Title

Fatty acid analysis of Neisseria gonorrhoeae, Neisseria meningitidis, and Egyptian strains by gas liquid chromatography

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Publication Date

1982

Peer reviewed|Thesis/dissertation

Fatty Acid Analysis of *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and Egyptian strains by Gas Liquid Chromatography

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF CLINICAL LABORATORY SCIENCE

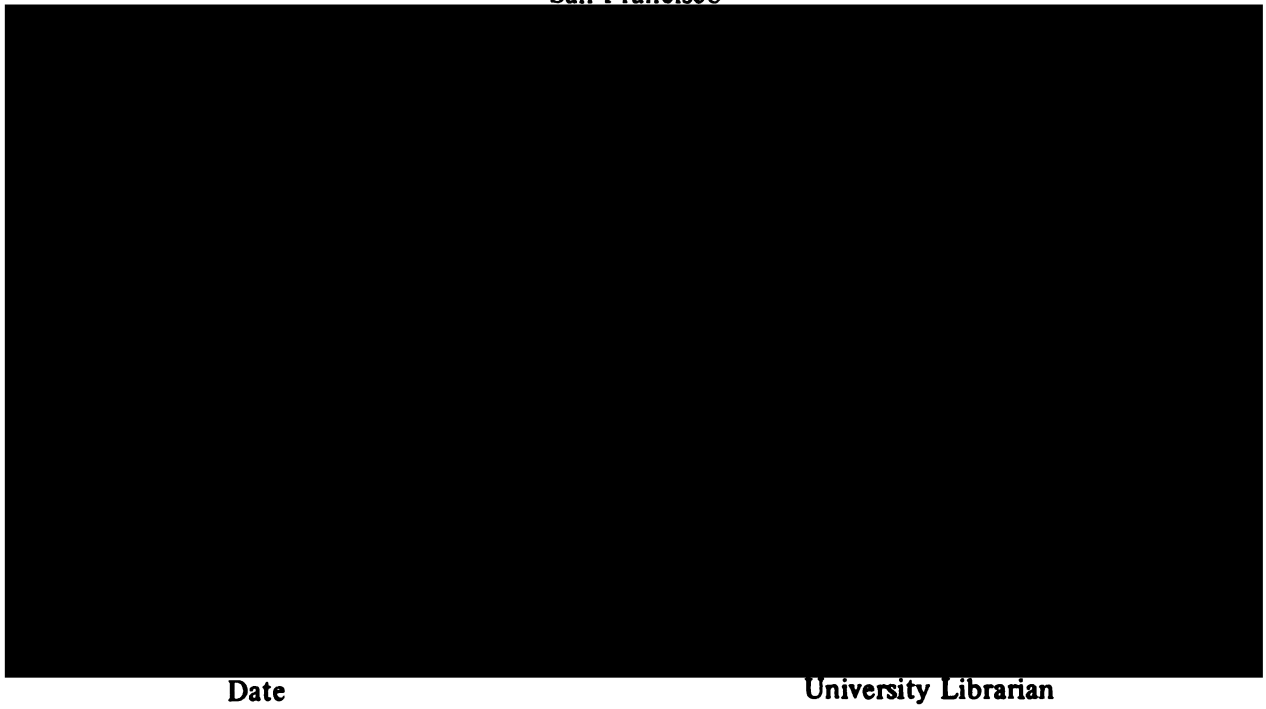
in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco



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ABSTRACT

Gas liquid chromatography (GLC) has been applied in this study to analyze the cellular fatty acid compositions of Neisseria gonorrhoeae, Neisseria meningitidis, and Neisseria species of indeterminant identity (Egyptian strains). All of these organisms are shown to have the following eight fatty acids: lauric acid, 3-hydroxydodecanoic acid, myristic acid, 3-hydroxytetradecanoic acid palmitoleic acid, palmitic acid, oleic acid, and stearic acid. Palmitoleic acid and palmitic acid are the most abundant fatty acids in the organisms studied. The fatty acid composition of the six different colonial types of gonococcus described by Swanson are shown in this study to have very similar cellular fatty acid profiles. On the other hand, the seven meningococcus serogroups are shown here to be relatively diverse in their fatty acid composition. The fatty acid profiles of Egyptian strains were found very similar to CF 1034 (Group X) meningococcus. GLC fatty acid analysis of Neisseria gonorrhoeae and Neisseria meningitidis show that these two organisms can not be differentiated from one another on the basis of fatty acid content.

The presence of a relatively large amount of 3-hydroxydodecanoic acid is characteristic of the pathogenic Neisseria. This can be used to distinguish pathogenic from non-pathogenic organisms.

ACKNOWLEDGEMENTS

I wish to thank the members of my thesis committee for their continued encouragement and advice throughout this study: to Dr. W. Keith Hadley for his valuable advice and generosity of time; to Dr. J. Brooks for providing challenging ideas and for his helpful suggestions; and to Dr. John James for his expert technical contribution throughout the development of this work.

I wish to express my deep gratitude and sincere appreciation to Dr. David Yajko, for his helpful editorial assistance, exceptional guidance and constant support in my academic year.

I thank Ms. Claudia Fenner and Debbie Draper for their willing and expert technical assistance in laboratory.

Lastly, I wish to thank my husband Ming-Ching for his support and forbearance during the years that it has taken to finish my graduate study.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER I

INTRODUCTION

Neisseria are gram-negative cocci 0.6 to 1.0 m in diameter [1]. The organisms are usually seen in pairs with adjacent sides flattened. They are very susceptible to adverse environmental conditions such as drying, chilling, exposure to unfavorable PH or to sunlight.

Neisseria gonorrhoeae and Neisseria meningitidis are the two major medically important species of the genus Neisseria. The organisms are genetically very closely related, but the clinical manifestations of the diseases they produce are quite different. They inhabit the upper respiratory tract and other mucosal surfaces of the body. Most clinical laboratories use carbohydrates utilization tests to distinguish Neisseria gonorrhoeae from Neisseria meningitidis. This method of identification can take up to 48-72 hours before results are obtained. In this study, the fatty acid profile of these organisms will be examined by use of Gas Liquid Chromotography(GLC) to determine whether fatty acid analysis can be used by the clinical laboratories.

1. Neisseria gonorrhoeae

This organism was identified in 1879 by Neisser. The incidence of gonorrhoeae infection is currently estimated at about two million cases in the United States per year and over seven million cases worldwide. Neisseria gonorrhoeae has two major classes of antigens associated with its surface layers: the pili protein antigen, and a polysaccharide antigen that is found in the lipopolysaccharide of the cell wall. Neither of these, however, has been clearly characterized.

1.1 Classification of Neisseria gonorrhoeae

In Kellogg's studies on pili, Neisseria gonorrhoeae were classfied into four different groups: T_1, T_2, T_3, and T_4[2]. Types T_1 and T_2 produced small colonies on agar plates. They were able to cause urethal infection in four tested male volunteers after 69 in vitro passages. Large colony types T_3 and T_4 had selective advantage in vitro, but after 69 in vitro passages were unable to infect human volunteers. The T_1 and T_2 types are heavily piliated organisms. T_3 and T_4 types are non-piliated organisms.

Swanson studied the opacity and piliation of Neisseria gonorrhoeae, and has classified the organism into six different colonial variants[3]: P^{++}_{op}, P^{++}_{Tr}, P^{+}_{op}, P^{+}_{Tr}, P^{-}_{op} and P^{-}_{Tr}. Opaque colonies are composed of gonococci that display extensive intracellular adherence and appear aggregrated. This is associated with increased colony friability, more marked colony highlight reflectance patterns and increased colony doming or thickness. Gonococci forming opaque colonies have one or more cell wall surface proteins not found in organisms from transparent colonies.

A dissecting microscope can be used to differentiate gonococci into six different colonial variants on a 22 hour old plate. The colonial variants have the following appearances:

P^{++}_{op}: very dark, golden with opalescence, smooth edge, diameter of 0.5 - 1.0 mm with a dark ring around each colony. These organisms are highly piliated.

P^{++}_{Tr}: very light, transparent, smooth edge. Diameter of 0.5 - 1.0 mm. Blue gray in color with no or reduced opalescence.

These organisms have a dark ring around each colony, and they are highly piliated.

P^{+}_{op}: dark, golden with opalescence, smooth edge. Diameter of 1.0 mm, without a dark ring around each colony. These organisms have fewer pili than P^{++} strains.

P^{+}_{Tr}: light, transparent, smooth edge. Diameter of about 1.0 mm. Blue gray, without a dark ring around each colony. Fewer pili than P^{++} strains.

