A study of beta-hemolytic streptococcus group A from school children and their families, and patients with pharyngitis, acute rheumatic fever and rheumatic heart disease in Chiang Mai

by

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CONTENT

ABSTRACT

The results showed that the isolation rate of group A streptococci in 1,611 sore throat patients who visited Maharaj Nakorn Chiang Mai Hospital and Maternal and Child Health Center Region V, Chiang Mai during November 1984 to December 1985 was 6.39%. The recovery of group A streptococci from 181 old cases of acute rheumatic fever and/or rheumatic heart disease patients was 2.21%. From the 23 new case of ARF and/or RHD patients, the isolation was 17.39%. In school children, the recovery rate varied from 0.99% to 15.73% depended on the school and the time of the isolation. Group A streptococci in both patients and school children predominate in the age group 5-14 years.

Among 103 strains of group A streptococci in sore throat patients, T-3/13/B3264, T-5/11, T-8/25/Imp19, T-4/28 and untypable strains were identified in the following proportions: 19.42%, 18.45%, 17.48%, 15.53% and 12.62% respectively. In the new case of ARF and/or RHD patients, each of T-5/12/27, T-8/25/Imp19, T-6 and an untypable strain was found. Only one strain of T-3/13/B3264 and three strains of T-8/25/Imp19 were isolated from the throats of old cases of ARF and/or RHD patients. In healthy school children, T-49 and untypable strains predominate. For pyoderma patients, T-11 was the predominant type. Oral penicillin treatment in school children who were carries of group A streptococci failed to eradicate the organism in

9.18% of cases wtih 10 days. All group A streptococci isolates were susceptible to chloramphenicol, penicillin, ampicillin, erythromycin, cefalothin and cefoxitin. The mean values of MIC/MBC of group A streptococci from sore throat patients, ARF and/or RHD patients, school children and pyoderma patients were 0.022/0.025, 0.024/0.026, 0.024/0.031, and 0.027/0.031 ug/ml, respectively. Only two members from two different families were positive for group A streptococci. The serum penicillin levels in six patients with ARF and/or RHD patients reached peak levels on the first day of benzathine penicillin G(BPG) intramuscular injection, and then decreased rapidly. After 14 days of penicillin administration, two out of six patients had lower penicillin level than the MIC values of group A streptococci. The half-life values of serum penicillin in these six patients were 10.50, 7.00, 9.00, 6.25, 10.25 and 7.25 days. Only 47.83% of group A streptococci positive school children and 33.33% ARF and RHD patients had an increase in ASO titer.

INTRODUCTION

The bacterial group Streptococcus pyogenes (Lancefield group A) is an important pathogen for man. S.pyogenes is found in all climatic zones of the world and belongs to the family Streptococcaceae. It is gram-positive, lacks cytochrome and is coccoidal in shape. S.pyogenes usually grows in chains of various lengths in liquid media but usually form tetrads in solid media.

On blood agar plates group A hemolytic streptococci may form any one of three colony types, designated mucoid, matt, and glossy. Mucoid colonies are formed by strains which produce large capsules: the abundance of hyaluronic acid gel gives the colony a glistening, watery appearance. The flatter, rougher, matt colonies were originally thought to reflect the production of M protein, but they are simply dried out mucoid colonies: as the gel becomes dehydrated the surface of the colony shrinks and becomes roughened[1,2].

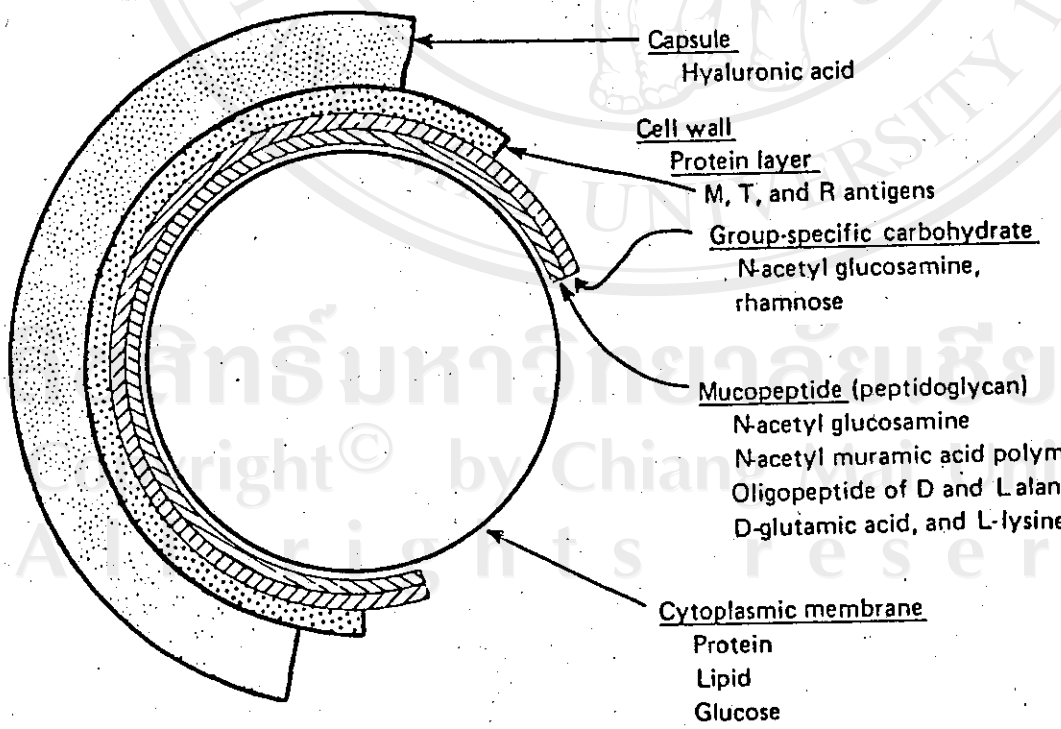
Glossy colonies are smaller; they are formed by cells that do not generate hyaluronate or do not retain it as a capsular gel. Group A streptococci produce several antigens, some of which are shown in Table 1. Generally, the outermost part of group A streptococci cell surface is the capsule, composed of hyaluronic acid. Beneath the capsule is the cell wall, which, in a simplified way, can be visualized as having a three-layer structure. The outermost cell-wall layer contains lipoteichoic acid and a number of protein components, namely M antigen, T antigen,

R antigen, MAP (M-associated protein, or so-called non-type-specific protein), SOF (serum opacity factor) and the Fc-binding factor. The second layer consists of polysaccharide, and the third layer consists of peptidoglycan(Figure 1).

