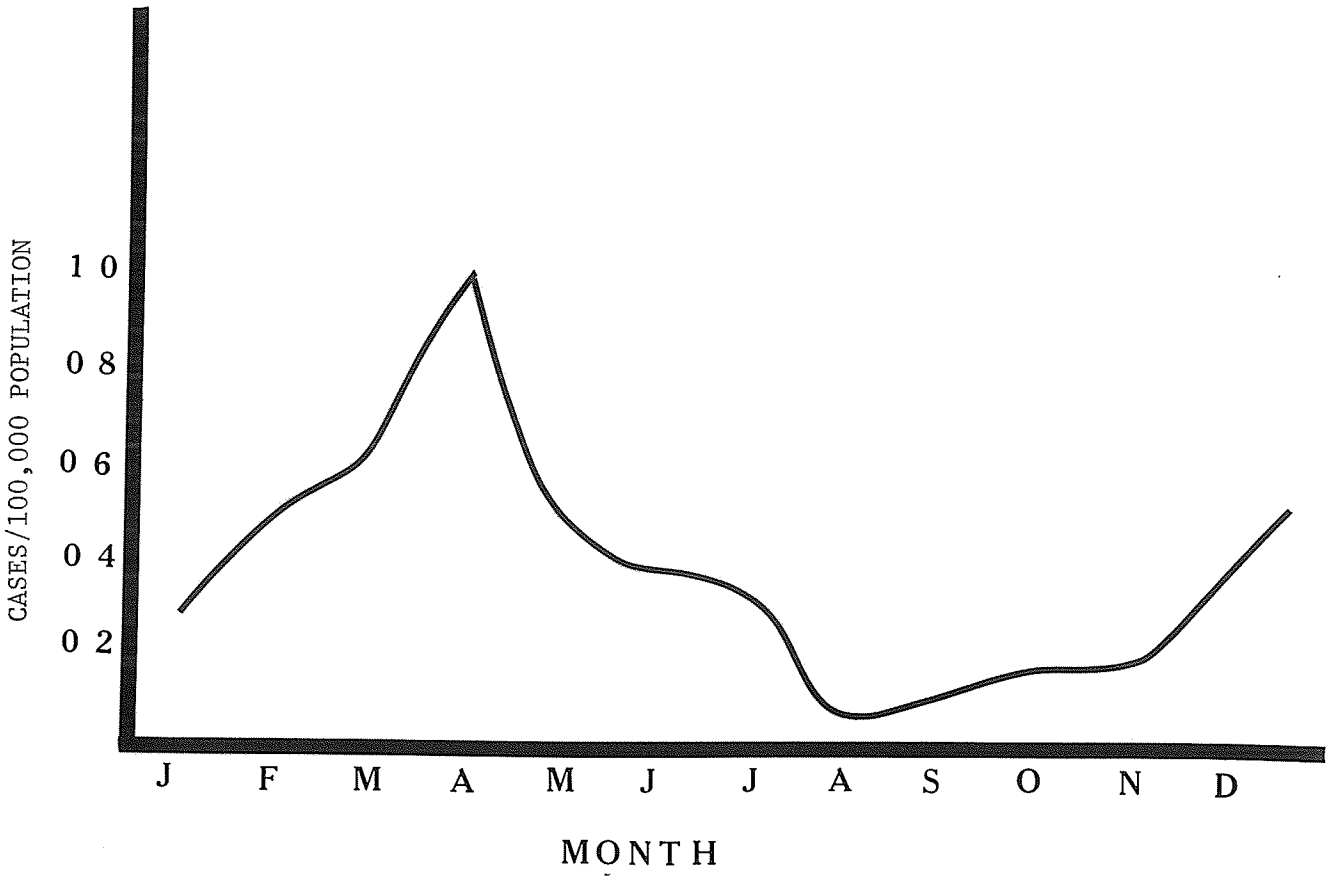
Meningococcal infections in Manitoba have shown a seasonal trend. The average monthly incidence of meningococcal disease is shown in Figure 2. The four months, February, March, April and May have shown

TABLE II

LABORATORY DATA ON 135 CASES OF MENINGOCOCCAL DISEASE WITH ONSET OF ILLNESS IN THE INTERVAL JANUARY 1, 1970 TO MAY 31, 1972

	1970	1971	1972 (Jan. 1 to May 31)
Sero Group A strains identified	5	23	9
Sero Group B strains identified	2	7	6
Sero Group C strains identified	0	9	7
1. Total number of cases with bacteriological confirmation	7	39	22
2. Total number of cases with serological evidence of Group A or Group C disease without bacteriological confirmation	1	8	4
3. Total number of cases with clinical diagnosis of meningococcal meningitis or meningococcal septicemia without serological or bacteriological confirmation	30	15	9

FIGURE 2.



AVERAGE MONTHLY INCIDENCE OF MENINGOCOCCAL CASES

FOR THE INTERVAL JAN. 1, 1970 TO MAY 31, 1972.

the highest average incidence with a peak in cases occurring in April. The lowest incidence of cases occurred in mid-summer with a gradual rise in incidence in the fall and winter months.

The age-specific rates for the 135 cases are shown in Figure 3. The highest incidence of cases is shown to have occurred in the 0 - 12 month age group, after which there is a progressive decrease in the attack rate. After age 15, the incidence of cases for all age groups remained below the incidence for the province. In the 20 to 70 year age span, the highest incidence of cases was noted in the 60 - 65 age group.

Table III summarizes the distribution of cases of meningococcal disease among the Indian and non-Indian population of Manitoba. The population of all Manitoba Indians is recorded as 31,526. This figure includes Indians who do not have association with a particular band and who are included in the population of rural municipalities, local district governments, cities, towns, villages or unorganized territories of residence. The distribution of cases has not been random for these two populations with the incidence of cases among Indians being from 10 to 20 times greater for each of the three years indicated.

From October 1, 1971 to May 31, 1972, meningococcal disease occurred in 24 families living in the Winnipeg area. These families were placed into categories depending upon the occupation of the head of the household. Table IV summarizes the data obtained on these 24