P^{-}_{op}: dark, opaque, edge not well visualized. The colony integrity is disrupted by the loop,and sizable chunks of the colony are visible after disruption(unlike mucoid colonies of the transparent strains). Diameter greater than 1 mm. No pili.

P^{-}_{Tr}: very light, transparent, edge not well visualized. Smooth flat colony. No pili.

Different gonococcal variants can also be distinguished by light reflection of the colonies. At approximately 45^{o} angle of incident illumination, P^{-} colonies have no high light(NH), P^{+}_{Tr} colonies have single high light(SH), while P^{++}_{Tr}, P^{+}_{op} and P^{++}_{op} colonies have double high light(DH). At approximately 40^{o} angle of incident illumination, P^{-} colonies have no high light, and $P++_{Tr}$ colonies have single high light. P^{+}_{op} colonies have single to double high light, and P^{++}_{op} colonies will have double high light. (Table 1.1)

Four colonial types of Neisseria gonorrhoeae were described by Kellogg[2] based upon pilation and opacity. However, Swanson classified gonococci into six different colonial variants. Characteristics of

Table 1.1 Light Reflection of Gonococcal Colonies

Approx. angle of illumination	Highlight patterns observed for colonies with differing phenotypes				
	P^-	P^+_{Tr}	P^{++}_{Tr}	P^+_{op}	P^{++}_{op}
45^o	NH	SH	DH	DH	DH
40^o	NH	NH	SH	SH-DH	DH

DH, Double highlight; SH, single highlight;
NH, no highlight.

the two schemes of classification are listed in Table 1.2.

1.2 Virulence factors in Neisseria gonorrhoeae

There is a firm evidence that gonococcal pili directly mediate attachment to cell surfaces[7]. Gonococci with pili are more able to attach to human mucosal surfaces than non-piliated gonococci[4]. Therefore, heavily piliated organisms are more virulent than the less or non-piliated organisms. Pili have a subunit molecular weight of 19,000 $\pm$ 2,500[7]. There is no recognizable terminal attachment organelle to the cell. Pili are antigenically diverse in different strains of gonococcus. Payne used a chicken embryo model to study the pathogenesis and immunology of experimental gonococcal infection This study showed that the ability of gonococci to acquire iron in vivo is a significant factor in virulence. The more virulent colony types appear to have an enhanced ability to compete with the host for iron. This ability may be related to the presence of pili. Another phenomenon is that the more piliated a gonococcus is, the more resistant it is to the bactericidal effect of host serum[5]. The reason for this is unknown. D. W. Thomas[6] studied the interaction of gonococci with phagocytic leukocytes from men and mice, and showed that pili probably help to prevent phagocytosis. However, pili are not the only factor involved in attachment. Other factors may play a role as well. For example, sugar residues on the gonococcal lipopolysaccharide can bind to the host cell membrane to help initiate an infection. Certain surface proteins (M.W.=24,000-30,000) of gonococci have also been postulated to play a role in adhesion[46].

The gonococcal cell envelop like all gram-negative organisms is

Table 1.2 Comparison of the Two Different Classification Schemes for Gonococci by Kellogg and Swanson

Piliation	Color	Opacity	Size	Highlight Pattern (45^{o} angle)	Colonial Morphology	Edge of Colony	Swanson	Kellogg
++	dark	op	$\leq$ 0.5mm	DH	doming	with dark ring	P^{++}_{op}	T2
++	light	Tr	$\leq$ 0.5mm	DH	doming	with dark ring	P^{++}_{Tr}	T2
+	dark	op	.5-1.0mm	SH	convex	smooth	P^{+}_{op}	T1
+	light	Tr	.5-1.0mm	SH	convex	smooth	P^{+}_{Tr}	T1
-	dark	op	>1mm	NH	flat	diffused	P^{-}_{op}	T3
-	light	Tr	>1mm	NH	flat	diffused	P^{-}_{Tr}	T3,T4

composed of three distinct layers: an inner cytoplasmic membrane, a middle peptidoglycan cell wall, and an outer membrane. The outer membrane contains lipopolysaccharide and a variety of proteins plus phospholipids. Lipopolysaccharide appears to play a role in the interaction of gonococci with human serum, perhaps by acting as a receptor for bactericidal antibody [8].

Gonococci may contain a polysaccharide capsule. Recently, three groups have described a gonococcal surface factor[9,10], which excludes india ink, stains with polysaccharide-specific dyes, and appears to inhibit phagocytosis. But the identity and significance of the gonococcal capsules remain to be determined.

Gonococci are not known to produce an extracellular toxin analogous to the enterotoxin or diphtheria toxin. Much of the toxicity resulting from gonococcal infection is probably due to the endotoxic effects of lipopolysaccharide.

Various characteristics of gonococcal strains such as Kellogg colony type 1 or type 2 morphology[12], the possession of pili[13], serum reistance[14], marked sensitivity to penicillin[15], and requirement for arginine, hypoxanthine, and uracil[16,17], have been associated with virulence. However, except for serum resistance and the possible role of pili in enhancing attachment to host cells[18,19, 20], none of these factors per se seems likely to play an active role in virulence.

1.3 Infections caused by Neisseria gonorrhoeae

Gonococcal infections include:

1. Uncomplicated genital gonococcal infections in men. Acute

anterior urethritis in the male [21].

2. Asending genital gonococcal infection in man. Local spread of urethral gonococcal infection in men has been reported to result in protatitis, seminal vesiculitis, epididynmitis, and inguinal lymphadenitis.
3. Uncomplicated genital gonococcal infection in women. The columnar epithelium of the endocervix is the primary site of urogenital infection in the female.
4. Asending genital gonococcal infection in women. Acute asending gonococcal infection acounts for more serious morbidity and economic loss than all other forms of gonococcal infection combined. Between 10% and 17% of women with gonococcal infection develop acute salpingitis [22]. Approximately 20% of such women subsequently have impaired fertility [23].
5. Disseminated gonococcal infection(DGI). Disseminated gonococcal infection has been estimated to occur in 1% to 3% of patients with gonorrhoeae[24,25], and probably is the most common cause of infectious arthritis in the United States. Host factors that appear to increase the likelihood of dissemination include pharngeal infection, menstruation, pregnancy, complement deficiency and possibly liver disease [26]. Dermatitis is present in 59% to 77% of the patients with DGI [27]. Most patients have 5 to 30 lesions which are located primarily on the extremities.
6. Oropharyngeal gonococcal infection.
7. Anorectal gonococcal infection.

8. Pediatric gonococcal infection.

2. Neisseria meningitidis

Neisseria meningitidis has the same general characteristics on gram strain as Neisseria gonorrhoeae. They both can grow on Thayer Martin media and utilize glucose. However, the meningococcus can ferment maltose while the gonococci can not. Also, Neisseria meningitidis produces a polysaccharide capsule which has been characterized. Based on their polysaccharide capsules, Neisseria meningitidis strains can be classified into seven different groups: A, B, C, D, X, Y, Z, 29E, and 135. . . The groups can be identified on the basis of agglutination reactions. Groups A, B and C are responsible for the great majority of clinically recognized diseases [1]. The organisms establish themselves in the membranes of the nasopharynx and enter the body via the upper respiratory tract. The incubation time before the onset of disease is several days to one week.

2.1 Infections caused by Neisseria meningitidis

Infections caused by Neisseria meningitidis include:

1. Bacteremia without sepsis: upper respiratory illness.
2. Meningococcemia without meningitis: patients show leukocytosis, skin rashes, generalized malaise, weakness, headache, and hypotension.
3. Meningitis with or without meningococcemia: patients have headache, fever, meningeal signs, and with a cloudy spinal fluid.
4. The meningoencephalitic presentation: these patients show meningeal signs with cloudy spinal fluid.

3. Egyptian Strains

Egyptian strains were isolated from patients' eye infections in Egypt and were obtained from Dr. Julius Schacter. They are oxidase positive, gram-negative diplococci. The microscopic morphology and biochemical reactions are very similar to pathogenic Neisseria. Several typical biochemiacl reactions for Neisseria gonorrhoeae, Neisseria meningitidis and Egyptian strains are listed in Table 1.3.

Eight different strains were used in this study. They have opaque or transparent colonial morphology. In this study, gas liquid chromatography was used to obtain fatty acid profiles of these strains.