Table 1 Streptococcal antigens

Cellular	Extracellular
Group-specific polysaccharide	Streptolysin O(SLO)
M-protein	Streptolysin S(SLS)
M-associated protein(MAP)	DNAse A,B,C,D
Peptidoglycan	Hyaluronidase
R & T proteins	NADase
Protoplast membrane	Streptokinase
Lipoteichoic acid(LTA)	Pyrogenic toxins
Serum opacity factor(SOF)	
Fc-binding factor	

Figure 1. Cell structure of group A streptococci



The fimbriae are located at the outermost layer of the cell wall and have two determinants. One of these is lipoteichoic acid (LTA), which enables the microorganisms to adhere to the epithelial cells of the human oral mucosa by means of its fatty acid moiety. The other fimbriae determinant is M protein. This M protein is a virulence factor in that it is responsible for resistance to phagocytosis. In addition, M protein is the type-specific substance in group A streptococci. The M protein is likely to have two moieties, one with precipitinogen activity and the other with antiphagocytic activity. The former is composed of smaller, type-specific molecules while the latter consists of an assembly of molecules attaining a molecular weight of around 30,000 daltons. These moieties are biologically distinct and are separable. At present, almost 70 M types can be differentiated in group A streptococci.

T antigens are resistant to pepsin and trypsin, and are acid and heat stable. Some T antigens are restricted to a single M type, while others may be shared by several M types (Table 2). T antigens are not associated with surface fuzz or virulence. Antibodies to T antigens are not protective.

Another antigen, M-associated protein, is found in all M protein-containing group A streptococci and some strains of group C and G, but not in M-negative strains. Antibody response to M-associated proteins are usually highest in patients with acute rheumatic fever.

Table 2 Relation of T patterns to M types

T Complex	M Types Bearing T Complex
1	1
2	2
3/13/B3264	3,13,33,39,41,43,52,53,56
8/25/Imp.19	2,8,25,55,57,58
5/11/12/27/44	5,11,12,27,44,59,61
14/49	14,49
15/17/19/23/47	15,17,19,23,30,47,54

The innermost part of streptococci is cytoplasm which contains a complex of nucleoproteins, nucleic acids, and other proteins, some with enzymatic activity.

An extracellular product, streptolysin O (SLO) is inactivated by oxygen. However, this inactivation can be reversed by reducing agents, such as cysteine or 2-mercaptoethanol. SLO is irreversibly inactivated by cholesterol. This hemolysin has immunologic cross-reactivity and properties similar to the oxygen-labile hemolysins of pneumococci, clostridia, and bacilli.

In vitro, SLO is toxic for red blood cells, white blood cells and various other cell types. In vivo, intravenous injection of SLO in animals may cause sudden death. Following pharyngeal or systemic infections, SLO induces a brisk antibody response, usually within 10 to 14 days. Immune responses to SLO following skin infection are considerably lower than those following pharyngeal infection. Another extracellular product, streptolysin S (SLS), is an oxygen-stable molecule. This hemolysin has been shown to be produced near the cell membrane. SLS causes

hemolysis by direct cell-to-cell contact or by transmission via the carrier molecules. The molecular weight of SLS is less than 20,000. SLS is lytic for red blood cells, white blood cells, bacterial protoplasts and L forms. SLS is responsible for the surface hemolysis seen on blood agar, and those occasional strains that lack SLS may appear nonhemolytic on surface growth.

The pyrogenic exotoxins (erythrogenic toxins), appear to be synthesized as a consequence of infection with temperate bacteriophages. Classically, much attention has been given to the scarlet fever rash which is induced by this toxin. However, pyrogenicity appears to be the primary effect of the erythrogenic toxins. Dermal reactivity is, at least in part, secondary to host hypersensitivity. There are at least three different serotypes of erythrogenic toxins (A, B, and C), with molecular weights of 8,000 daltons, 17,500 daltons, and 13,200 daltons, respectively. Pyrogenic exotoxins are heat labile, but are stable to acid, alkali, and pepsin. The type C pyrogenic toxin causes increased permeability of the blood-brain barrier to endotoxin of bacteria and exerts its pyretic effect by direct action on the hypothalamus.

Nucleases A,B,C, and D are extracellular enzymes, which presumably assist in the generation of substrates for growth. They are produced by most group A streptococci. Nucleases A and C have only DNase activity, while B and D also possess RNase activity. All have molecular weight of 25,000 to 30,000 daltons and require calcium and magnesium

for optimal activity. DNase B is produced by group A and a few strains of group C and G streptococci. Other enzymes, such as DNase A,C and G, are produced not only by group A but also by other group of streptococci. Antibody titers to DNase B are of great value in the serodiagnosis of skin infection, where the SLO response may be blunted.

Clinical significance

In human, Streptococcus pyogenes is a well known cause of pharyngitis, tonsillitis, sinusitis, lymphadenitis, arthritis, osteomyelitis, endocarditis and meningitis. Pneumonia is a rare occurence that is associated with a preceding viral illness. In the child under 4 years of age, upper respiratory infection may be subacute, with rhinorrhea as the only manifestation. On the contrary, school age children are acutely ill with fever, sore throat, exudative tonsillitis, and cervical adenitis. Erythrogenic strains may produce scarlet fever, in which the streptococcal infection is accompanied by skin rashes.

Approximately 3 percent of patients may develop rheumatic fever within 5 weeks of a pharyngeal infection with S. pyogenes. The clinical manifestations of rheumatic fever include carditis, polyarthritis, chorea, erythema marginatum, and subcutaneous nodules. Unlike rheumatic fever, acute glomerulonephritis may follow either a pharyngeal or a skin infection with group A streptococci. A limited number of M and T serotypes are recognized as nephritogenic strains and are responsible for the majority

of acute glomerulonephritis cases. The latent period for nephritis is 10 days after throat infections. In patients with skin infections, elevated anti-DNase B titers occur more frequently than antistreptolysin O titer. The diseases and their sequelae remain a major problem in the developing nations of the world, where overcrowding and poor hygiene are still prevalent[3].

There are not so many studies of group A streptococci from patients, especially in rural areas. This study therefore stresses on the basic data of group A streptococci from school children and patients. This will lead to further in depth studies of this group of organisms.

LITERATURE REVIEW

Group A streptococcus is considered to be a causative bacterial agent of pharyngotonsillitis. Clinical symptoms occur in several grades; normal throat, nonexudative, mildly exudative, and severely exudative. Because of this wide range of clinical symptoms, culture is the best way to rule out this etiologic agent. Healthy Group A streptococcus carriers can spread the organism by close contact. Susceptible persons may develop pharyngotonsillitis, and some of these cases may subsequently develop poststreptococcal inflammatory diseases such as rheumatic fever, rheumatic heart disease and acute glomerulonephritis. Thus, streptococcal infection is an important health problem. The best way to prevent it is to eradicate the organism from carriers and the patients.