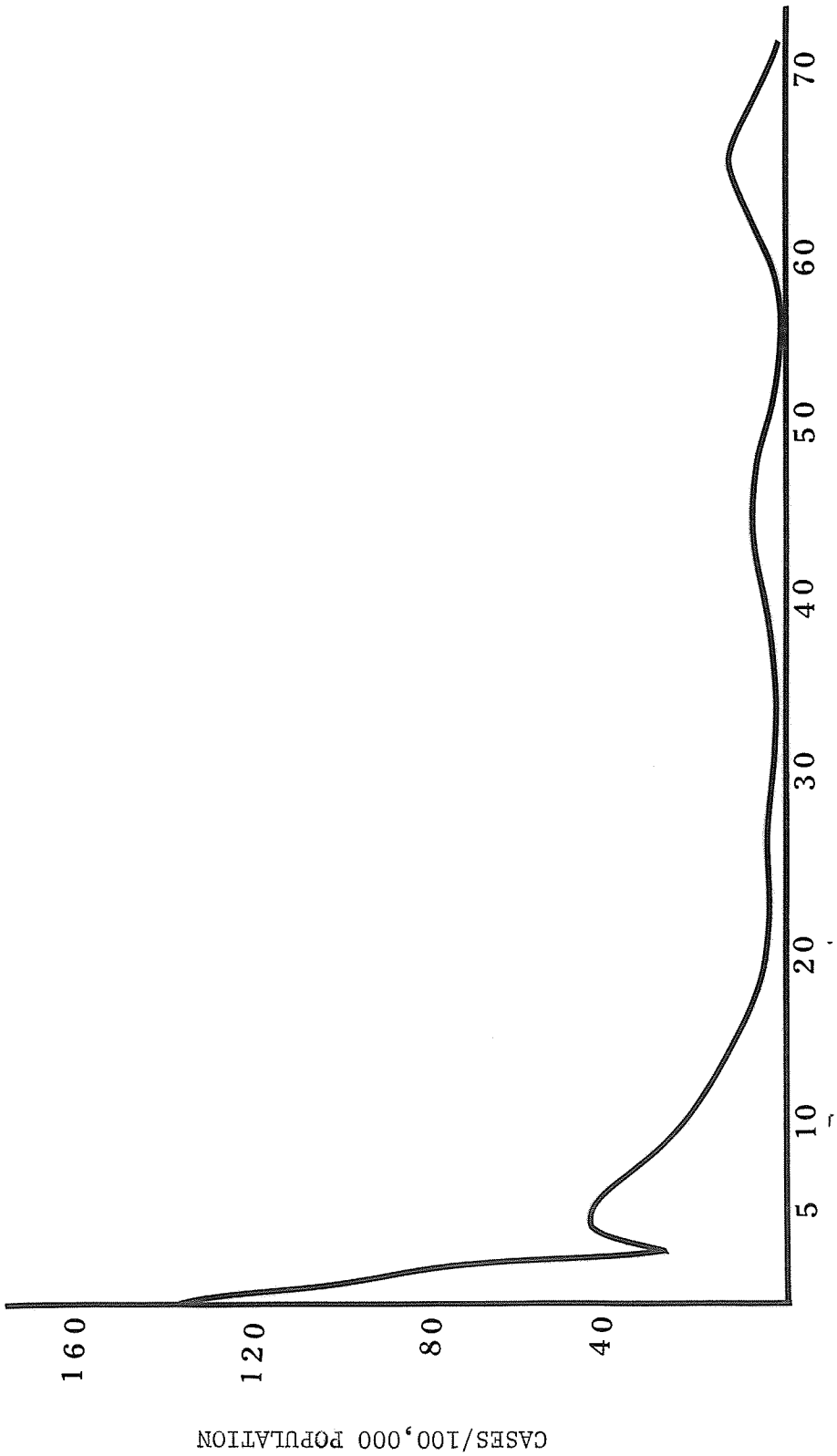


FIGURE 3. AGE RELATED MENINGOCOCCAL DISEASE ATTACK RATE Jan. 1, 1970 - May 31, 1972.

TABLE III

INCIDENCE OF MENINGOCOCCAL DISEASE AMONG INDIAN AND NON-INDIAN RESIDENTS OF MANITOBA

JANUARY 1, 1970 - MAY 31, 1972

	INDIAN		NON-INDIAN	
	cases	incidence*	cases	incidence
1970	15	47.57	23	2.33
1971	23	68.78	40	4.05
1972 (Jan. 1-May 31)	7	22.20	28	2.83

* cases per 100,000 population

population figures compiled by the Continuing Programs Secretariat Planning and Priorities Committee of Cabinet, Government of Man., in co-operation with the Health Services Commission.

TABLE IV

DISTRIBUTION BY OCCUPATIONAL GROUP OF ALL CASES

OF MENINGOCOCCAL DISEASE IN THE WINNIPEG AREA

OCTOBER 1, 1971 - May 31, 1972

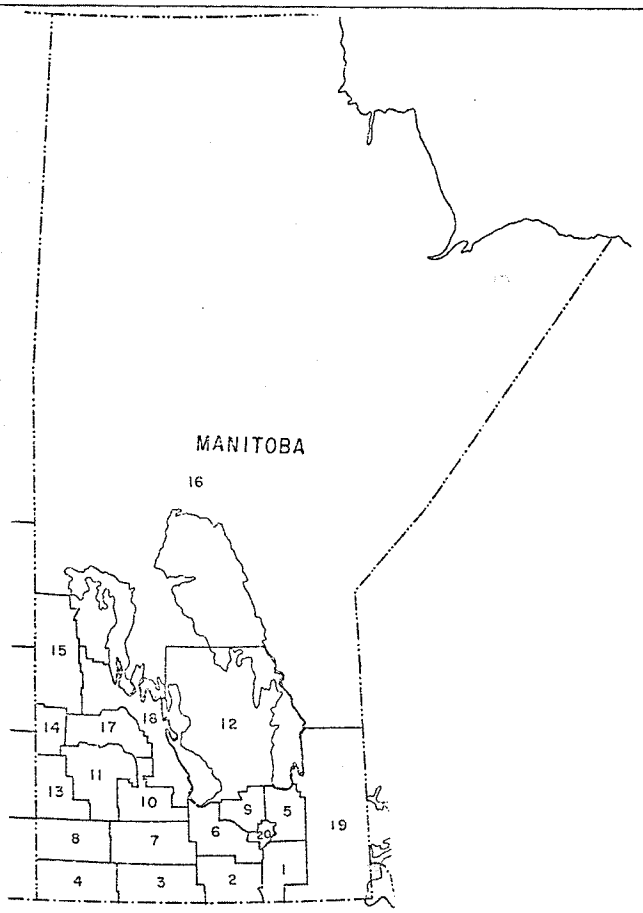
OCCUPATION OF FAMILY HEAD	NO. OF CASES	PER CENT OF CASES
GROUP I (welfare, unemployed)	13	54
GROUP II (labourer, semiskilled)	10	42
GROUP III (skilled, professional)	1	4

families. Group 1 included all individuals who indicated they were either unemployed or receiving welfare at the time of the patients' admission to the hospital. Group II included occupations that could be classed as semi-skilled or general labour. Group III included skilled and professional workers. Thirteen of the 24 cases, or 54% occurred in families in which the head of the household was either unemployed or receiving welfare. These cases occurred during a period in which not more than 6 per cent of the labour force in this province was unemployed. This data would indicate that meningococcal disease has not been randomly distributed among the families of the employed and the unemployed.

Figure 4 shows the incidence of 135 cases of meningococcal disease by census tract from January 1, 1970 to May 31, 1972. Those divisions not listed in the table had no reported cases during this interval. From this data it is apparent that census division 16 was the area of highest incidence during 1970. In 1971, the incidence of cases in division 12 rose from 7.3/100,000 population to 63.9/100,000 population. The other area showing a high rate of infection during 1971 was division 15. For the first five months of 1972, division 16 continues to have a high incidence of disease. Although a number of areas have shown infection rates equal or slightly greater than the average for the province, the highest concentration of cases per population, has occurred in the three northern census divisions.

FIGURE 4

INCIDENCE OF MENINGOCOCCAL DISEASE BY CENSUS TRACT FOR RESIDENTS OF MANITOBA, JANUARY 1, 1970 - MAY 31, 1972.



CENSUS DIVISION	CASES			INCIDENCE*		
	1970	1971	1972	1970	1971	1972
No. 1	0	1	2	0	3.29	6.59
No. 5	0	2	1	0	6.10	3.05
No. 6	1	2	2	3.26	6.52	6.52
No. 10	1	0	0	5.31	0	0
No. 12	2	17	1	7.50	63.90	3.75
No. 13	0	0	1	0	0	8.84
No. 15	0	2	0	0	15.31	0
No. 16	26	6	9	37.57	8.66	13.00
No. 18	0	1	0	0	7.47	0
No. 19	0	1	1	0	5.15	5.15
No. 20	7	26	15	1.29	4.81	2.77

*cases per 100,000 population. Population of census division, 1971 census of Canada, Statistics Canada.