4. Areas of Study

The objective of this thesis work is to use gas liquid chromatography to determine the fatty acids profile of different Neisseria species and Egyptian strains. It consists of the following areas of study:

1. Study the similarities of differences in fatty acid composition among the six Neisseria gonorrhoeae colonial variants. Previous studies using GLC to analyze the cellular fatty acid composition of Neisseria gonorrhoeae were reported by Wiseman [38,40], Lewis [33], Kellogg [2,12], and Brooks [48,49]. They all used the Kellogg classification (T_1, T_2, T_3, T_4) scheme. The outer membrane variants of the cell wall were not taken into consideration. In this thesis work, the fatty acid profile of Neisseria gonorrhoeae strains were studied by applying Swanson's classification ($P{+}{+}_{op}$, $P{+}{+}_{Tr}$, $P{+}_{op}$, $P{+}_{Tr}$,

Table 1.3 Carbohydrate Utilization Tests of *Neisseria gonorrhoeae*, *Neisseria menigitidis*, and Egyptian Strains

	Neisseria gonorrhoeae	*Neisseria menigitidis*	Egyptian Strains
Glucose	+	+	+*
Maltose	-	+	-
Sucrose	-	-	-
Lactose	-	-	-

* weak and delayed

P^-_{Op}, P^-_{Tr}), and a cellular fatty acid profile for each colonial type was determined.

2. Study the similarities or differences in fatty acid composition among different isolates of Neisseria gonorrhoeae.
3. Compare the fatty acids profile of the lipopolysaccharide component with the total cellular fatty acids composition. This may give indirect evidence for the presence or absence of the capsule of Neisseria gonorrhoeae.
4. Study the fatty acid composition of the seven serogroups of Neisseria menigitidis. First, compare the fatty acid profile of N. gonorrhoeae with that of N. menigitidis. Determine whether the separation of the two species can be based on the results of the gas liquid chromatography. Second, compare the fatty acids profile of the seven serogroups of Neisseria menigiditis in order to see whether fatty acid composition correlates with differences in virulence.
5. Study the Egyptian strains. Determine the fatty acid composition of these strains, and compare the data among the Egyptian strains. Compare the data from all the Egyptian strains with the data from all the gonococcus strains and all the meningococcus serogroups to determine their similarities to either gonococcus or meningococcus.
6. Study the short chain fatty acids (metabolites) of N. gonorrhoeae . First, compare the data among six different colonial variants. Determine whether there are quantitative or qualitative differences. Second, based on the composition of

the fatty acids, determine whether toxic fatty acids are being produced by the gonococcus.

CHAPTER II

MATERIALS AND METHODS

1. Media

The growth medium used for all the cellular fatty acids studies of Neisseria gonorrhoeae, Neisseria meningitidis, and Egyptian strains was clear PPT agar medium. It contains per liter: protese No.3, 7.5g; thiotone peptone, 7.5g; sodium chloride, 5.0g; K_2HPO_4, 4.0g; KH_2PO_2, 1.0g; soluble starch, 1.0g; Nobel agar,12g; and IsoVitaleX. The IsoVitaleX should be freshly thawed. The pH after autoclaving was 7.4±0.1. This is critical for the growth of gonococcus. All media were stored at room temperature until used.

Dr. James' modification of Catlin Neisseria medium in liquid form was used to harvest short chain fatty acids of gonococcal metabolism. This medium was prepared as follows: Catlin Neisseria chemically defined medium w/o glycerin, 18.2g; H_2O 950ml; IsoVitaleX, 10ml; Glucose, 6.5g; K_2HSO_4, 7.4g; Sodium Bicarbonate, 1.0g; Glycerol, 0.91g; adjust volume to 1000 ml. The pH before adding IsoVitaleX and filter sterilization was 7.4±0.1. Medium was stored at 4°C until needed. The original formulation of Catlin broth medium was tested in this study. However, the growth of each colonial variant would not stay in its original phenotype. For example: $P{+}{+}_{op}$ organisms after 22 hours growth in Catlin broth medium at 35°C in a shaking water bath would become $P{+}{+}_{Tr}$. Dr. James' modification of Catlin Neisseria defined medium can stablize the growth of certain variants.

2. Organisms

2.1 Neisseria gonorrhoeae

F62 strains: the cultures were obtained from Dr. Swanson's Lab. According to different opacity and piliation characteristics, the organisms were classfied as $P{+}{+}_{op}$, $P{+}{+}_{Tr}$, $P{+}_{op}$, $P{+}_{Tr}$, $P{-}_{op}$ and $P{-}_{Tr}$, with the aid of a dissecting microscope after 20 hours of growth on clear PPT agar medium. These variants were passed daily.

Other gonococcal strains: $1436P{+}{+}_{Tr}$ and $1436\ P{+}{+}_{op}$ were obtained from patients at U. C. Hospital on June 13, 1979. $1446\ P{-}_{op}$ and $1446\ P{-}_{Tr}$ were obtained from patients at U. C. Hospital on January 28, 1980. $1420\ P{-}_{op}$ and $1420\ P{-}_{Tr}$ were obtained from the microbiology laboratory of U. C. Hospital on February 14, 1980. The above organisms were stored at $-70^{o}C$ until used. A gram stain was done before every experiment to check the purity.

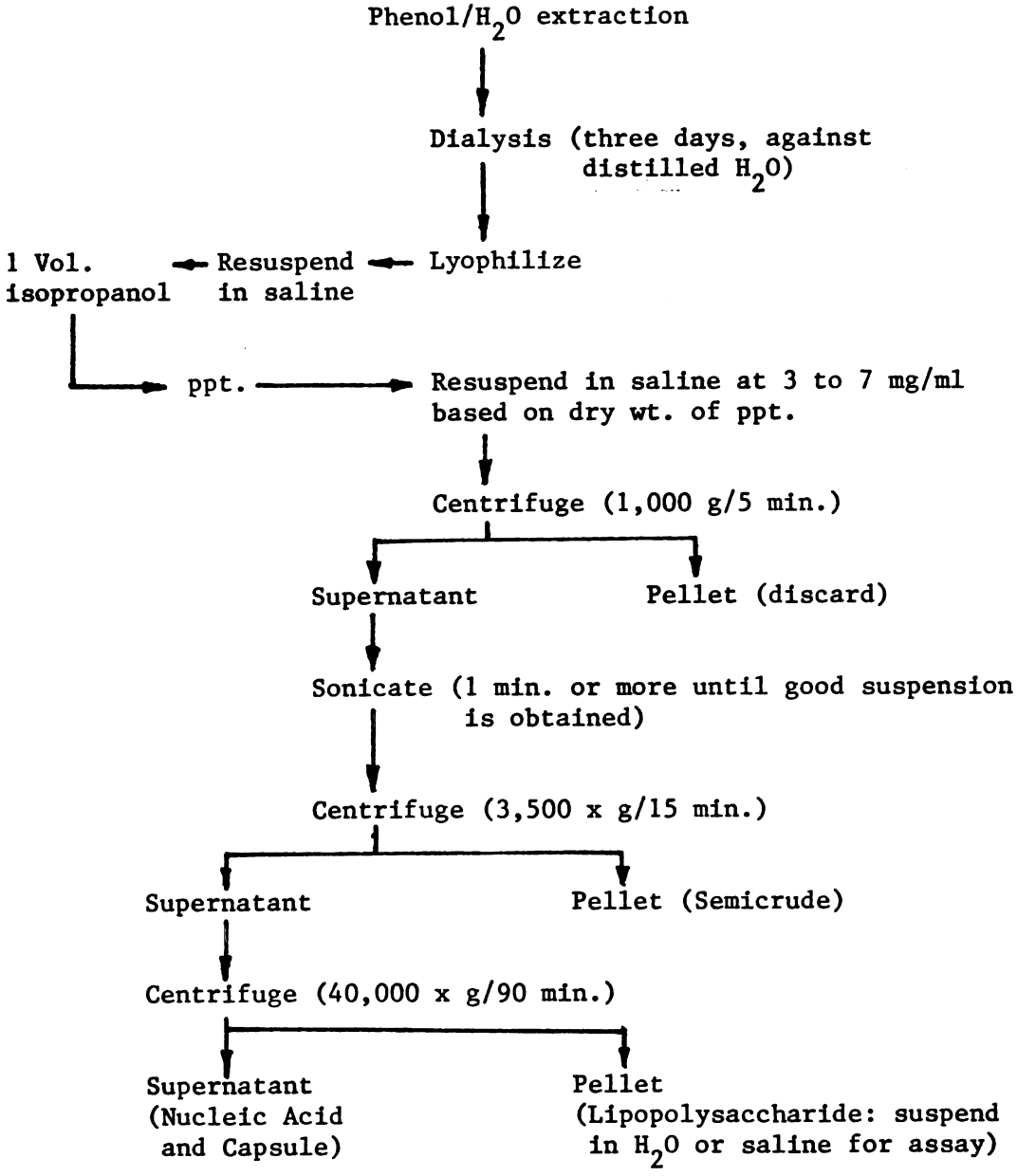
2.2 Neisseria meningitidis

Strains CF 1030 (group A), CF 1031 (group B), CF 1032 (group C), CF 1033 (group D), CF 1034 (group X), CF 1035 (group Y), and CF 1036 (group Z) were obtained from the Center for Disease Control. All of the strains were stored at $-70^{o}C$ until used. Gram stains were done before each experiment to check the purity of the culture. Strains 7mg (group C), M137 (group C), M139 (group A), CF 1038 (groupA), and M1797 (group B) were obtained from Dr. Vedros' laboratory.