It was found that the prevalence of the carrier state varies widely. Carrier rate depends on the classes of the students studied and the examinations,[4] the age groups and seasonal factors.[5,6,7]

The result of throat culture studied by Kaneko et al[1] in Japan showed that there was a great variation of carrier rate among various classes and examinations. The carrier rate of Group A streptococci was 28.5% on the average, but varied from 10 to 71% per examination per class. Kaneko et al could not detect Group A streptococci from environmental sources. So, streptococci seemed to be transmitted from child to child, by droplets; and their being together in one

class played an important role in this transmission. The most prevalent serotypes in their study were T4 and T12. (This result differed from studies done in the Philippines where T-3-13-B3264 was the most prevalent type among asymptomatic carriers[8].

In patients with scarlet fever in Thailand, the predominant type of group A streptococci was T55(M55)[9]. This type of group A streptococci has been reported to cause acute glomerulonephritis more often than acute rheumatic fever[10].

With regards to age group, Tupasi et al.(1877) in the phillipines[5] studied Group A streptococcus in school children and found that the highest incidence of isolation was in the 5-6 year age group. The most frequently occuring T-type was T-13.

Hoffman (1985)[7] found that the rate of asymptomatic throat carriage of Group A beta-hemolytic streptococci (GABHS) was 10.9% in patients =<14 years of age, 2.3% in patients between 15 and 44 years old, and 0.6% in patients >=45 years old.

Limson (1977)[6] found that the beta-hemolytic streptococcus carrier rate was low in the "dry" months of the year, January-February.

Although antistreptolysin O (ASO) titer is widely used to confirm infection with group A streptococci, it is limitated in that it is positive in only 80% of upper respiratory tract infected patients, 88.5% of active

rheumatic patients and 53% of pyoderma patients. In addition, infections with non-group A streptococci were also associated with elevated ASO titers.[11] In contrast, a long period of penicillin administration resulted in a decrease of ASO response.[12]

The serotypes of streptococci involved in outbreaks of acute rheumatic fever(ARF) and skin infection in many countries were quite different. In the United Kingdom and in the United States of America, the predominant serotypes were 1, 3, 5, 6, 14, 18, 19, 24, 27 and 29[1,10,13]. The predominant serotypes from sore throat cases in Bangkok, Thailand showed T 4/28, T 3/13/B3264, T 8/25/Imp19[14], but the number of isolates was small.

Treatment of streptococcal infections of the pharynx or tonsils is directed toward eradication of group A streptococci, which appears to be essential for the prevention of rheumatic fever. Penicillin is considered the drug of choice. The recommendation is 25 to 50 mg/kg/day in 2,3 or 4 divided oral doses(200,000 to 400,000 units orally 4 times daily) for ten days. Patients who are hypersensitive to penicillin may be treated with erythromycin 20 to 40 mg/kg/day in 2,3, or 4 divided oral doses(0.25 to 0.5 g 4 times daily) for 10 days[15,16]

For preventing recurrences of rheumatic fever, either a repetitive oral penicillin regimen or monthly injections of long-acting penicillin G benzathine can be used. It was reported that the group receiving injections still had a streptococcal infection attack rate of 0.6%[17]. The

penicillin serum level of group A streptococcal pharyngitis patients varied from 18 to 30 days.[18]. Peak serum concentrations in patients receiving benzathine penicillin injection were from 0.01 to 0.06 µg/ml and in those receiving oral penicillin were 3 to 20 µg/ml[19].

Wide variations in the sensitivity to penicillin of strains of group A streptococci were observed with minimal inhibitory concentration of penicillin varying from 0.003 to 0.05 ug/ml[20].