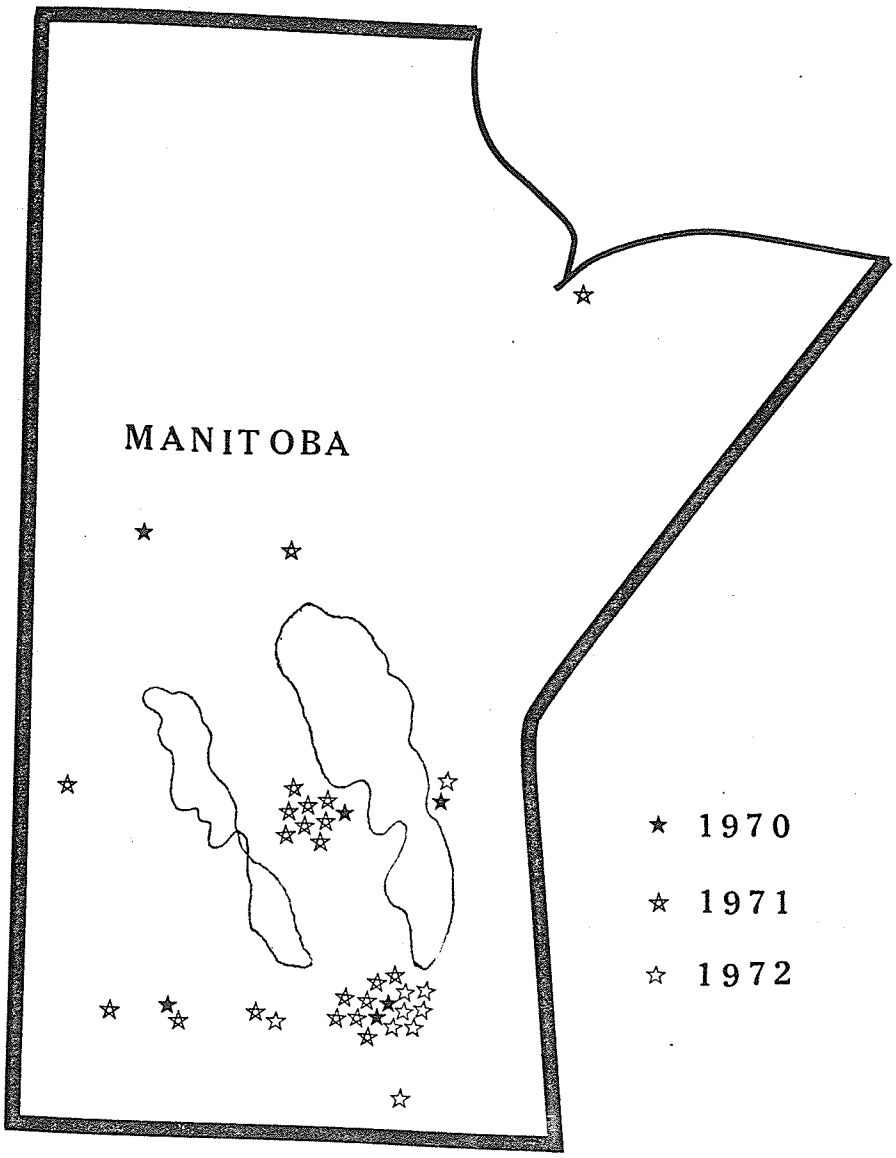
Figure 5 shows the geographical distribution of all group A isolates from January 1, 1970 to May 31, 1972. The majority of isolates from 1970 were not typed making an interpretation difficult, however, there appears to be a drift from north to south of this sero-group. In 1971, group A was isolated from various areas in northern Manitoba with two significant clusters, one in the Interlake District, in which all isolates for which the serogroup is known were group A, and a second cluster in the city of Winnipeg. The majority of the isolates in 1972 have been from the Winnipeg area.

DISCUSSION

The increase in meningococcal disease in this province cannot be attributed to any change in the age associated risk of disease. Similarly, the seasonal variation of disease resembles that of the United States. Bennett et al (1969) reported the peak of cases to occur in March, a month earlier than the peak in Manitoba cases, however, the difference in climate may account for this.

A significant point is the great difference in the incidence of disease among Indian, as contrasted to the non Indian segment of this province. Geographical data indicates that increased incidence of meningococcal disease has occurred primarily in the northern areas of the province where the majority of the Indian reserves are located. This may account in part for the disproportionate number of cases in this ethnic group. The cases observed in the native population in Winnipeg may be extensions of those occurring in the northern areas.

FIGURE 5.



GEOGRAPHICAL DISTRIBUTION OF ALL GROUP A CASES FOR MANITOBA, JAN. 1, 1970 - MAY 31, 1972

A disproportionate number of cases have appeared among the low income families. This point warrants further study for the determination of a more specific causal relationship.

V. NASOPHARYNGEAL CARRIAGE OF MENINGOCOCCUS

With the introduction of serogroup A meningococcus into the community as well as a rising incidence of meningococcal disease, the question of the risk of infection associated with exposure to a patient arises. Since meningococcal meningitis and septicemia are rare events compared to nasopharyngeal carriage, a determination of asymptomatic carriers among contacts of patients, serves as a better indication of the risk of infection than does a seach for association between cases. Nasopharyngeal carriage is considered to be a true infection (Feldman, 1966) accompanied by antibody response (Goldschneider et al, 1969).

The objective of this portion of the investigation was to determine infection rates as indicated by nasopharyngeal carriage of meningococci, among persons at various levels of association to the proband. The sampling frame was the city of Winnipeg, extended to include residents living within a 25 mile radius of the city.

Nasopharyngeal specimens were collected from all members of households in which a case of meningococcal disease occurred. Household contacts were defined as all persons who had slept in the home within three days prior to the patient's admission to hospital. The study included all bacteriologically confirmed cases occurring within the sampling frame from October 1, 1971 to June 1, 1972.

For the same eight months, using the same sampling frame, nasopharyngeal specimens were collected from all members of households in which non-meningococcal C.N.S. infections had occurred.

Household members were defined again as anyone who had slept in the home within three days prior to a patient's admission to the hospital.

During the last three months of this study, nasopharyngeal specimens were collected from neighbours of the family in which an index case of meningococcal disease had occurred. Neighbours were defined as all members of the households located on the immediate left and on the immediate right of the residence of the index case.

All nasopharyngeal specimens were examined for the presence of Neisseria meningitidis and all strains isolated were serogrouped using specific antisera.

The results of these surveys are shown in Table V. In the first group, families in which meningococcal disease had occurred, 54 of the 92 members had positive nasopharyngeal cultures. This is compared to a 7 per cent carriage found among the neighbours of these families, and 11 per cent carriage found in members of families in which C.N.S. infection, other than meningococcal, had occurred.

In families with cases of meningococcal disease, 50 of the 54 carriers identified, or 92.5 per cent, carried the same sero-group as the index case. Group A carriage was detected only in families with Group A disease.

The age related distribution of nasopharyngeal carriage among the 54 carriers found in the families of the index cases is presented in Table VI. As shown previously (Table V) the overall carriage

TABLE V

NASOPHARYNGEAL CARRIAGE OF MENINGOCOCCUS IN FAMILIES

FAMILY TYPE		NUMBER OF FAMILIES	NUMBER OF INDIVIDUALS	PER CENT WITH POSITIVE CULTURE
I	Index	19	92	58.7
	index case			
	Group A	9	45	57.8
	Group B	3	20	65.0
	Group C	7	27	55.5
II	Neighbour	9	28	7.1
III	Other CNS infection	10	35	11.4

TABLE VI

NASOPHARYNGEAL CARRIAGE OF MENINGOCOCCUS IN FAMILIES OF PROBAND (INDEX CASE)

DISTRIBUTION BY AGE

AGE	NUMBER	PER CENT WITH POSITIVE CULTURE
20+	36	58.3
15 - 19	13	61.5
10 - 14	10	60.0
5 - 9	21	57.1
4 or less	11	61.5
Males	40	60.0
Females	52	57.6

rate for this group was 58.7 per cent. Age related distribution of carriers, as determined by this survey, indicate that the carriage rate for each of the five age categories is the same as the overall carriage rate. Rates varied only from 57.1 per cent for the 5-9 age group to 61.5 per cent for the 15 to 19 and the 4 and under age groups. Males were found to be carriers as frequently as females.

The age related distribution of cases in this province indicate that the risk of disease is the greatest for the five and under age group. However, once the organism is introduced into a home, our data indicates that the risk of infection is equal for all ages.