2.3 Egyptian strains

Eg 1, Eg 2, Eg 3, Eg 4, Eg 5, Eg 6, Eg 7 and Eg 8 were originally obtained from Dr. Schacter's laboratory. All of the strains were stored at $-70^{o}C$ until used.

3. Lipopolysaccharide purification



The above purification procedure was reported by Dr. Douglas of UCSF. The semicrude and pure pellet were suspended in saline, and were stored in a freezer at -70°C before use.

4. Gas Liquid Chromatography

4.1 Principle of Gas Liquid Chromatography

Separation of fatty acids by gas chromatography is carried out in the elution mode, i.e., the vaprous sample is introduced as a "plug" into the carrier gas stream at the inlet of the column. The carrier gas, called the mobile phase, flows through the column continuously and the sample components, described as solutes, are distributed between the mobile phase and the finely distributed liquid stationary phase in the column. As a result of the sweeping action of the carrier gas flow and the distribution of the solutes between the two phases to different extents (depending on their "affinity" to the stationary phase), the sample components emerge as more or less separated bands at the column outlet, and are recorded as peaks on the chromatogram.

4.2 Description of the gas chromatograph

Analysis was performed on a Varian Model 1200 gas chromatograph, eqipped with a hydrogen flame detector and Bio-Rad Laboratory Model 1310 strip chart recorder. The instrument was equipped with a coiled glass column with outside diameter of 0.25 inch, inside diameter of 2mm, and length of 10 feet. The column was packed with: (1) 3%SP-2100 DOH on 100/120 supelcoport(Supelco Inc., Bellefonte, PA) for cellular fatty acid separation (C 11:0 - C 20:0). (2) 15% SP-1220/1% H_3PO_4 on 100/120 chromosorb W AW.(Supelco, Inc., Bellefonte, PA) for the separation of volatile fatty acids and non-volatile methylated fatty acids.

4.3 Operating parameters

(1) Bacterial cellular fatty acids:

Detector temperature: 250°C.

Column temperature: Programmed for initial temperature of 150°C to final temperature of 225°C at a rate of 4°C/min.

Injectionport temperature: None (direct injection).

Carrier gas: Nitrogen.

Flow rate: 25 ml/min.

Range: 8 x 10^{-10}afs.

Hydrogen: 25ml/min.

Chart drive: 0.5 inch/min.

(2) Analysis of volatile fatty acids and non-volatile methylated fatty acids:

Detector temperature: 155°C.

Column temperature: 155°C (isothermal).

Carrier gas: Nitrogen.

Flow rate: 25 ml/min.

Range: 4x10^{-10}afs.

Hydrogen: 25 ml/min.

Air: 300 ml/min.

Chart drive: 0.5 inch/min.

4.4 Operation

(1) A newly packed column was conditioned for 24 hours at a temperature which was 50°C higher than the final temperature of the routine set up.

(2) There was a 20 minute dwell between each injection for the "dirty" residue to elute.

(3) Column was washed by organic solvent (ex: methanol, choloform)

between each run.

(4) Nitrogen gas flow was turned on all the time to prevent deterioration of the column.

(5) Gas flow rate was tested daily (including N_2, H_2 and air).

(6) Gas tight syringe was washed in organic solvent 4-5 times after every usage.

4.5 Measurement

In order to compare the fatty acid profile of each tested organism, every elution peak was measured for the whole chromatogram. There are two methods of measurement approach, namely (1) Peak area measurement, and (2) Peak height measurement. As shown in Figure 2.1, the line AC is the base line for a elution peak ADC. The area enclosed between peak and base line is the peak area. The distance from the baseline to peak maxima, BD, is the peak height.

The peak area measurement has the following disadvantages:

(1) It is time consuming. The measurement of "Height x Width at half height" is required.

(2) Peak areas are less dependent than peak height under current operating conditions (samples are less than 10 μg).

(3) According to Varian manual, plots of peak height vs. sample size have a more greater linear range than corresponding plots for peak area.

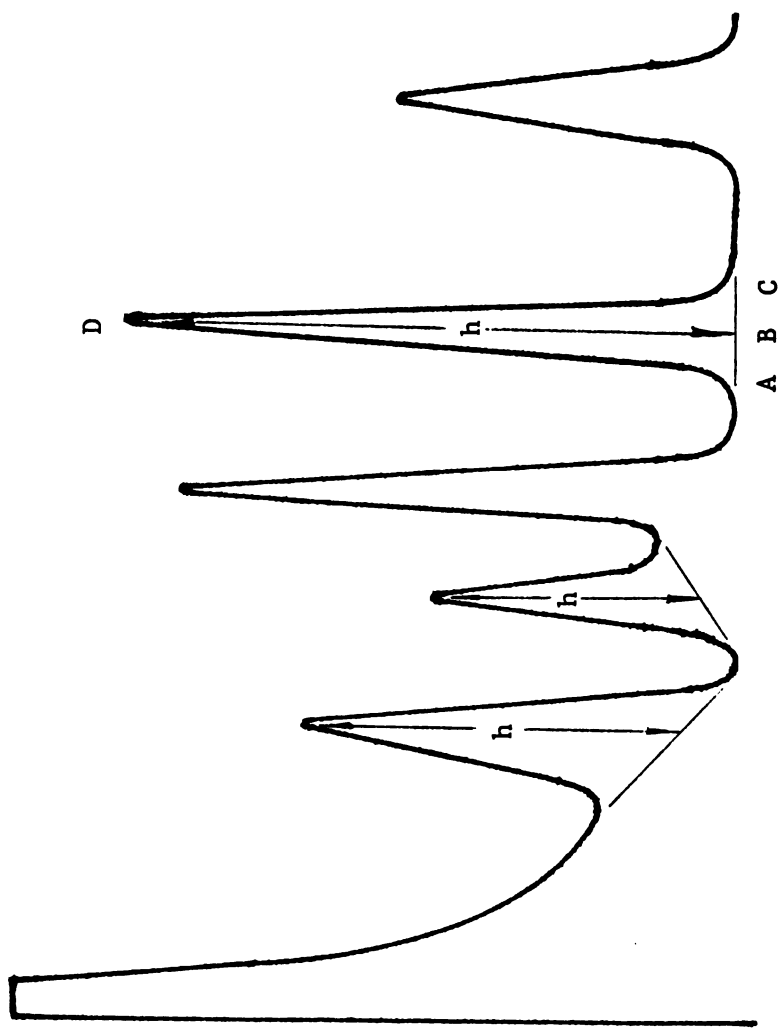


Fig. 2.1 Peak Measurement

Therefore, the peak height measurement was used throughout this study.

Peak height was measured in mm. In a chromatogram, we divide each single peak height by the sum of all the peak heights to get a percentage of this particular fatty acid derivative (methyl ester) relative to the total cellular fatty acid methyl esters. All the comparisons of results discussed in the following chapter are based on percentage calculation described above.

4.6 Preparation of Bacterial Strains for Gas Liquid Chromatography

All the organisms (Neisseria gonorrhoeae, Neisseria meningitidis and Egyptian strains) were grown under 5% CO_2 at 35°C for 18-20 hours with optimal humidity. For every test, there was a media control. The procedures for obtaining fatty acids are as follows:

Method I - Cellular fatty acid composition

(1) A gram stain was done to check the purity of the organisms.

(2) Glass slides and a platinum loop were used to scrape the cells from three plates of 20 hour cultures. Cells were suspended in 4.5 ml distilled water in a small tube and standardized to 500 Klett units.

(3) Two 0.5 ml suspension aliquots were transferred separately into two 4 ml teflon top vials with 0.5 ml 20% NaOH in 50% methanol solution.

(4) The bacterial cell suspensions were saponified for 40 minutes at 100°C in a water bath.

(5) The pH was lowered to 2.0 with 6N HCl.

(6) The methyl esters of the long chain fatty acids were then formed by adding 1 ml of 10% boron trichloride-methanol reagent (w/vol), and the mixture was heated for 5 minutes at 80°C in a water bath.