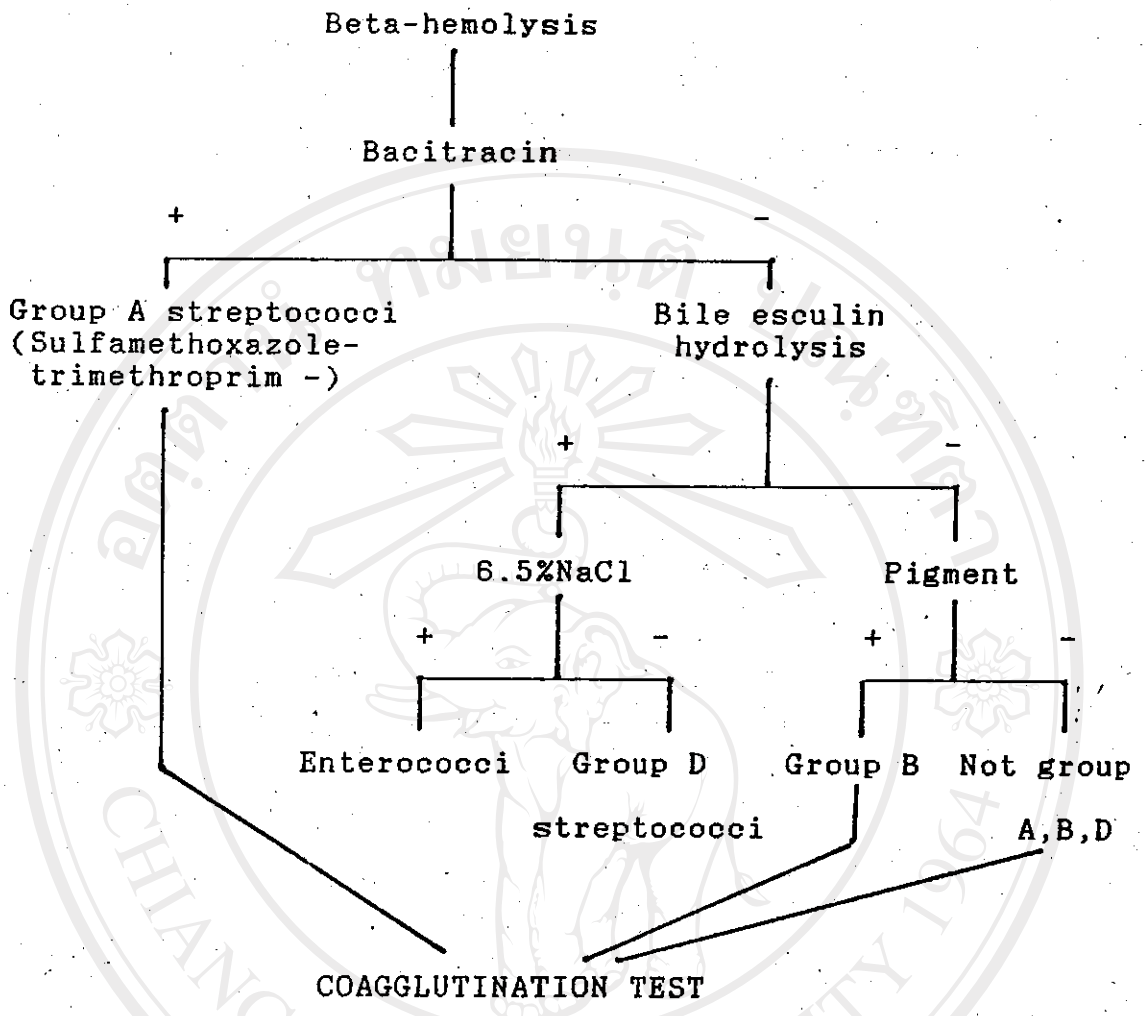
MATERIALS AND METHODS

1. Sample size: Five hundred school children from four government primary schools in Chiang Mai, Thailand, were studied three times in June, October, and February. Specimens from patients with sore throat, rheumatic fever, rheumatic heart disease and skin infection attending Maharaj Nakorn Chiang Mai Hospital were collected during a one year period.

2. Specimens collection: Two throat swabs from each child or patient were collected and brought back (without transport medium) for culturing on blood agar within three hours of collection[1].

3. Bacterial culture: Two swabs were rubbed over an area of 2x3 centimeters on each half of two blood agar plates and were streaked with a sterile wire loop in four directions in order to isolate the microorganisms into single colonies. One plate was incubated anaerobically at 35°C overnight, while another plate was incubated in candle jar(3-5% CO_2) at the same temperature. Identification of the beta-hemolytic streptococci was done using the following tests: bacitracin differential disk (0.04 µg/ml)(Difco) and trimetroprim-sulfamethoxazole disk for group A streptococci, pigment medium (islam medium)[21,22] for group B streptococci, and bile esculin agar and 6.5% NaCl for group D and enterococci according to scheme I. All beta-hemolytic streptococci were confirmed by coagglutination test (Phadebact).

Scheme I Key for beta-hemolytic streptococci identification



4. T-typing : All group A streptococci were cultured in 5 ml Todd-Hewitt Broth (TH broth) overnight at 30°C and were then centrifuged to sediment the bacteria. This sediment was resuspended in 0.5 ml TH broth and treated with two drops of 5% trypsin solution(Difco 1:250) and one drop of 0.04% phenol red solution as an indicator. The suspensions were adjusted to pH 8.0 by using 0.2N NaOH. The suspension was incubated in a water bath at 37°C for 1 hour with shaking at

every 15 minutes. Finally, the pH of the solution was adjusted to pH 7.0 by using 0.2N HCl[1]. For agglutination, polyvalent antisera and monovalent antisera produced at Toho University, Japan, were used. The slide agglutination reaction was carried out: one drop of bacterial suspension was added to one drop of polyvalent antisera and subsequetly, the corresponding monovalent antisera. The agglutination reaction was evaluated within 1 minute or at a maximum of 2 minutes.

5. Treatment: The streptococcal carriers were physically examined by pediatrician and were treated with 200,000 units of oral penicillin four times daily.

6. Follow-up on the effectiveness of treatment: Throat swab cultures were done from carriers at day 5 and day 10 after treatment. If positive results were obtained, further treatment was given for five more days.

7. Family studies: Throat swabs were taken from the family members of the carriers

8. Drug susceptibility test: The agar disk diffusion test was used to detect the susceptibility of the beta-hemolytic streptococci to penicillin, chloramphenicol, tetracycline, co-trimoxazole, ampicillin, erythromycin, cefalothin and cefoxitin. All disks were stored at $0^{o}C$ and were placed on the test plate at room temperature one hour before use[23,24,25].

Three to ten colonies of the overnight culture were picked with a sterile wire loop and inoculated into a test tube containing 0.5 ml of TH broth and were incubated for

two to five hours to obtain a bacterial suspension of moderate cloudiness. The suspension was then diluted, if necessary, with saline solution or TH broth to a visual equivalent to McFarland No. 0.5. The bacterial suspension was spread evenly in three directions on the surface of Mueller-Hinton blood agar plate with a sterile cotton swab. Excess suspension was removed from the swab by rotating the swab against the side of the tube before the plates were seeded. After the inoculum had dried for three to five minutes, the disks were placed on the agar plate with flamed forceps and gently pressed down to ensure contact. Plates were incubated either immediately, or within 15 minutes at 35°C to 37°C. After overnight incubation, the diameter of the inhibition zone was measured in millimeters with calipers in three directions.

The zone diameters were averaged and interpreted as resistant (=<11 mm), intermediate (12-21 mm) and susceptible (>=22 mm).

9. Determination of the Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC):(26,27,28,29,30)

All strains of group A streptococci isolated were tested for the MIC by using the micromethod. In order to determine the MBC, the culture suspension(10 µl) of the wells showing no growth was transferred to a blood agar plate. The plate was incubated in candle jar at 35°C to 37°C overnight. The MBC read as the least concentration of

penicillin which produced complete (99.8%) killing of the inoculum.

10. Determination of penicillin serum level by cylinder-plate bioassay with Sarcina lutea ATCC 9341[31] :

Five milliliters of blood were drawn by aseptic technique from each of the six rheumatic heart disease patients and were collected in sterile test tubes without anticoagulant. The sera were separated from clotted blood and tested for penicillin level as soon as possible. Mueller-Hinton agar (Difco) was prepared according to the direction and adjusted the pH to 6 by pH paper. Six milliliters of melted agar were put into screw cap test tube (16x125 mm) were and autoclaved. The medium was allowed to cool down in a water bath to 50°C. A 1:10 dilution of overnight culture of Sarcina lutea ATCC 9341 was then added to the 6 ml of MH agar at 50°C. The content was thoroughly mixed by low speed vortex mixer and poured into a sterile petri dish (15x100 mm). The dish was tilted back and forth to distribute the seed agar evenly and was then placed on a level surface while the seed agar harden. Six cylinders were placed onto the seeded agar. Every alternate cylinder was filled with the reference concentration of penicillin(0.03 μg/ml) whereas the remaining cylinders were filled with the tested sera. The standard penicillin concentration of 0.008,0.015,0.06,0.08 and 0.12 μg/ml were simultaneously employed in order to establish a standard curve for each assay. In order to construct the standard curve, the zone diameter was plotted onto the semilogarithmic paper and was

transformed into the linear regression line.