The purpose of the second part of the survey was to determine meningococcal carriage rates among nursing personnel who had been directly responsible for the care of patients with meningococcal disease. From October 1, 1971 to April 30, 1972, 20 cases of meningococcal disease were admitted to the 4N ward of the Winnipeg Children's Hospital. Twelve of these were identified as group A meningococcal disease. Nasopharyngeal specimens were collected from 15 members of the nursing staff on this ward. All 15 had been employed on 4N from October 1, 1971 to April 30, 1972, and all had contact with patients during that period. The results of these cultures indicated only one which carried meningococcus, this was identified as sero-group Y. None of the 20 cases admitted to 4N from which meningococcus was isolated and sero-grouped were attributed to Group Y.

On October 7, 1971, nasopharyngeal specimens were collected from 60 second year medical students. In contrast to the nursing staff from 4N, none of these students had any known patient contact. Of these 60, two were found to be carriers of meningococcus. Both students carried non-groupable strains.

In November , 1971, an 8 month old child from a Hutterite colony was admitted to the Winnipeg Children's Hospital with Group A meningococcal meningitis. This colony was located within our study area and provided an opportunity to determine infection rates, following development of disease, within a well defined social order.

The 93 residents of the colony occupied 12 homes. Although the family living areas were self-contained, contact of families with one another is much greater than would be expected inthe conventional rural community. All meals are eaten in a common dining hall and all work is carried out as a co-operative effort. The children attended a school on the colony.

The total population of the colony was asked to participate in the survey. Nasopharyngeal specimens were collected from all but three of the 93 residents. Five individuals who were visiting at the colony, as well as the resident teacher, were included in the survey.

Results of these cultures (Table VII) indicated that 6 members of the colony were carriers of group A meningococcus. Four of these

TABLE VII

NASOPHARYNGEAL CARRIAGE OF MENINGOCOCCUS

SURVEY OF HUTTERITE COLONY

Number living on colony	93
Number refused to participate	1
Number absent on day of survey	2
Visitors (included in survey)	5
Teaching staff	1
TOTAL NUMBER CULTURED	96

Serogroups isolated

Group A	6
Group B	2
Non Groupable	5

Carrier rate for colony (all groups)	13.5%
Carrier rate for colony (Group A)	6.2%
Carrier rate for family of index case (Group A)	80%

six were members of the household in which the index case had occurred. Two other adult males were also found to be carriers of group A. All of the adult males who were carriers of group A had recently worked in the Interlake area, where part of the land owned by the colony is located. There are no permanent homes on this property, but the men working there share common living facilities.

Although there was considerable amount of mingling for meals, work and school activities, high risk of infection was limited to the immediate family of the proband.

The final part of the carrier study dealt with the risk of infection within schools where students have been in contact with a case or known carrier of Group A meningococcus. Table VIII summarizes the data from four classrooms. Of the 131 students cultured, 33 had contact with a case of group A meningitis, the other 98 had contact with a known carrier of group A meningococcus.

Twenty-one of the 131 cultures were positive for meningococcus, none of which, however, were group A. The sero-groups isolated were predominantly group B and group Y. Twelve were group B, 4 were group Y, one group X, one group C and three isolates were ungroupable with our antisera.

The extent of contact with respect to time could not be determined for these students. The student from the Grade II class attended school the day before she was admitted to the hospital.

TABLE VIII

NASOPHARYNGEAL CARRIAGE OF MENINGOCOCCUS CLASSROOM SURVEY

GRADE	ASSOCIATION	NO. OF STUDENTS	NO. WITH POSITIVE CULTURE GROUP A	
II	Case Group A	33	4	0
II	Carrier Group A	29	3	0
I	Carrier Group A	33	10	0
X	Carrier Group A	36	4	0
	TOTAL	131	21	0

CARRIER RATE 16.1%

SEROGROUPS ISOLATED	
Group B	12
Group X	1
Group Y	4
Group C	1
Non Groupable	3

However, the length of time which the remaining three students were carriers before their classmates were surveyed is not known.

In a second survey, an attempt was made to determine the risk of infection in the classroom over a period of time. Two Grade I classes were cultured monthly for three months. The results are recorded in Table IX. In classroom A, one student was a known carrier of group A meningococcus, and had positive nasopharyngeal cultures for two months before the first class survey was taken. Repeated cultures indicated that he continued to carry group A meningococcus for the three months that his classmates were under observation.

Classroom B was chosen as a control. It was located in a school attended by students of approximately the same ethnic and socio-economic distribution as classroom A. Both classrooms had similar carrier rates; none of the students became carriers of group A during the three months that they were under observation. The presence of a carrier in the class for at least five months with no acquisitions occurring during that time suggests that transmission must occur only rarely.

Transmission of meningococcus undoubtably must occur in the community, we have, however, not been able to demonstrate any increased risk of infection for nursing staff or students who are associated with cases or carriers of group A. It is apparent that for all

TABLE IX

NASOPHARYNGEAL CARRIAGE OF MENINGOCOCCUS SCHOOL SURVEY

SCHOOL A

LENGTH OF CONTACT	ASSOCIATION	NO. PRESENT	NO. WTTH POS. CULTURE
Three months	Carrier Gr. A	16	0
Four months	Carrier Gr. A	23	2
Five months	Carrier Gr. A	18	2
	TOTAL	57	4

AVERAGE CARRIER RATE 7%

No. of Group A - 0

SCHOOL B

SURVEY	ASSOCIATION	NO. PRESENT	NO. WITH POS. CULTURE
1.	No known Group A	17	1
2.	No known Group A	23	2
3.	No known Group A	23	3
	TOTAL	63	6

AVERAGE CARRIER RATE 6.5%

No. of Group A - 0

three sero-groups, the risk of infection is high in families in which a case of meningococcal disease has occurred.

VI. A COMPARISON OF THE EFFICACY OF SULFISOXAZOLE AND MINOCYCLINE IN THE ERADICATION OF THE CARRIER STATE

Sulfonamides are currently being used in this province as prophylactic therapy for all members of families in which a case of meningococcal disease has occurred. However, in the United States, roughly half of all group B strains isolated at the N.C.D.C. from 1966 to 1969 required concentrations in excess of 1.0 mg./100 ml. of sulfadiazine to inhibit growth. Similarly, by 1969, approximately 90 per cent of all Group C strains were resistant to sulfadiazine. (Bennett et al, 1969). Group A disease has occurred only rarely in the United States during the past decade, however, the isolation of a sulfonamide resistant strain in Seattle, Washington (Clark, 1971) is ominous. Group A strains in North America could follow the same trend toward sulfonamide resistance that have been noted in serogroups B and C.

It appeared that further information was required concerning the real effectiveness of prophylactic sulfonamide in this community as well as an evaluation of an alternate form of therapy. The purpose of this portion of the study was to compare the efficacy of sulfisoxazole and minocycline in the eradication of the carrier state.

Our study began in October 1971, and included members of households of all cases of meningococcal disease which occurred within a 25 mile radius of the city of Winnipeg. When a patient with meningococcal disease was admitted to hospital, the family was contacted and posterior nasopharyngeal specimens were collected

from all members of the household. This included anyone who had slept in the home within three days prior to the patient's admission to the hospital.

Following the collection of the nasopharyngeal specimens, all family members began therapy which was assigned according to the first letter of the surname. "A" to "M" inclusive received minocycline, (100 mgm. b.i.d.) for four days; "N" to "Z" inclusive received sulfisoxazole, (500 mgm. q.i.d.) for four days.

Nasopharyngeal specimens were collected from all household members immediately after completion of therapy, at the end of two weeks, and again at the end of three months. All specimens were examined for the presence of Neisseria meningitidis and all strains isolated were serogrouped.

A total of 75 individuals had nasopharyngeal cultures positive for Neisseria meningitidis. Of these 75, 29 were treated with sulfisoxazole and 46 were treated with minocycline. Five persons receiving minocycline, and one who had received sulfisoxazole, failed to complete the prescribed therapy and were not included in the study.