(7) The fatty acid methyl esters were then extracted from the cooled mixture with 2 ml of chloroform/hexane (1:4). A few drops of saturated NaCl solution were added to enhance the separation. A second extraction with 2 ml of solvent removed only trace amounts of methyl esters (98% by the first extraction, less than 1-2% by second extraction). The upper layer solvent containing the fatty acid methyl esters were carefully removed by Pasteur pipet, and were combined into a vial with a teflon top cap to prevent reaction between the solvent and the cap. The sample was evaporated to a volume of approximately 0.2 ml under a gentle stream of air.

(8) A small amount of Na_2SO_4 is added to remove moisture, and the methyl esters are analyzed by gas liquid chromatography.

(9) A bacterial fatty acids standard was run on a daily basis before any analysis. The bacterial fatty acids were identified by the comparison of the retention times of their esters on the appropriate column.

(10) Inject 4 µl of the methyl ester sample into the column, and keep the remainder at -70°C.

Method II - Cellular fatty acid composition

(1) Prepare a gram stain from two identical 20 hour plates.

(2) Harvest the organisms with glass slides and a platinum loop. Suspend into 0.5 ml distilled water in a 4 ml small vial with teflon cap.

(3) Add 0.5 ml of 20% NaOH in 50% methanol solution to each vial.

(4) Saponification, acidification, dehydration, and standardization were the same as in Method I.

Method III - Volatile fatty acids and alcohol metabolites

(1) Warm "James' modified Catlin broth medium" to 35°C in a water bath.

(2) Pipet 5 ml medium into each tube (for each organism, make two tubes) and add one loopful of 20 hr growth of organisms from PPT media. Vortex lightly to break large clumps of organisms. Incubate at 35°C in a shaking water bath for 20-22 hours.

(3) Make a gram stain to check the purity of the organism.

(4) Acidify culture to pH2 or below using approximately 0.1 ml 50% aqueous H_2SO_4(v/v).

(5) Pipet 2 ml of the acidified culture into a conical centrifuge tube. Add 1 ml ethyl ether and stopper the tube. Mix ether and culture by inverting the tube 20 times. Pour the ether layer into a small test tube. Add anhydrous $MgSO_4$ or $CaSO_4$ to equal approximately one-half the volume of ether in the tube, stopper, and let stand at least for 10 minutes.

(6) Inject 14 µl of the ether extract into the GC column (run the bacterial standard parallel to the test).

Method IV - Methyl derivatives of non-volatile acids of Neisseria metabolism

(1) Growth of the organisms and acidification of the culture are the same as Method III.

(2) Pipet 1 ml of the original acidified culture into a test tube (for each organism, there were two tubes in the test). Add 2 ml methanol and 0.4 ml of 50% H_2SO_4. Stopper, and heat at 55°C in a temperature bloc for 30 minutes. Add 1 ml water and 0.5 ml chloroform, replace stopper, mix by gentle inversion of the tube 20 times.

(3) Pipet the chloroform layer (underneath the aqueous layer) into a small vial. Inject 14 1 of the chloroform extract into the GLC column. The remainder was stored at -70°C for later studies.

Method V - Fatty acid composition of lipopolysaccharide

Crude and semicrude lipopolysaccharide was prepared as described earlier and stored at -70°C. Pipet 1 ml of each and process as described in Method I.

4.7 Method of Analysis

To compare the similarities or differences among fatty acid profiles, the statistical method "t-Test" was used in this study to decide whether the difference between sample means was significant.

For every two comparable organisms, a t value was calculated by

$$t = \frac{(\bar{x} - \bar{x}')}{S_{(\bar{x}-\bar{x}')}}$$

where $(\bar{x} - \bar{x}')$ is the observed difference of sample means, and $S_{(\bar{x}-\bar{x}')}$ is the estimate of its standard error.

For each value of t, we can calculate the value of P (level of significance) according to the following equation [50, 51] :

$$P = 1 - \phi(t) + Z(t) \times \left[\tfrac{1}{4}\, t(t^2+1)V^{-1} + \frac{1}{96}\, t(3t^6-7t^4-5t^2-3)V^{-2} + \frac{1}{384}\, t(t^{10}-11t^8+14t^6+6t^4-3t^2-15)V^{-3}\right]$$

where

$$Z(t) = \frac{(\sqrt{2\pi})^{-1} e^{-\frac{1}{2}t^2}}{\phi(x)}$$

and

$$\phi(x) = (\sqrt{2\pi})^{-1} \int_{-\infty}^{x} e^{-\frac{1}{2}r^2}\, dr$$

and V = degree of freedom

A proper value of P was selected as the criterion for accepting or rejecting the hypothesis that the organisms are similar. Due to the relatively small degrees of freedom of all the tests, a value of 0.1 was chosen for P. The similarity of a fatty acid between two organisms is accepted if P value is equal to or greater than 0.1.

CHAPTER III

RESULTS

1. Background

Tentative identification of each elution peak by gas liquid chromatography was established by comparing with a bacterial fatty acid standard mixture, which contains 23 fatty acid methyl esters, obtained from Sulpelco Inc. (Table 3.1). The chromatogram of this standard mixture is shown in Figure 3.1. Methyl esters of fatty acids containing 10 to 20 carbon atoms can be identified in the extract of whole bacteria. A typical chromatogram is shown in Figure 3.2.

In this fatty acid analysis study, PPT agar medium and James' modification of Catlin broth medium were tested as controls to determine whether fatty acids were present in the uninoculated media. Neither of them showed any peak in the chromatogram. This suggested that there was no measurable contaminant in the media which would interfere with the test results. For every analytical test, a parallel test was done to check the reproducibility and to evaluate the accuracy of the data.

Fatty acid analysis was done by GLC on the following organisms:

(1) Gonococcus: F62 gonococcus (P^{++}_{op}, P^{++}_{Tr}, P^{+}_{op}, P^{+}_{Tr}, P^{-}_{op}, P^{-}_{Tr}), 1420 gonococcus (P^{-}_{op}, P^{-}_{Tr}), 1436 (P^{++}_{op}, P^{++}_{Tr}), 1446 (P^{+}_{op}, P^{+}_{Tr}).

(2) Meningicoccus: CF 1030(A), CF 1031(B), CF1032 (C), CF 1033(D), CF 1034(X), CF 1035(Y), M 139(A), M 1797(B), M 137(C), and 7mg(C).

(3) Egyptian strain: EG-1, EG-2(Tr,op), EG-3, EG-5, EG-7, and EG-8.

Table 3.1 Composition of bacterial fatty acid standard mixture listed in order of elution on 3% SP-2100 DOH

Shorthand Designation	Name
1. 11 : 0	Methyl Undecanoate
2. 2-OH 10 : 0	Methyl 2-Hydroxydecanoate
3. 12 : 0	Methyl Laurate (Dodecanoate)
4. 13 : 0	Methyl Tridecanoate
5. 2-OH 12 : 0	Methyl 2-Hydroxy Dodecanoate
6. 3-OH 12 : 0	Methyl 3-Hydroxy Dodecanoate
7. 14 : 0	Methyl Myristate (Tetradecanoate
8. a-15 : 0	Methyl 12-Methyl Tetradecanoate
9. 15 : 0	Methyl Pentadecanoate
10. 2-OH 14 : 0	Methyl -2-Hydroxy Tetradecanoate
11. 3-OH 14 : 0	Methyl -3-Hydroxy Tetradecanoate
12. 16 : 1	Methyl Palmitoleate(9-hexadecenoate)
13. 16 : 0	Methyl Palmitate(hexadecanoate)
14. a-17 : 0	Methyl 14-Methyl Hexadecanoate
15. 17: 0	Methyl dl-cis-9,10-Methyl Hexadecanoate
16. 17 :0	Methyl Heptadecanoate
17. 2-OH 16 : 0	Methyl 2-Hydroxyhexadecanoate
18. 3-OH 16 : 0	Methyl 3-Hydroxyhexadecanoate
19. 18 : 1	Methyl Oleate (octadecenoate)
20. 18 : 0	Methyl Stearte (octadecanoate)
21. 19 : 0	Methyl dl-cis-9,10-Methylene-octadecanoate
22. 19 : 0	Methyl Nonadecanoate
23. 20 : 0	Methyl Arachidate(eicosanoate)

* In the bacterial fatty acid standard mixture designation, the first number gives the number of carbon stoms in the fatty acid, and the secondindicates the number of double bonds.(29)