In order to determine the concentration of penicillin in the sample, the mean value of the three zone diameters of each sample was corrected by adding algebraically the mean diameter of the sample to the difference between the diameter of the correction point (i.e., 0.03 µg/ml in the test plate) and the mean of the three values obtained from the reference concentration (i.e., 0.03 µg/ml in the standard plate) that was tested in parallel. This corrected mean was projected from the abscissa of the standard line for each assay and the corresponding point on the ordinate indicated the apparent concentration of penicillin in the serum[1].

11. Antistreptolysin O titer: The ASO titer was determined by the tube method[1].

RESULTS

Prevalence of beta-hemolytic streptococcus group A from school children, sore throat, ARF and/or RHD patients

The recovery of beta-hemolytic streptococcus group A from 1,611 sore throat patients who visited Maharaj Nakorn Chiang Mai Hospital and Maternal and Child Health Center Region V, Chiang Mai, during November 1984 to December 1985 was 6.39%(Table 1). In 181 old cases of acute rheumatic fever(ARF) and/or rheumatic heart disease patients(RHD), the recovery rate was 2.21%. In 23 new cases of ARF and/or RHD patients, it was 17.39%.

When the patients were divided into four age groups: 0-4, 5-9, 10-14 and >15 years old, the distribution of group A streptococci in the four age groups of sore throat patients was 3.15%, 11.95%, 13.38%, and 5.88%, respectively. In new cases of ARF and/or RHD patients, the recovery rate was 0%, 26.08%, 73.91% and 0%, respectively. In old cases of ARF and/or RHD patients, Group A streptococcus was mostly isolated from patients over 10 years old (Table 2).

The isolation of group A streptococci from children attending four primary schools was shown in Table 3. The prevalence of group A streptococci varied according to the time of the survey and varied from school to school. The frequency of the isolation was higher in June and February than in October. Furthermore, group A streptococci was found mostly in the 5-14 age group as shown in Table 4.

T-patterns of group A streptococci in school children and patients

The T-pattern of 103 isolates of group A streptooocci recovered from sore throat patients was shown in Table 5. T-3/13/B3264 was the predominant type. In four group A positive new cases of ARF and/or RHD patients, each of T-5/12/27, T-8/25/Imp19, T-6 and an untypable strain was found. Only one isolate of T-3/13/B3264 and three isolates of T-8/25/Imp19 in old case of ARF and/or RHD patients were isolated. In school children, T-49 and nontypable streins predominated (11.22% and 29.59%, respectively). For pyoderma patients, T-11 showed the predominant type(17.28%) (Table 5).

The result of oral penicillin treatment in school children

After 5 days of oral penicillin treatment, 9.19% of group A positive school children still harboured the organisms. Among school children, group A streptococci were completely eradicated after 15 days of treatment, but group C or G streptococci were eliminated after 10 days of treatment (Table 6).

Throat swabs were also taken from members of 50 families of group A positive school children. Only two persons from two different families were positive for group A streptococci.

Drug susceptibility test

Isolated group A streptococci were tested for

susceptibility to various antibiotics by the agar disk diffusion test. The result showed that chloramphenicol, penicillin, ampicillin, erythromycin, cefalothin and cefoxitin completely inhibited all group A strains. A few of them was susceptible to tetracycline and co-trimoxazole (Table 7).

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of group A streptococci from school children and patients

The mean values of MIC of group A streptococci from sore throat, ARF and/or RHD, wound infection patients and school children to penicillin were 0.022±0.007, 0.024±0.007, and 0.024±0.011 µg/ml,respectively. For MBC, the mean values were 0.025±0.007, 0.026±0.006 and 0.031±0.015 µg/ml, respectively. There were no significant differences between the MICs, MBCs and MBC/MIC of group A streptococci isolated from patients and school children(Table 8).

Pharmacokinetics of serum penicillin in RHD patients

The serum level of penicillin was determined in six ARF patients, one female and five male, at 1 hour, 1 day, 7 days, 21 days, and 28 days after an intramuscular injection of 1.2 million units of benzathine peniclin G (Table 9 and Figure 2). The peak level of penicillin in all patients was on the first day of the injection. After 14 days of penicillin injections, the penicillin level in 2 out of 6 patients' was lower than the protective level against group

A streptococci (i.e., compared with the mean MIC value). After 21 days of the prophylaxis the penicillin level of three patients was lower than the MIC mean value, and after 28 days of the prophylaxis, all of the six ARF patients had the penicillin level lower than the MIC mean value. The serum half-life values of penicillin in these six patients were 10.50, 7.00, 9.00, 6.25, 10.25, and 7.25 days.

Antistreptolysin O titer in patients and students'

The mean values (±standard error,S.E.) of ASO (in Todd Units) from group A streptococci positive school children and ARF and/or RHD patients were 509±60 Todd Units and 555±284 Todd Units, respectively. During the same period, group A streptococci negative school children and patients had mean ASO value of 381±71 TU for group C positive, 388±91 TU for group G positive and 332±33 TU for patients. The normal value of ASO in the Northern Thai population is 250 Todd Units. Therefore, 47.83% of the group A positive school children and 33.33% of group A positive ARF patients had higher antistreptolysin O titer than the normal value. In addition, some of group A streptococci negative school children and patients also had higher titers of ASO than normal value(53.33% and 28%, respectively)(Table 10).

DISCUSSION

Prevalence of group A streptococci in patients and school children

The recovery rate of group A streptococci from 1,611 sore throat patients in this study was 6.39%. In other studies, the recovery rate varied from 5 to 35 percent according to the age group and season[16,32,33,34,35,36]. The low percentage of isolation in this study may be because the subjects in this study were did not only have exudative sore throat but also nonexudative sore throat causative agents may not have been group A streptococci. Roose et al (1985)[37] found that there was a highly significant correlation between redness in the oropharynx ($p=0.004$) and a positive culture for group A streptococci. The patients with streptococci group A also had marked redness of the tonsils in comparison with those with a negative culture ($p=0.015$). In addition, sore throat patients in this study were mostly younger than five years (61.08%). Ardati and Dajani (1980)[38] showed that various group A streptococci adhered poorly to the buccal epithelial cells of children younger than three years. The factors affecting the adherence capacity were:

1) the ratio of group A streptococci to epithelial cells (they found that the maximum adherence (70%) was seen with a ratio of 1000:1).

2) the time for optimum adherence, which was within the

first 15 minutes of incubation. (and did not increase during an additional two-hour incubation).

The prevalence of group A streptococci in sore throat patients in our study varied from 3.15, 11.95, 13.38 to 5.88 percent from age groups 0-4, 5-9, 10-14 to >=15 respectively. Thus, those aged 5 to 14 years had the highest group A streptococci isolation rate. Recovery of group A streptococci was unusual in children under 5 years old and young adults. The higher recovery rate with 5-9 and 10-14 age groups may reflect the fact that these subjects are mostly primary school children and are from low income families, whose knowledge of personnel hygiene may not be sufficient to protect themselves from spreading the organism. Group A streptococci were more frequently isolated from new cases of ARF and/or RHD patients than from old cases. It may be that old case patients were on penicillin prophylaxis, but that some positive cases may have been in contact with group A streptococci at the time when the penicillin serum level was lower than the MIC or MBC of the organisms.