Data obtained on the remaining 69 individuals is shown in Table X. Sulfisoxazole reduced the carrier rate by 75 per cent immediately following therapy in the 28 individuals treated. The seven carriers with positive cultures after treatment were colonized with strains of meningococcus resistant to 1 mgm per cent of sulfisoxazole. All carriers of sulfisoxazole sensitive strains had

TABLE X

EFFECT OF SULFISOXAZOLE AND MINOCYCLINE ON NASOPHARYNGEAL CARRIAGE OF MENINGOCOCCUS

No. with positive culture	Antibiotic	
	Sulfisoxazole	Minocycline
On initiation of therapy	28	41
On completion of therapy	7*	0
Per cent reduction	75	100
2 weeks after initiation of therapy	5/19	5/41
Per cent reduction	75	88
90 days after initiation of therapy	5/19	8/36
Per cent reduction	75	75

*7 strains resistant to sulfisoxazole, not included in the remainder of the data.

negative cultures immediately following therapy. At the end of two weeks, five of the 19 carriers of sensitive strains had positive cultures. The remaining 14 individuals continued to have negative nasopharyngeal cultures for the 90 days they were under observation, representing approximately 75 per cent reduction in carrier rate of sulfisoxazole sensitive strains.

None of the 41 individuals treated with minocycline had positive cultures immediately following therapy. At the end of two weeks, there was an 88 per cent reduction in carriage and 75 per cent reduction in carriage at the end of 90 days. Of the eight individuals who were found to be carriers of meningococcus 90 days after the initiation of therapy, seven were of the same sero-grouping as they had carried before minocycline had been taken. One individual, initially colonized with serogroup A, carried serogroup Y two weeks after therapy, and continued to carry group Y for the remainder of the 90 days that he was under observation.

Eighty-one strains of Neisseria meningitidis isolated from patients and asymptomatic carriers during the course of this study were tested for antibiotic susceptibility. Antibiotic sensitivities were determined for penicillin, sulfisoxazole, minocycline and rifampin.

From Table XI, it can be seen that all serogroups were inhibited by 1.0 mcg/ml of minocycline with a mean M.I.C. of 0.22 mcg/ml.

TABLE XI

MINIMUM INHIBITORY CONCENTRATION FOR MINOCYCLINE (MCG/ML.)

SEROGROUP	0.5	0.25	0.125	0.067
Group A				
34 strains	0	28	2	4
Group B				
23 strains	0	17	6	0
Group C				
24 strains	0	21	3	0
TOTAL TESTED	0	66	11	4
81 strains				

None of the 81 strains tested were resistant to rifampin (Table XII) which may reflect the very limited extent to which this drug has been used in Manitoba. All strains were inhibited by 0.067 mcg/ml with a mean M.I.C. of 0.021 mcg/ml. Similarly, all strains tested were shown to be sensitive to penicillin. All were inhibited by 1 mcg/ml and the mean M.I.C. was 0.22 mcg/ml. (Table XIII).

All 34 strains of group A meningococcus were sensitive to sulfisoxazole. However, six of the 23 strains of group B required concentrations greater than 1 mgm% of sulfisoxazole to inhibit growth. Group C strains showed an even greater tendancy to resistance; 21 of the 24 strains tested required concentrations in excess of 1 mgm% to inhibit growth. (Table XIV).

It would appear that although no strains resistant to penicillin, rifampin, or minocycline were detected, resistance of sero-groups B and C to sulfisoxazole is quite prevalent in this province.

DISCUSSION

Of the 81 strains of meningococcus for which antibiotic sensitivities were determined, one-third were found to be sulfisoxazole resistant. This high prevalence of resistant strains greatly reduces the efficacy of this drug and emphasizes the importance of sero-grouping and sensitivity testing. Vaccines are currently being developed (Artenstein et al, 1970), however, until these are available for general use it is necessary to continue the search for an alternative for sulfidiazine in the chemoprophylaxis of resistant strains of meningococcus.

TABLE XII

MINIMUM INHIBITORY CONCENTRATION FOR RIFAMPIN (MCG/ML.)

SEROGROUP	0.125	0.067	0.033	0.016	0.008	0.004	0.002
Group A							
34 strains	0	4	11	12	3	4	0
Group B							
23 strains	0	1	7	3	10	2	0
Group C							
24 strains	0	2	3	5	12	2	0
TOTAL TESTED							
81 strains	0	7	21	20	25	8	0

TABLE XIII

MINIMUM INHIBITORY CONCENTRATION FOR PENICILLIN (MCG/ML.)

SEROGROUP	1.0	0.5	0.25	0.125	0.06	0.03	0.01
Group A							
34 strains	0	2	23	1	6	2	0
Group B							
23 strains	0	1	12	4	6	0	0
Group C							
24 strains	0	3	18	1	1	1	0
TOTAL TESTED							
81 strains	0	6	53	6	13	3	0

TABLE XIV

MINIMUM INHIBITORY CONCENTRATION FOR SULFISOXAZOLE

SEROGROUP	<1 mgm%	1 mgm%	>1 mgm%
Group A			
34 strains	34	0	0
Group B			
23 strains	17	0	6
Group C			
24 strains	3	0	21
TOTAL TESTED			
81 strains	54	0	27

Deal et al (1969) summarized the results of various therapeutic trials of oxytetracycline, erythromycin, penicillin G, ampicillin, ethoxzolamide and procaine penicillin. None of these were as effective in eliminating N. meningitides from the nasopharynx as sulfadiazine before resistant strains became prevalent.

All of the 81 strains of meningococcus from this province were found to be sensitive to 1 mcg/ml of penicillin, and this antibiotic remains highly efficient in the treatment of meningococcal disease. However, development of disease has been noted in patients receiving prophylactic penicillin (Deal et al, 1969; Tobin, 1956). During the course of this study, development of meningococcal meningitis attributed to Group C N. meningitides was observed in one patient receiving oral penicillin.

In addition, we have noted the development of a secondary case of meningococcal meningitis in a 16 month old child following prophylaxis with 125 mgm b.i.d. sulfisoxazole for three days. The interval from the primary to the secondary case was 10 days. Both cases were attributed to sulfisoxazole resistant group B meningococcus.

Studies of the effectiveness of rifampin have been encouraging, and the 81 strains isolated in Manitoba were all shown to be sensitive. However, reports of rapid development of resistant strains following therapy with this drug (Guttler et al, 1971) indicate that it would have limited use as a widespread prophylactic.

Results from this study as well as that reported by Devine (1970) and Guttler et al (1971) indicate that minocycline is a

potentially useful prophylactic antimicrobial agent. It was equally effective for all sero-groups and at the end of 90 days was effective in eradicating the carrier state approximately 75 per cent of the time. This was the same rate of reduction achieved when sulfisoxazole was used to treat sulfisoxazole-sensitive strains of meningococcus.

In view of these encouraging results further trials are warranted using larger experimental populations. Special attention should be paid to the rate of development of resistant strains.

VII. DETECTION OF GROUP A ANTIBODY WITH THE INDIRECT HEMAGGLUTINATION TEST

A limitation encountered in the study of the epidemiology of the meningococcus is the inability to identify the etiology of the infection in many instances. The appearance of sero-group A meningococcus in this province, and the development of sulfonamide resistance within the various sero-groups, emphasizes the importance of a serological method that is sero-group specific and sensitive enough to detect the asymptomatic carrier, as well as identify the etiology of a clinical case.

This portion of the study describes the antibody response following asymptomatic carriage of group A meningococcus as well as antibody response following group A associated disease, as detected by the indirect hemagglutination test.

One or more serum samples were collected from 46 patients with bacteriologically confirmed meningococcal disease. Of the 46 cases, 23 were group A, 13 were group C, and 10 were group B associated disease.

Figure 6 indicates the specificity of the HA test to detect group A antibody. This graph shows the HA titres obtained with the sera of 46 patients against group A antigen. The sera of patients with group B and C disease show little or no response to the group A antigen, and none have titres ≥ 32.