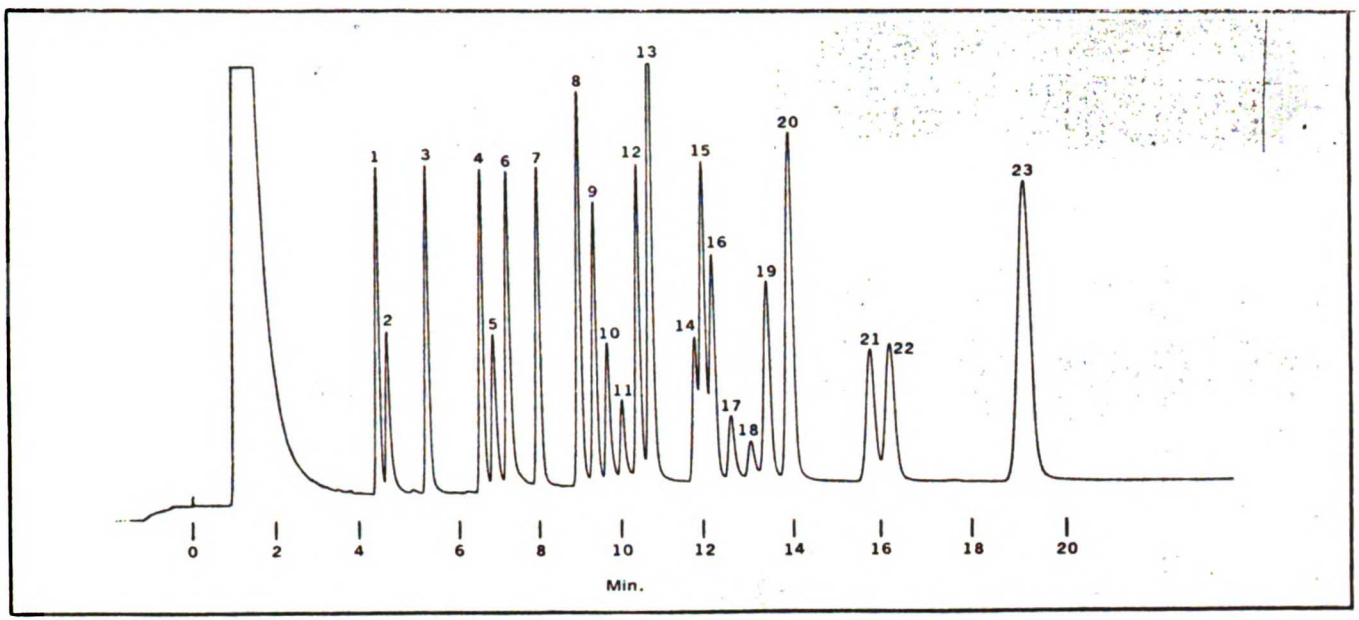


Fig. 3.1 Chromatogram of Bacterial Fatty Acid Standard (For the identification of the peaks, see Table 3.1)

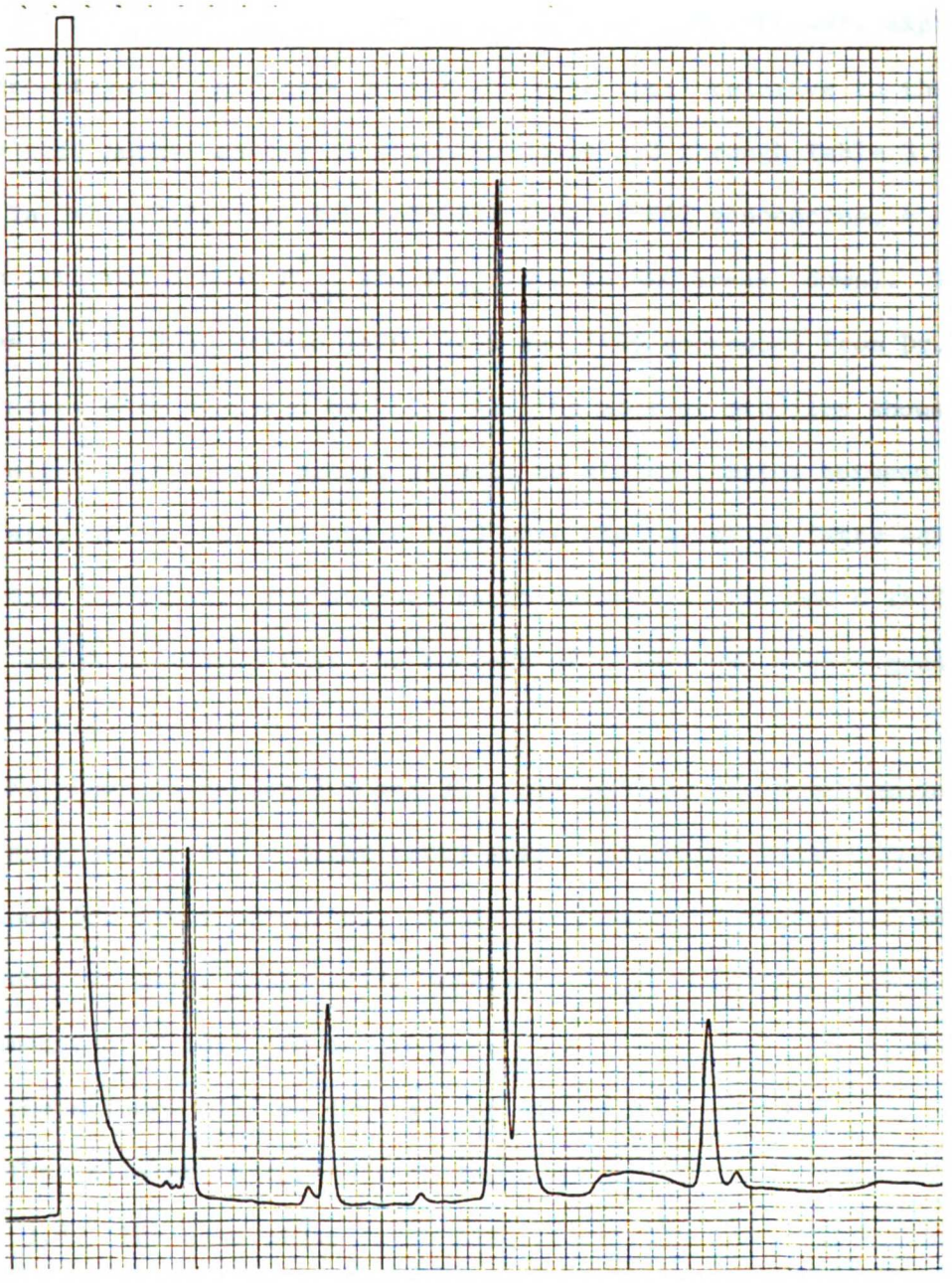


Fig. 3.2 Chromatogram of Eg-3.

(4) lipopolysaccharide of F62 gonococcus and 1278 gonococcus.

2. Peak height relative to the total fatty acid methyl esters

Cellular fatty acid chromatograms obtained by GLC were expressed by percentage of each peak height (or peak area) relative to the total fatty acid methyl esters as shown in Table 3.2 through Table 3.13.

In Table 3.2, for each colonial type of F62 gonococcus, 4 or 5 tests were run except P^{+}_{op} and P^{+}_{Tr} (due to GLC break down). In Tables 3.3 to 3.5, strain 1420, 1436 and 1440 gonococci from Dr. Brook's laboratory were analyzed by GLC. All the elution profiles showed the same eight fatty acids peaks, as well as similar ratios between every two fatty acids. C 16:1 and C 16:0 were the two major fatty acids of the eight measured. The average ratio between C 16:1 and C 16:0 is 1.28, which indicates that the content of C 16:1 was always greater than C 16:0.

As shown in Tables 3.6 and 3.7, six serogroups of meningococci were analyzed by GLC. Eight fatty acids (identical to gonococci) were obtained for every serogroup. As in gonococcus, C 16:1 and C 16:0 were the two major fatty acids among the eight measured. However, the amount of C 16:1 can be greater, equal to or less than C 16:0. More comparisons will be done at the end of this chapter.

The cellular fatty acids of Egyptian strains are listed in Table 3.8. The same eight fatty acids were obtained as in gonococcus and meningococcus, and C 16:1 and C 16:0 were the two major fatty acids. Except for Eg-7, the content of C 16:1 was found greater than C 16:0. The ratios between C 12:0 and C 14:0 are very close to that in meningococcus (Group X) CF1034 (Table 3.9).