In school children, group A streptococci predominated in the 5-14 year age group in comparison with younger or older age groups. Some of them were sibling or good friends who had close contact (i.e., they used the same cups for drinking water). They may transmit the organism by hands, droplets or utensils. The personnal hygiene of students varied from school to school and from class to class; this

may result in the variation of group A streptococci isolated from different schools and classes in the study of Kaneko et al[4] and our study.

T-patterns of group A streptococci in patients and school children

The predominant T-types in sore throat patients in our study were T-3/13/B3264, T-8/25/Imp19, T-5/11, and T-4/28. Some of these T-types were also found predominated in the study of Dharmasakti, et al.[14](T-4/28, T-3/13/B3264 and T-8/25/Imp19). These types were not only found in the throat but also in skin as in the study of Wannamaker (1970)[12]. However, the interesting strains were the untypable strains which showed up predominantly in our results (17.24%). This A further study is required to find out the association between carriage of their organisms and these strains.

Only two strains of group A streptococci from students' families were isolated. Their T-types are distinct from isolates obtained from the children. Therefore, the students might get the organisms from other family members or from close friends at school. Within family transfer of organisms did not therefore seem to be important.

The result of oral penicillin treatment in school children

It can be inferred from Table 6 that group C and group G streptococci are easier to eradicate than group A streptococci. Green et al (1969)[39] found a good correlation between poor compliance and treatment failure.

In our study, therefore, the students' compliance was checked by counting the remaining penicillin tablets at every visit. Only one student failed to take the drug. In this case, the bacterial culture and the drug administration were restarted and then the data were included. There were no differences in the MIC values of the isolates obtained from the failed treatment students (9.18%,Table 6) and the other isolates. Failure of treatment might be due to the fact that the penicillin level in tonsillar tissue is not sufficient to eradicate the organisms. Higher dosage of penicillin may give a better response[37].

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of penicillin G for group A streptococci

The MIC and MBC of group A streptococci of the sore throat strain were not significantly different from the ARF or RHD strains. The MIC and MBC values can be used as guidelines for the effectiveness of treatment by comparing with penicillin serum levels for patients.

Pharmacokinetics of penicillin in RHD patients

A major problem found in RHD patients is that some of them had a recurrent infection from group A streptococci even though they were on penicillin prophylaxis.[40] Therefore, we are questioning whether the serum penicillin level is sufficient for protecting against reinfection. In studying the pharmacokinetics of serum penicillin from six

patients, it was found that the rate of penicillin reduction varied from patient to patient. This may depend on many factors, such as body size and composition, distribution of drug through fluid compartments, differential binding by serum proteins, and rate of metabolism and excretion. All these determinants are subjected to much individual and temporal variation such as genetic and environmental factors, consequences of disease, concomitant administration of other drugs[41] and doses of penicillin received[40].

The average duration of demonstrable penicillin activity in the plasma in our result is more than 28 days, but at this time, the level is always lower than the MIC and MBC of group A streptococci. This finding is similar to that of Wright et al (1959)[42]. From these results, the penicillin prophylaxis schedule in RHD patients should be reconsidered.

The antistreptolysin O (ASO) titer

Only 47.83% of group A streptococci positive school children and 33.33% of ARF and RHD patients had increased ASO titer. In contrary, Burdash et al (1986)[43] found that 85% of patients with acute rheumatic fever have an increase ASO titer. The low incidence of increased ASO titer in ARF/RHD patient may reflect the low number of ARF patients and recurrently infected RHD patients, rather than the low incidence of streptococcal infection or low level of immune response. A group A positive patient who has an increased

ASO titer to 1,250 TU was an ARF patient, but others were RHD patients. Some of the school children who have increased ASO titer were sufferred from mild symptoms of sore throat. Their sore throat may caused by group A streptococci. Actually, ASO determination is still a valuable test to support the diagnosis of ARF and acute streptococcal sore throat, but some false positive may occur in patients infected by other groups of beta-streptococci and the titer may not increase in most patients with skin infections. Therefore, the detection of an elevation of the antibody level to other antigens should be used to confirm the diagnosis of RHD, prolonge carriage of group A streptococci and skin infection, such as the antideoxyribonuclease test (ADNase) or antipolysaccharide antigen[44,45,46].

SUMMARRY

This study focused on the basic data of group A streptococci in school children, sore throat patients, rheumatic fever and rheumatic heart disease patients. These basic data will lead to more in depth studies of group A streptococci in Thailand.

The results showed that the isolation rate of group A streptococci in 1,611 sore throat patients who visited Maharaj Nakorn Chiang Mai Hospital and Maternal and Child Health Center Region V, Chiang Mai during November 1984 to December 1985 was 6.39%. The recovery of group A streptococci from 181 old cases of acute rheumatic fever and/or rheumatic heart disease patients was 2.21%. From the 23 new case of ARF and/or RHD patients, the isolation was 17.39%. In school children, the recovery rate varied from 0.99% to 15.73% depended on the school and the time of the isolation. Group A streptococci in both patients and school children predominate in the age group 5-14 years.

Among 103 strains of group A streptococci in sore throat patients, T-3/13/B3264, T-5/11, T-8/25/Imp19, T-4/28 and untypable strains were identified in the following proportions: 19.42%, 18.45%, 17.48%, 15.53% and 12.62% respectively. In the new case of ARF and/or RHD patients, each of T-5/12/27, T-8/25/Imp19, T-6 and an untypable strain was found. Only one strain of T-3/13/B3264 and three strains of T-8/25/Imp19 were isolated from the throats of old

cases of ARF and/or RHD patients. In healthy school children, T-49 and untypable strains predominate. For pyoderma patients, T-11 was the predominant type. T-patterns in our group A streptococci isolates were quite scattered and some of them were complex types. This result was similar to T-types that were studied in the Philippines but differed from T-types in Japan or western countries. This should have a further study to find out the cause of these complex types.

Oral penicillin treatment in school children who were carries of group A streptococci failed to eradicate the organisms in 9.18% of cases with 10 days. The patient who infected with group A streptococci should take a full dose of antibiotics and follow-up the effectiveness of treatment by culture the organism.