Hemagglutination titres in sera obtained from patients with group A disease against group A antigen are presented in Figure 7. Sera obtained from three patients was undated. The composite graph

FIGURE 6.

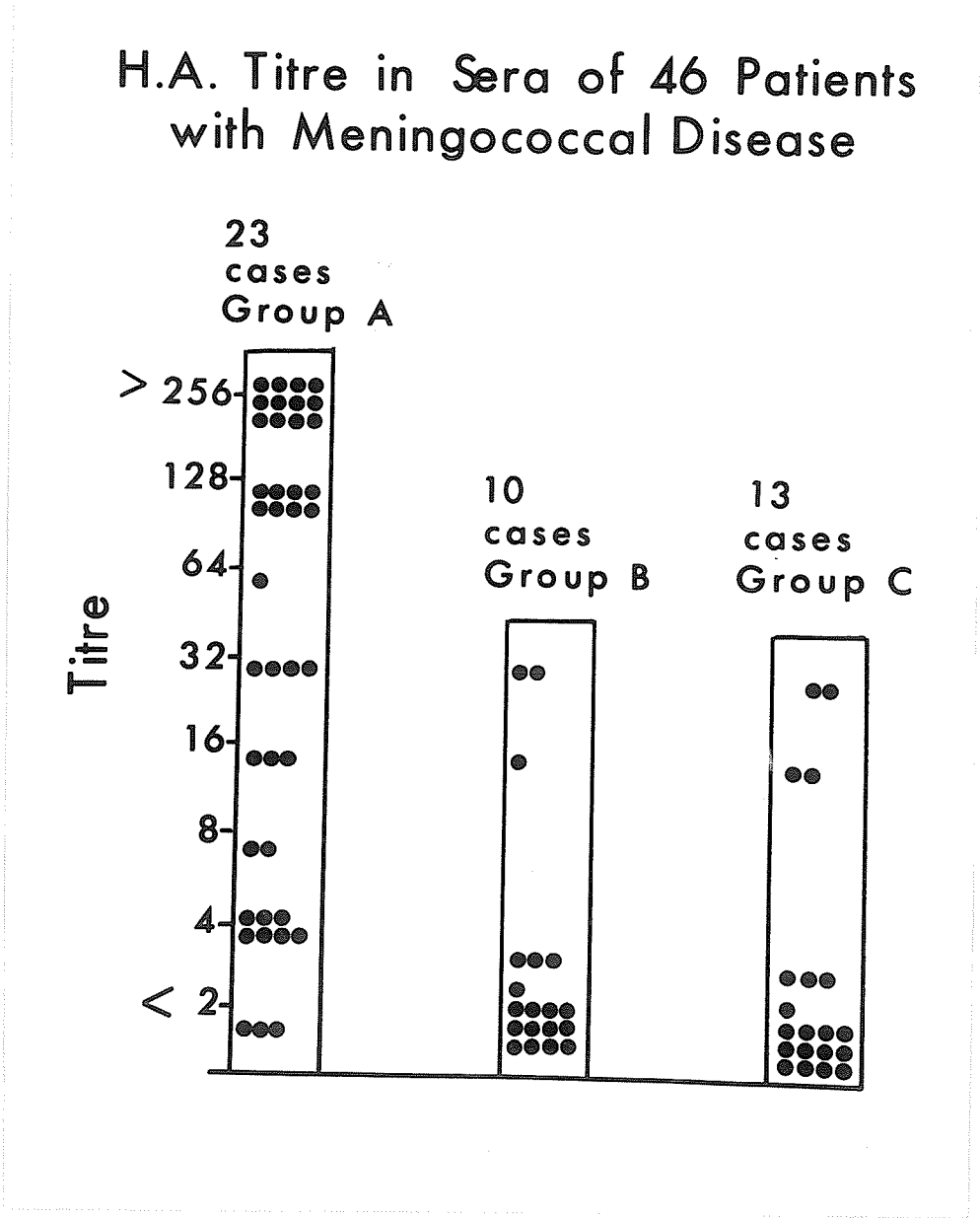
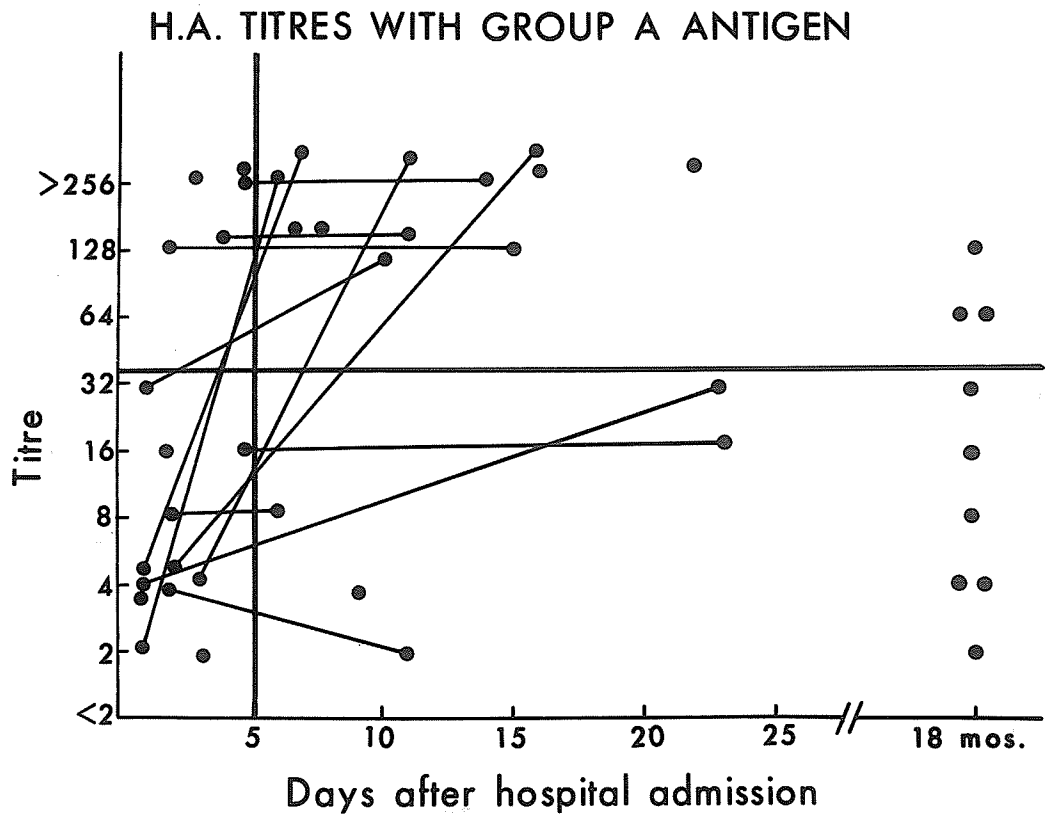

H.A. Titre in Sera of 46 Patients with Meningococcal Disease
23 cases Group A
10 cases Group B
13 cases Group C
Titre
> 256
128
64
32
16
8
4
< 2

FIGURE 7.


H.A. TITRES WITH GROUP A ANTIGEN
>256
128
64
32
16
8
4
2
<2
Titre
5
10
15
20
25
18 mos.
Days after hospital admission

represents the hemagglutination titres determined in 34 serum samples collected from the remaining 20 patients at the indicated time after hospital admission. During the first three days the majority of sera had antibody titres less than 32, two of the 12 sera collected during this period yielded titres greater than 256. Five patients had hemagglutination titres ≤ 32 after the fifth day of illness. The remaining 15 patients had titres ≥ 128 after five days.

Sera was obtained from nine patients approximately 18 months after the onset of illness. These patients had titres ranging from 4 to 128. Two of these patients with HA titres of 16 and 64 carried group A meningococcus in the nasopharynx at the time the sera was obtained. This drop in hemagglutination titre following disease appears to be independent of asymptomatic carriage of the organism.