Table 3.2 Fatty Acids Analysis of F62 Gonococcus

	Peak height (%) relative to total cellular methyl ester									
	P^{++}_{op}		P^{++}_{Tr}		P^{-}_{op}		P^{-}_{Tr}		P^{+}_{op}	P^{+}_{Tr}
	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ		
12 : 0	8.19	2.43	8.9	0.9	11.69	2.55	12.0	4.65	9.13	6.1
3-OH 12 : 0	0.54	0.16	1.09	0.88	1.33	1.12	1.13	0.7	0.4	1.9
14 : 0	8.27	0.89	8.94	0.69	9.55	0.88	7.7	0.97	8.8	8.4
3-OH 14 : 0	0.7	0.83	0.35	0.1	0.53	0.46	0.45	0.34	0.4	0.5
16 : 1	40.85	6.27	39.8	6.75	42.4	4.9	43.68	2.7	42.0	42.5
16 : 0	28.18	4.21	28.05	4.85	32.8	5.56	29.9	1.12	31.2	31.0
18 : 1	7.9	0.98	6.7	1.17	6.75	2.53	8.25	2.2	8.0	8.9
18 : 0	0.25	0.1	0.15	0.1	0.15	0.1	0.24	0.2	0.4	0.5

Table 3.3 Fatty Acids Analysis of 1420 Gonococcus

	Peak height (%) relative to total cellular methyl ester		
	$P-_{Tr}$		$P-_{op}$
	$\bar{X}$	σ	
12 : 0	12.77	3.04	13.8
3-OH12 : 0	1.01	0.19	2.1
14 : 0	12.1	1.49	11.0
3-OH14 : 0	0.77	0.09	0.7
16 : 1	31.6	1.91	30.5
16 : 0	26.1	1.85	27.3
18 : 1	15.33	2.3	14.0
18 : 0	0.63	0.12	0.6

Table 3.4 Fatty Acids Analysis of 1436 Gonococcus

Peak height (%) relative to total cellular methyl ester

	P^{++}_{Op}		P^{++}_{Tr}	
	$\bar{X}$	σ	$\bar{X}$	σ
12 : 0	11.38	1.47	11.23	1.33
3-OH 12 : 0	0.69	0.17	0.79	0.66
14 : 0	9.88	1.3	9.03	1.9
3-OH 14 : 0	0.2	0.1	0.57	0.38
16 : 1	35.9	2.02	37.0	0.92
16 : 0	31.25	2.09	30.6	2.4
18 : 1	9.28	2.51	7.2	0.35
18 : 0	0.48	0.19	0.6	0.26

Table 3.5 Cellular Fatty Acids Analysis of 1446 Gonococcus

Peak height (%) relative to total cellular methyl ester

	$P-_{Op}$		$P-_{Tr}$	
	$\bar{X}$	σ	$\bar{X}$	σ
12 : 0	14.5	1.1	14.5	2.5
3-OH 12 : 0	0.8	0.1	0.85	0.2
14 : 0	10.8	0.7	11.2	2.5
3-OH 14 : 0	0.45	0.07	0.6	0.1
16 : 1	34.2	4.53	30.75	2.5
16 : 0	28.8	0.35	26.0	5.6
18 : 1	10.0	4.6	15.8	3.1
18 : 0	0.35	0.07	0.55	0.07

Table 3.6 Cellular Fatty Acids of Meningococcus

Peak height (%) relative to total cellular methyl ester

		CF1030(Gr.A)		CF 1031(Gr.B)		CF 1032(Gr.C)	
		$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
	12 : 0	17.35	5.1	13.6	0.0	15.0	0.0
3-OH	12 : 0	1.15	0.21	1.0	0.0	0.55	0.21
	14 : 0	13.45	2.19	11.85	0.21	12.0	0.0
3-OH	14 : 0	0.45	0.07	0.45	0.07	0.25	0.07
	16 : 1	33.5	2.12	34.5	0.71	32.0	1.41
	16 : 0	30.0	4.24	32.5	0.71	32.0	1.41
	18 : 1	4.0	1.4	5.9	0.41	5.15	0.21
	18 : 0	0.55	0.21	0.3	0.14	1.05	0.35

Table 3.6 Cont'd.

		Peak height (%) relative to total cellular methyl ester					
		CF 1033(Gr.D)		CF 1034(Gr.X)		CF 1035(Gr.Y)	
		$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
	12 : 0	13.0	2.83	22.5	10.6	14.3	0.99
3-OH	12 : 0	0.1	0.0	1.1	0.5	0.95	0.71
	14 : 0	1.65	0.7	8.95	1.9	8.0	0.85
3-OH	14 : 0	1.5	0.14	0.5	0.14	0.15	0.07
	16 : 1	30.5	0.7	34.0	2.83	33.25	1.05
	16 : 0	39.25	4.6	25.0	4.2	33.25	2.47
	18 : 1	13.75	1.77	7.5	3.5	9.4	0.85
	18 : 0	1.2	0.28	1.2	1.13	0.5	0.42

Table 3.7 Cellular Fatty Acids of M139, M1797 7mg, and M137 Meningococcus

		Peak height (%) relative to total cellular methyl ester							
		M 139(Gr.A)		M 1797(Gr.B)		7 mg(Gr.C)		M 137(Gr.C)	
		$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ	$\bar{X}$	σ
	12 : 0	11.3	2.4	15.9	0.49	10.7	1.27	8.0	0.0
3-OH	12 : 0	0.88	0.17	0.68	0.25	0.41	0.08	0.68	0.45
	14 : 0	17.0	0.0	13.2	5.37	9.2	1.13	12.2	1.13
3-OH	14 : 0	0.25	0.21	0.55	0.07	0.95	0.07	0.85	0.21
	16 : 1	35.15	1.63	27.5	3.54	30.25	1.77	36.75	1.24
	16 : 0	29.7	0.99	33.2	8.77	37.0	1.41	31.9	0.14
	18 : 1	4.75	0.78	8.15	5.4	9.55	0.63	9.55	3.61
	18 : 0	0.95	0.78	1.15	0.07	1.56	0.05	0.5	0.14

Table 3.8 Cellular Fatty Acids Analysis of Egyptian Strain

Peak height (%) relative to total cellular methyl ester

		Eg-1		Eg-2(Op)		Eg-2(Tr)		Eg-3	
		$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
	12 : 0	17.3	9.48	10.18	3.12	10.23	2.36	11.3	1.84
3-OH	12 : 0	0.55	0.21	0.79	0.12	0.88	0.82	0.6	0.14
	14 : 0	6.45	1.2	6.84	0.96	6.14	1.57	6.85	0.92
3-OH	14 : 0	0.45	0.35	0.28	0.14	0.25	0.08	0.37	0.04
	16 : 1	33.5	2.12	39.2	2.75	37.45	2.84	37.2	0.28
	16 : 0	32.0	2.83	32.4	1.25	32.0	4.69	34.5	0.71
	18 : 1	9.45	3.46	8.73	1.47	11.5	4.43	7.25	0.35
	18 : 0	0.65	0.21	0.62	0.3	0.8	0.38	0.75	0.35

Table 3.8 Cont'd

Peak height (%) relative to total cellular methyl ester

		Eg-5		Eg-7		Eg-8	
		$\bar{x}$	σ	$\bar{x}$	σ	$\bar{x}$	σ
	12 : 0	15.5	1.63	14.42	3.6	21.05	2.33
3-OH	12 : 0	0.6	0.0	0.85	0.49	0.7	0.14
	14 : 0	8.8	2.4	6.1	0.5	8.6	0.28
3-OH	14 : 0	0.3	0.0	0.6	0.1	0.4	0.0
	16 : 1	32.4	0.85	33.5	6.36	34.6	3.11
	16 : 0	28.5	0.71	35.0	4.24	28.13	2.65
	18 : 1	12.8	2.26	6.0	2.83	5.7	2.12
	18 : 0	0.6	0.14	0.3	0.14	0.36	0.05

Table 3.9 The Ratios of C12:0 to C14:0 of Menigococcus, gonococcus and Egyptian Strains

Organism	C12:0/C14:0 (%of peak height)
CF1030	1.29
CF1031	1.14
CF1033	7.88
CF1034	2.5**
CF1035	1.79
CF1032	1.25
F62P++Op	0.99
F62P++Tr	0.99
F62P+Op	1.03
F62P+Tr	0.68
F62P-OP	1.22
F62P-Tr	1.55
Eg-1	2.68
Eg-2	1.48(Op);1.76(Tr)
Eg-3	1.66
Eg-5	1.76
Eg-7	2.36
Eg-8	2.44

** CF 1034 (Gr X) meningococcus has a ratio of 2.5 between C12:0 and C14:0 which is much closer to all the Egyptian strains comparing with gonococcus and other meningococcus.