All group A streptococci isolates were susceptible to chloramphenicol, penicillin, ampicillin, erythromycin, cefalothin and cefoxitin. The mean values of MIC/MBC of group A streptococci from sore throat patients, ARF and/or RHD patients, school children and pyoderma patients were 0.022/0.025, 0.024/0.026, 0.024/0.031, and 0.027/0.031 µg/ml, respectively. From the ratio between the MBC and MIC values, all group A streptococci isolates showed no peniclin tolerant or resistant phenomenon. Therefore, penicillin should be a drug of choice for treatment of patients infected by group A streptococci and no allergy to this drug.

Only two members from two different families were positive for group A streptococci. This number is too small to make any conclusion. A further study should be made together with the study of T-pattern of group A streptococci from the children.

The serum penicillin levels in six patients with ARF and/or RHD patients reached peak levels on the first day of benzathine penicillin G(BPG) intramuscular injection, and then decreased rapidly. After 14 days of penicillin administration, two out of six patients had lower penicillin level than the MIC values of group A streptococci. The half-life values of serum penicillin in these six patients were 10.50, 7.00, 9.00, 6.25, 10.25 and 7.25 days.

Only 47.83% of group A streptococci positive school children and 33.33% ARF and RHD patients had an increase in ASO titer, but the number of ARF patients in our study was too small to make a conclusion of this test for diagnostic value. It should have a study of serodiagnosis for rheumatic heart disease diagnostic confirmation and a predictive value.

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Table 1 Prevalence of group A streptococcus from sore throat patients and acute rheumatic fever (ARF) and/or rheumatic heart disease(RHD) patients

Subject	No.of case	No. of group A streptococcus isolated (%)
Sore throat	1,611	103(6.39)
Old case of ARF and/or RHD	181	4(2.21)
New case of ARF and/or RHD	23	4(17.39)
Total	1,815	111(6.12)

Table 2 Age group distribution of group A streptococcus in 1,611 sore throat and 204 ARF and/or RHD patients

Age groups (years)	Sore throat		Old case of ARF		New case of ARF	
	No. patients (%)	No. group A (%)	No. patients (%)	No. group A (%)	No. patients (%)	No. group A (%)
0-4	984 (61.08)	31 (3.13)	0	0	0	0
5-9	385 (23.90)	46 (11.95)	29 (16.02)	0	6 (26.09)	1 (16.67)
10-14	157 (9.75)	21 (13.38)	87 (48.07)	1 (1.15)	17 (73.91)	3 (17.65)
>=15	85 (5.28)	5 (5.88)	65 (35.91)	3 (4.62)	0	0
Total	1,611 (100)	103 (6.39)	181 (100)	4 (2.21)	23 (100)	4 (17.39)

Table 3 The prevalence of group A streptococcus from school children

Date	School	No.children	No.GAS isolated (%)
June	A	211	11(5.21)
	B	134	20(14.92)
	C	101	8(9.92)
	D	117	9(7.69)
October	A	197	0
	B	144	0
	C	101	1(0.99)
	D	101	2(1.98)
February	A	178	28(15.73)
	B	128	15(11.72)
	C	97	9(9.28)
	D	112	2(1.79)
Total		1,621	104(6.42)

GAS = group A streptococcus

Table 4 The distribution of group A streptococcus in various age groups of 560 school children

Age group	No.children (%)	No.group A isolated (%)
0-4	10(1.79)	0
5-9	316(56.43)	50(50.00)
10-14	231(41.25)	49(49.49)
>=15	3(0.54)	1(1.01)
Total	560(100.00)	100(100.00)

Table 5 T-pattern of group A streptococcus from school children and patients

T-pattern	School children	Sore throat	Pyo-derma	ARF and/or RHD		
				Old case	New case	Total
	No.(%)	No.(%)	No.(%)	No.(%)	No.(%)	(%)
1	3(3.06)	3(2.91)	1(1.23)	0	0	7(2.41)
2	1(1.02)	0	0	0	0	1(0.34)
[3,13,B3264]						
3	3(3.06)	0	0	0	0	3(1.03)
13	4(4.08)	0	8(9.88)	0	0	12(4.14)
B3264	2(2.04)	0	4(4.94)	0	0	6(2.07)
3/13/B3264	4(4.08)	20(19.42)	3(3.70)	1(25.00)	0	28(9.65)
[4,28]						
4	2(2.04)	0	6(7.41)	0	0	8(2.76)
28	7(7.14)	0	2(2.47)	0	0	9(3.10)
4/28	0	16(15.53)	0	0	0	16(5.52)
[5,11,12,27,44]						
11	7(7.14)	0	14(17.28)	0	0	21(7.24)
12	1(1.02)	0	0	0	0	1(0.34)
44	1(1.02)	0	0	0	0	1(0.34)
5/11	0	19(18.45)	1(1.43)	0	1(25.00)	21(7.24)
Others	0	0	1(1.23)	0	0	1(0.34)
6	0	5(4.84)	4(4.94)	0	1(25.00)	10(3.45)
[14,49]						
49	11(11.22)	0	2(2.47)	0	0	13(4.48)
14/49	2(2.04)	8(7.77)	0	0	0	10(3.45)
[8,25,Imp19]						
Imp19	5(5.10)	0	9(11.11)	0	0	14(4.83)
25/Imp19	0	0	5(6.17)	0	0	5(1.72)
8/25/Imp19	7(7.14)	18(17.48)	2(2.47)	3(75.00)	1(25.00)	31(10.69)
9	2(2.04)	1(0.97)	0	0	0	3(1.03)
18	1(1.02)	0	0	0	0	1(0.34)
22	1(1.02)	0	0	0	0	1(0.34)
23	2(2.04)	0	4(4.94)	0	0	6(2.07)
Other complex	3(3.06)	0	8(9.88)	0	0	11(3.79)
NT	29(29.59)	13(12.62)	7(8.64)	0	1(25.00)	50(17.24)
Total	98(100)	103(100)	81(100)	4(100)	4(100)	290(100)

NT = nontypable

Table 6 The result of oral penicillin treatment in school children

Streptococcus (group)	Number	Number(%) failure of treatment after day:		
		5	10	15
A	98	9(9.18)	9(9.18)	0
C	9	1(11.11)	0	ND
G	12	5(41.67)	0	ND
Total	119	15(12.61)	9(7.56)	

ND = not done

Table 7 Sensitivity patterns of Streptococcus pyogenes from school children and patients

Subject	Number	Percent susceptible							
		C	T	ST	P	AM	E	CF	FX
School children	42	100	38.1	23.8	100	100	100	100	100
Sore throat, RHD,ARF	133	100	1.5	0	100	100	100	100	100
Pyoderma	66	100	5.9	0	100	100	100	100	100

Table 8 The penicillin minimal inhibitory concentration(MIC) and the minimal bactericidal concentration(MBC) of group A streptococci from school children and patients