HA titres against group C antigen in sera of 13 patients with group C disease are shown in Figure 8. As demonstrated with group A disease, hemagglutination titres rise rapidly during the first five days of illness. Two patients failed to have titres > 32 after the fifth day of illness.

The distribution of indirect hemagglutination titres in a group of 110 asymptomatic individuals is shown in Figure 9. The 41 asymptomatic carriers of meningococcus were divided according to the serogroup carried. The remaining 69 individuals had nasopharyngeal cultures negative for N. meningitidis. These are divided according to their association with cases of meningococcal disease. The control

FIGURE 8.

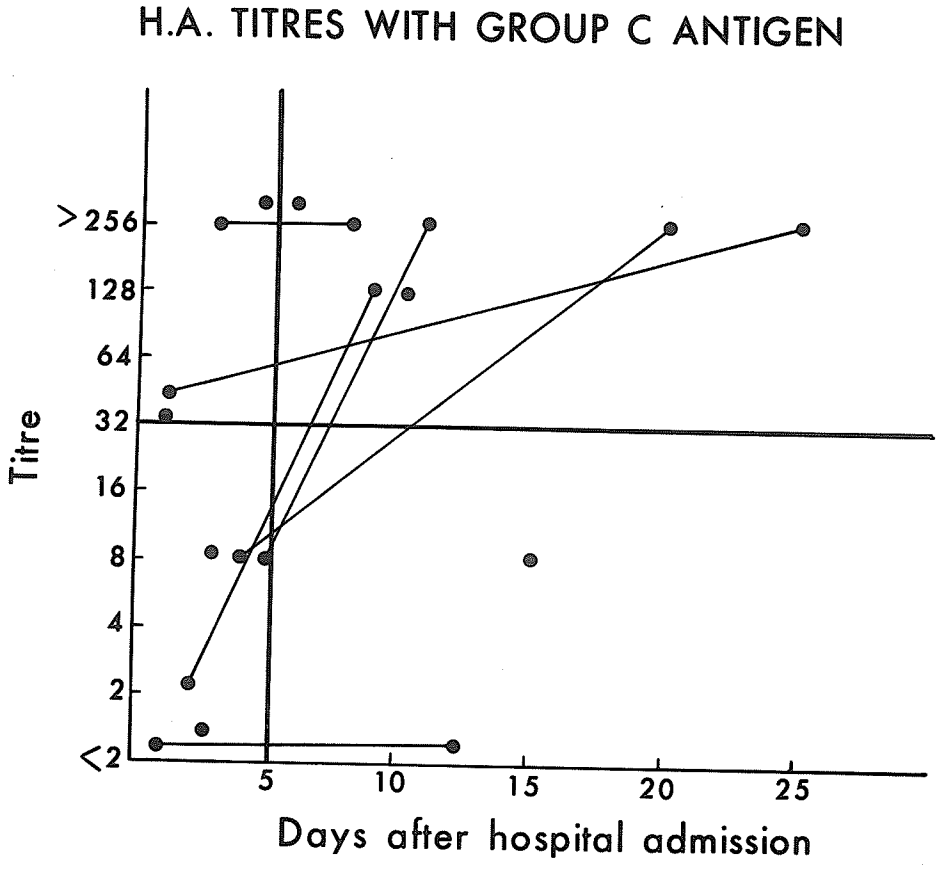
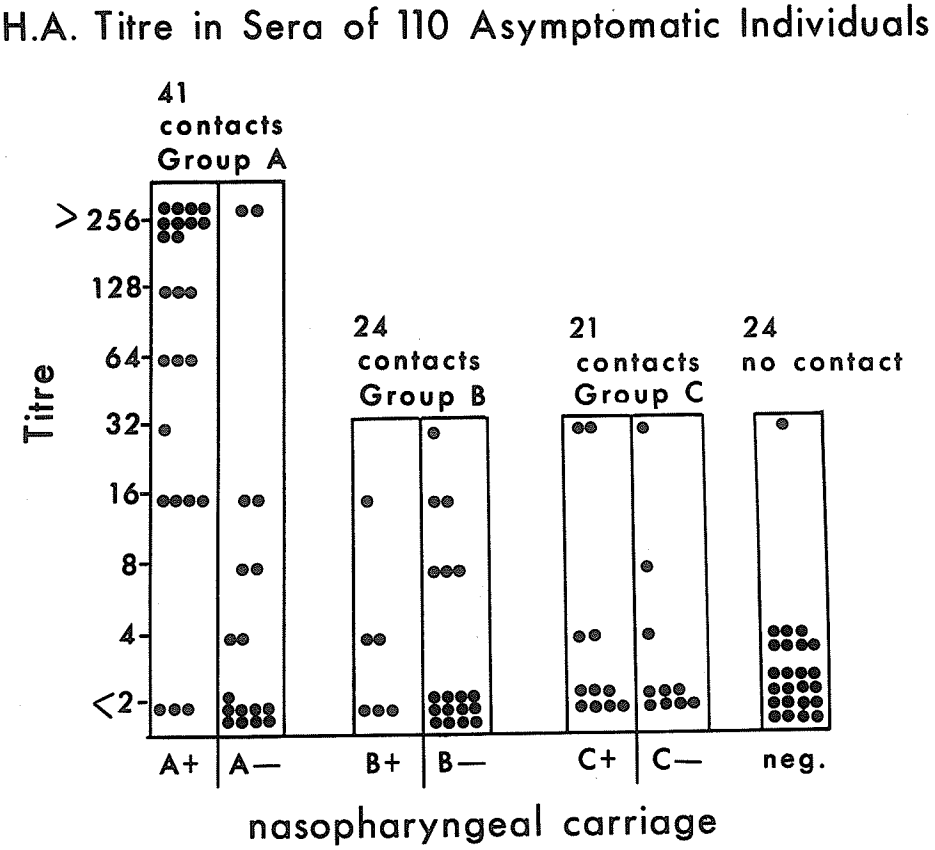

H.A. TITRES WITH GROUP C ANTIGEN
Titre
>256
128
64
32
16
8
4
2
<2
5
10
15
20
25
Days after hospital admission

FIGURE 9.


H.A. Titre in Sera of 110 Asymptomatic Individuals
41 contacts Group A
24 contacts Group B
21 contacts Group C
24 no contact
Titre
>256
128
64
32
16
8
4
<2
A+
A—
B+
B—
C+
C—
neg.
nasopharyngeal carriage

group were members of households in which there had been no known cases of meningococcal disease.

Fifteen of the 24 carriers of group A meningococcus (62%) had HA titres $\geq$32. None of the 17 carriers of group B or C meningococcus had hemagglutination titres greater than 32. The correlation of hemagglutination titre and negative nasopharyngeal cultures is also noted in households of group B cases, group C cases as well as the control group. Of these 52 individuals, all had titres $<$32. Two members of this group had nasopharyngeal cultures negative for meningococcus and HA titres $\geq$256. Both of these were members of households in which a case of group A associated disease had occurred.

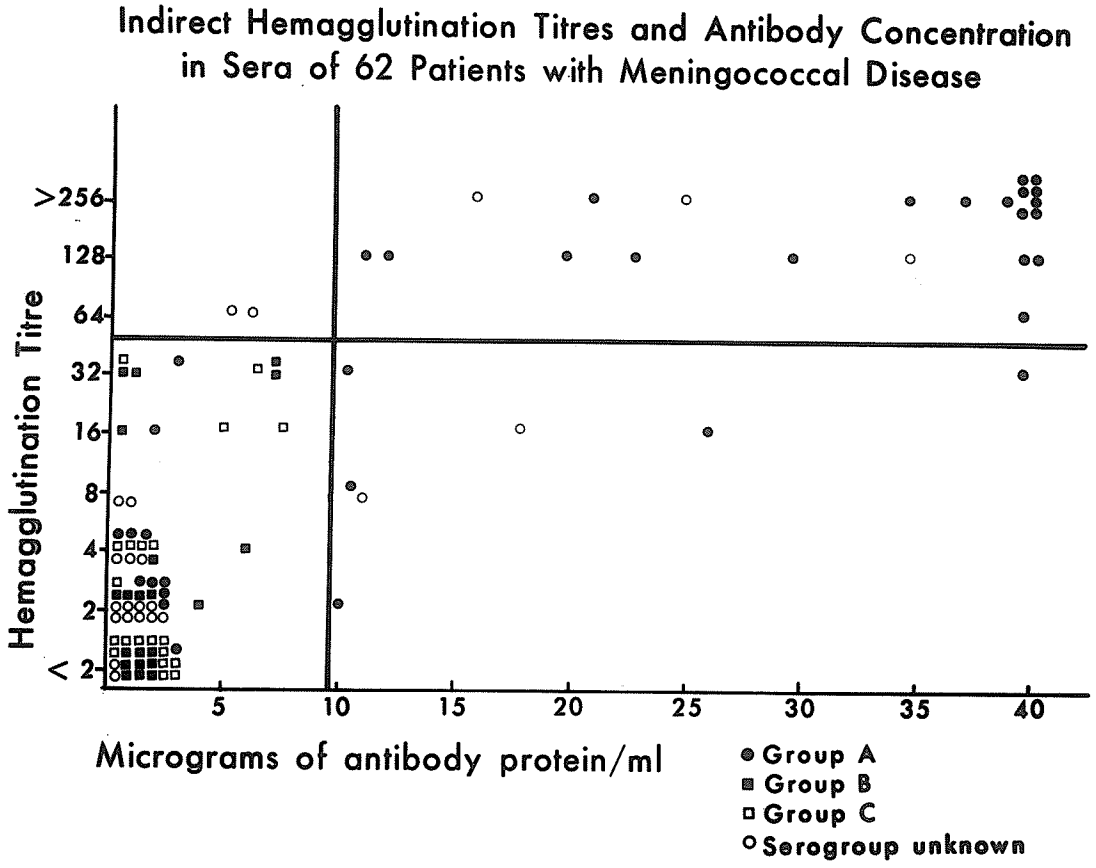
The correlation of group A antibody response as determined by the indirect hemagglutination titre and the quantitative determination of antibody by radioactive antigen binding capacity is presented in Figure 10. These are results obtained with 103 sera collected from patients with a clinical diagnosis of meningococcal meningitis.

The rank-correlation coefficient was used to test the hypothesis that the two variables were independent. (Dixon, 1969). This test has the advantage that no assumptions are made as to whether the two variables are normally distributed. The level of significance of this correlation was tested using the student t test

$(t= \sqrt{(n-2)\ r^2/(1-n^2)}\ ).$

A high correlation was found in the results of these two determinations of antibody response following clinical disease (r_s= .849 p .001). A high correlation for these two determinations

FIGURE 10.


Indirect Hemagglutination Titres and Antibody Concentration
in Sera of 62 Patients with Meningococcal Disease
Hemagglutination Titre
>256
128
64
32
16
8
4
2
< 2
5
10
15
20
25
30
35
40
Micrograms of antibody protein/ml
Group A
Group B
Group C
Serogroup unknown

in sera of 126 asymptomatic individuals was also noted (r_s= .475 p .001). These results were verified using the Goodman-Kruskal index of correlation for grouped data (Kendall, M.G. and Stuart, A. Advanced Theory of Statistics, 2nd Edition.). This test indicated a correlation of .884 and .592 for the antibody determinations in sera of cases and contacts of cases respectively. In five patients with bacteriologically confirmed group A disease, the HA titres remained $\leqslant$32 after the fifth day of illness. Table XV shows the distribution by age of HA titres following group A disease. All sera was collected after the fifth day of illness. Four of the five patients with little or no HA antibody were under the age of two years.

Two patients with group C disease failed to demonstrate HA titres against that antigen. Table XVI shows the distribution by age of HA titres obtained after the fifth day of illness in 10 patients with group C disease. One of the patients who failed to show any HA antibody was under the age of two however, 3 patients whose ages were two months, four months and one year, had hemagglutinating antibody titres $\geqslant$256.

Sera from patients with group B or C meningococcal disease had antibody concentrations which ranged from $\leqslant$.23 to 7.61 micrograms of antibody protein/ml as determined by the radioimmuno assay method using a purified group A antigen. By the fifth day of illness, all patients with group A disease had antibody concentrations 10 micrograms/ml with this technique. The distribution of antibody concentration

TABLE XV

DISTRIBUTION BY AGE OF TITRES OF HA ANTIBODY TO GROUP A ANTIGEN IN 17 PATIENTS WITH GROUP A DISEASE

Age	No. Tested	No. of sera* with indicated titre					
		≤ 4	8	16	32	64	≥ 128
8-24 mos.	5	2	1	1			1
2-8 years	7						7
$>$ 16 years	5				1		4

* all sera collected after 5th day of illness

TABLE XVI

DISTRIBUTION BY AGE OF TITRES OF HA ANTIBODY TO GROUP C ANTIGEN IN 10 PATIENTS WITH GROUP C DISEASE

Age	No. tested	No. of sera* with indicated titre					
		≤4	8	16	32	64	≥128
8-24 mos.	4	1					3
2-8 years	2		1				1
≥8 years	4						4

* all sera collected after 5th day of illness

by age is shown in Table XVII. The five patients in which HA antibody was not detected are shown. In most cases antibody concentrations were low, but detectable, reflecting the greater sensitivity and specificity of the radio immuno assay method.

Whether or not there is a patient-age relationship to the quantity of group A antibody produced is not clear from this data. This point warrants further investigation.

DISCUSSION

Hammond et al (1968), using an impure antigen to sensitize erythrocytes found that they required special treatment to achieve sensitization. However, this difficulty was not encountered in working with the impure group A antigen. The test was found to be group specific and sensitive enough to detect 75 per cent of the clinical cases of group A disease by the fifth day of illness. The somewhat lower sensitivity in detection of asymptomatic carriers of group A meningococcus (62 per cent) may be a reflection of the length of carriage at the time the sera was collected.

The indirect hemagglutination test was found to be a technically simple procedure and although not sensitive enough to detect all cases of group A disease, once standardized, could be a useful method in the study of the epidemiology of this organism.

TABLE XVII

ANTIBODY CONCENTRATION AS DETERMINED BY RADIOACTIVE ANTIGEN BINDING TECHNIQUE IN SERA OF 16 PATIENTS WITH GROUP A DISEASE

AGE		
8 - 24 months	2 - 8 years	>8 years
15.88*	>40	>40
15.83*	>40	11.5
10.07*	>40	10.79*
37.53*	>40	23.15
>40	>40	>40
	19.19	

All sera collected after the 5th day of illness

* HA titres ≤32

VIII. SUMMARY

There has been an increased incidence of meningococcal infections in Manitoba, beginning in 1970 and continuing into 1972. This is attributed to the introduction into the community of sero-group A meningococcus as well as an increased incidence of infection with the other endemic sero-groups. These cases have occurred with increased frequency among the native Indian population, and have shown a strong association with low income families. Most cases occurred in March, April, and May with the highest incidence of disease in children under the age of two years. Geographic plotting of cases show a gradual southerly drift.

Surveys to detect nasopharyngeal carriers have shown the risk of infection to be high in families in which a case of meningococcal disease had occurred, irrespective of the serogroup of the index case. Although risk of disease is high for the younger individual, the risk of infection in these homes was found to be equal for all age groups. In contrast to the high carriage rate among the families of cases,we were not able to demonstrate any increased risk of infection for nursing staff or students who are associated with cases or carriers of meningococcus.

Results of the comparison of minocycline and sulfisoxazole in the treatment of carriers show that minocycline is a promising prophylactic antimicrobial agent.

Evidence was presented that indicates the indirect hemagglutination test to be a useful epidemiological method in the study of group A associated meningococcal disease.

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