Subject & Group A Streptococcus	Number	Mean MIC+SD	Mean MBC+SD	MBC/MIC
School children	50	0.024 ± 0.011	0.031 ± 0.015	1.29
Sore throat	107	0.022 ± 0.007	0.025 ± 0.007	1.15
ARF and/or RHD	15	0.024 ± 0.007	0.026 ± 0.006	1.15
Skin infection(Pyoderma)	87	0.027 ± 0.024	0.037 ± 0.025	1.37

Table 9 : Serum penicillin level in various periods after 1.2 million units benzathine penicillin G injection

Patient no.	sex,age,weight	Serum penicillin level (μg/ml):						Half-life (T1/2) day
		1 hr	day 1	day 7	day 14	day 21	day 28	
1	F ,14 , 43.0	0.086	0.060	0.034	0.021	0.016	0.019	10.50
2	M , 9 , 19.0	0.167	0.201	0.097	0.047	0.028	0.021	7.00
3	M ,17 , 31.5	0.199	0.181	0.083	0.050	0.039	0.012	9.00
4	M ,13 , 50.7	0.104	0.176	0.025	0.039	0.013	0.003	6.25
5	M ,19 , 44.0	0.120	0.116	0.051	0.047	0.028	0.021	10.25
6	M ,13 , 40.5	0.116	0.108	0.012	0.015	0.011	0.004	7.25
$\bar{X} \pm$ S.D.		0.132 ±0.042	0.140 ±0.054	0.050 ±0.034	0.036 ±0.015	0.022 ±0.011	0.013 ±0.008	8.38±1.79
Range		0.086-0.199	0.060-0.201	0.012-0.097	0.015-0.050	0.011-0.039	0.003-0.021	6.25-10.50

F = female , M = male

age = years

weight = kilograms

$\bar{X} \pm$ S.D. = Mean $\pm$ Standard deviation

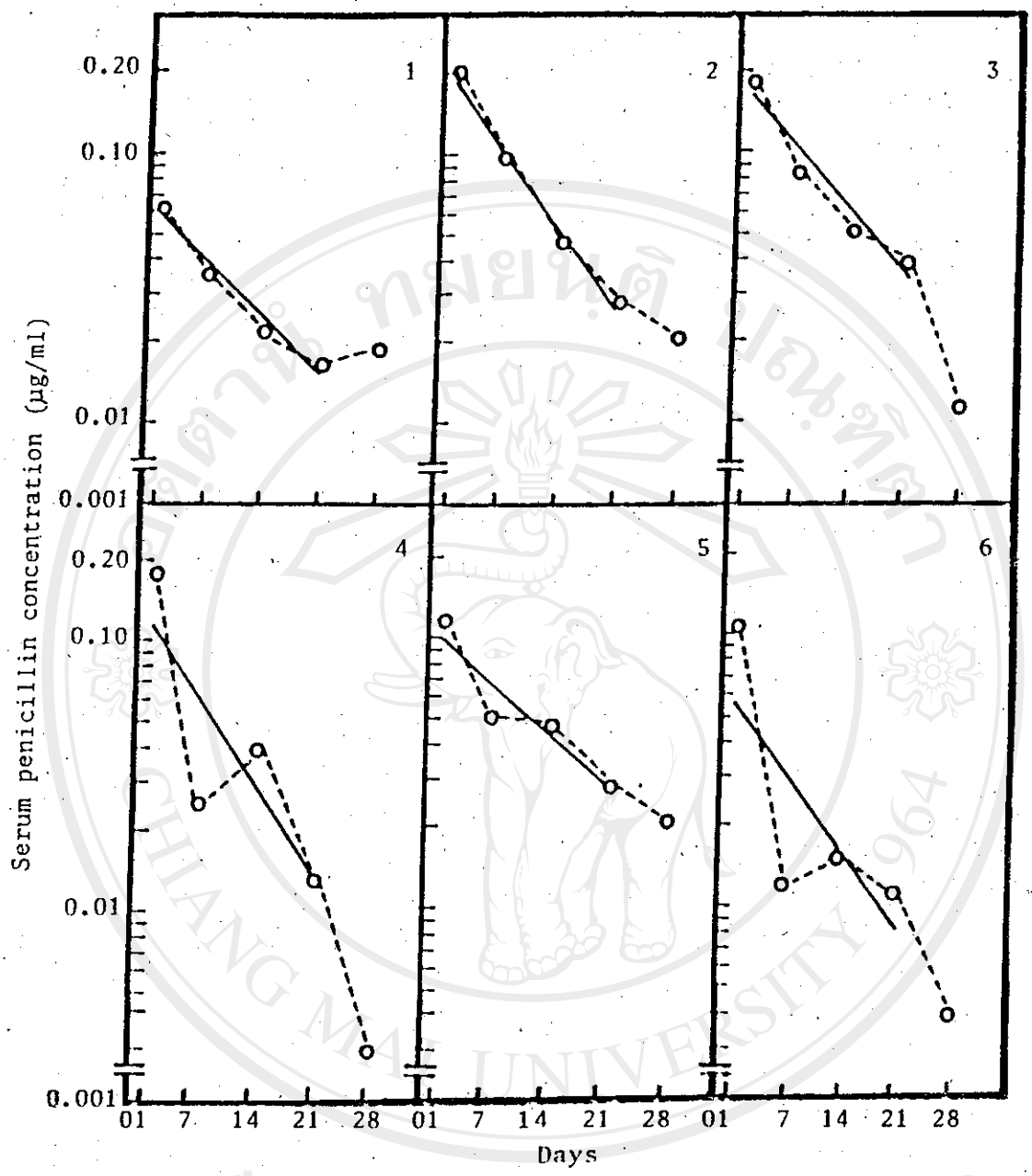

Figure 2 : Pharmacokinetic of serum penicillin concentration after 1.2 million units benzathine penicillin G intramuscular injection

Panel 1,2,3,4,5 and 6 represent the patient number 1,2,3,4,5 and 6 respectively.

o-------o = corection mean

——— = linear regression line

Table 10 The antistreptolysin O(ASO) titer from school children and patients' sera

Subject & Streptococcus (group)	Todd Unit											
	50	100	125	166	250	333	500	625	833	1250	Total	X+S.E.
School children												
A	1	0	1	9	13	3	5	2	3	9	46	509±60
C	0	0	0	2	0	1	2	1	0	0	6	381±71
G	0	0	0	1	2	1	1	0	1	0	6	388±91
ARF and/or RHD												
A	0	0	0	1	1	0	0	0	0	1	3	555±284
Not A	11	5	39	28	7	5	8	5	2	15	125	332±33
Total	12	5	40	41	23	10	16	8	6	25	186	