Fatty acid composition of LPS were compared in Tables 3.10 and 3.11. Two different strains of gonococci were analyzed. Four of the eight cellular fatty acids, C 12:0, C 14:0, C 16:1 and C 16:0, were found in both pure LPS and semi-crude LPS. 3-OH C 12:0, 3-OH C 14:0, C 18:1 and C 18:0 were not present in LPS, but in bacterial cells. C 18:1 and C18:0 were found attaching to the capsule of meningococcus. This is a clue of the presence of the capsule in gonococcus.

3. Statistical Analysis

In order to determine the differences or similarities between the fatty acid components of every two comparable organisms, the t-Test was used to interpret the results. Using the equation described in Chapter II, a P value (level of significance) was calculated for each t. Because the degrees of freedom of all the tests are small, a relatively large P value (0.1) was chosen as the criterion for acceptance or rejection.

3.1 Neisseria gonococcus

F62 P++ Op and F62 P++Tr have very similar fatty acid composition (Table 3.12). All the fatty acids have a high p value (1), but the oleic acid has a low p value (0.006), suggesting a significant statistical difference between opaque and transparent colony gonococci in the amount of this fatty acid.

F62 P-Op and F62 P-Tr have similar fatty acid composition (Table 3.12), except the myristic acid has a relatively low p value (0.009). These strains do not differ in their content of oleic acid. Thus, the difference in oleic acid content seen between P++Op and P++Tr strains may be unrelated to their difference in

Table 3.10 Fatty Acids Analysis of Lipopolysaccharids : F62 Gonococcus

Peak height (%) relative to total LPS methyl ester

	Transparent			Opaque		
	Pure LPS		Semi-Crude LPS	Pure LPS	Semi-Crude LPS	
12 : 0	81.4	57.0	45.5	40.5	1.0	2.3
14 : 0	1.32	0.1	0.1	5.0	1.0	2.3
16 : 1	4.4	10.0	5.0	7.1	1.0	2.3
16 : 0	12.3	33.3	50.0	47.6	97.0	93.2

Table 3.11 Fatty Acids Analysis of Lipopolysaccharide 1278 Gonococcus

Peak height (%) relative to total LPS methyl ester

	Transparent		Opaque	
	Pure LPS	Semicrude LPS	Pure LPS	Semicrude LPS
12 : 0	33.0	42.0	36.0	15.3
14 : 0	0.1	9.1	0.1	0.1
16 : 1	2.6	19.6	0.1	0.1
16 : 0	64.1	30.3	63.0	84.8

Table 3.12 Comparisons Between Heavily Piliated Opaque and Transparent Strains $P{+}{+}_{op}$ and $P{+}{+}_{Tr}$, and Between Non-piliated opaque and Transparent Strains $P-_{op}$ and $P-_{Tr}$

	F62 $P{+}{+}_{Op}$ / F62 $P{+}{+}_{Tr}$		F62 $P-_{Op}$ / F62 $P-_{Tr}$	
Fatty Acid	t	p	t	p
12 : 0	0.64	> 0.1	-0.1	> 0.17
3-OH 12 : 0	-1.41	0.1	0.32	> 0.17
14 : 0	-1.37	0.1	3.25	0.009*
3-OH 14 : 0	0.97	> 0.1	0.33	> 0.17
16 : 1	0.26	> 0.1	0.52	> 0.17
16 : 0	0.05	> 0.1	1.02	0.17
18 : 0	1.82	0.06*	1.03	0.17
18 : 0	0.71	> 0.1	0.82	> 0.17
Degree of Freedom	6		6	

* significantly different

opacity/transparancy.

The comparison between opaque and transparent gonococcus strains 1436 and 1446 gave the same result. There is no statistically significant difference in the fatty acid composition of these strains (Table 3.13). A comparison of the heavily piliated and non-piliated opaque variants of strain F62 shows that lauric acid and myristic acid are found in different ratios (Table 3.14). Similarity in fatty acid profile, however, is found between heavily piliated and non-piliated transparent variants of F62 strains.

Table 3.15 compares the heavily piliated opaque and transparent variants of strains F62 and 1436. The t-Test indicates that lauric acid is different in both opaque and transparent organisms. Other differences exist in myristic acid for the opaque variants, and in stearic acid for the transparent variants.

3.2 Egyptian Strains

As shown in Table 3.16, palmitoleic is the only fatty acid which shows statistically significant difference between EG-1 and EG-2 opaque strains. Lauric acid and palmitoleic acid are the two fatty acids showing differences between EG-1 and EG-2 transparent strains. In the comparison between EG-1 and EG-3, palmitoleic acid is the only one which shows a difference, and between EG-1 and EG-5, palmitic acid is different. Comparing EG-1 with EG-7, stearic acid is the only one found different in ratio. Between EG-1 and EG-8, three of the eight fatty acid measurements are found different in ratios.

Table 3.17 gives comparisons of fatty acid components between EG-3 and EG-5, EG-3 and EG-7, and between EG-3 and EG-8. In every

Table 3.13 Comparison between 1436P++Op and P++Tr; 1446P-Op and P-Tr

	1436 P++Op / 1436 P++Tr		1446 P-Op / 1446 P-Tr	
Fatty Acid	t	p	t	p
12 : 0	0.13	> 0.11	0.12	> 0.16
3-OH 12 : 0	0.68	> 0.11	-0.28	> 0.16
14 : 0	-0.69	> 0.11	-0.21	> 0.16
3-OH 14 : 0	-1.78	0.07*	-1.34	0.16
16 : 1	-0.68	> 0.11	0.95	> 0.16
16 : 0	0.44	> 0.11	0.69	> 0.16
18 : 1	1.39	0.11	-1.46	0.14
18 : 0	-0.74	> 0.11	-2.83	0.05*
Degree of Freedom	5		2	

* significantly different

Table 3.14 Comparison between Heavily Piliated Organisms and Non-Piliated Organisms of F62

	F62 P++Op / F62 P-Op		F62 P++Tr / F62 P-Tr	
Fatty acid	t	p	t	p
12 : 0	-1.99	0.05*	0.63	> 0.22
3-OH 12 : 0	-0.83	> 0.14	-0.31	> 0.22
14 : 0	-2.05	0.04*	-0.12	> 0.22
3-OH 14 : 0	0.37	> 0.14	0.57	> 0.22
16 : 1	-0.39	> 0.14	0.31	> 0.22
16 : 0	-1.21	0.14	0.82	0.22
18 : 1	0.85	> 0.14	0.56	> 0.22
18 : 0	0.63	> 0.14	0.75	> 0.22
Degree of Freedom	6		7	

* significantly different

Table 3.15 Comparison between F62 P++Op and 1436 P++Op; F62 P++Tr and 1436 P++Tr

Fatty Acid	F62 P++Op 1436 P++Op t	p	F62 P++Tr 1436 P++Tr t	p
12 : 0	2.25	0.03*	-2.77	0.02*
3-OH 12 : 0	1.3	0.12	0.64	> 0.15
14 : 0	2.03	0.04*	-0.09	> 0.15
3-OH 14 : 0	-1.13	0.15	-1.15	0.15
16 : 1	-1.46	0.10	0.70	> 0.15
16 : 0	1.35	0.12	-0.82	> 0.15
18 : 1	1.02	0.14	-0.70	> 0.15
18 : 0	1.27	0.12	-3.20	0.01*
Degree of Freedom	6		5	

* significantly different

Table 3.16 Comparison of Fatty Acid composition among Egyptian Strains Eg-1 and Eg-2(Op); Eg-1 and Eg-2(Tr); Eg-1 and Eg-3; Eg-1 and Eg-5; Eg-1 and Eg-7; Eg-1 and Eg-8

	Eg-2(Op) Eg-1		Eg-2(Tr) Eg-1		Eg-1 Eg-3	
Fatty Acid	t	p	t	p	t	p
12 : 0	-1.29	0.14	-1.58	0.09*	1.24	0.17
3-OH 12 : 0	1.65	0.1	0.54	> 0.14	< 1.0	> 0.18
14 : 0	0.41	> 0.14	-0.24	> 0.14	< 1.0	> 0.18
3-OH 14 : 0	-0.82	> 0.14	-1.23	0.14	0.47	> 0.18
16 : 1	2.42	0.05*	1.7	0.08*	3.45	0.04*
16 : 0	0.25	> 0.14	0.0	≫ 0.14	1.71	0.11
18 : 1	-0.34	> 0.14	0.56	0.14	1.15	0.18
18 : 0	0.12	> 0.14	0.50	0.14	0.50	> 0.18
Degree of Freedom	3		4		2	

* significantly different

Table 3.16 Cont'd

Fatty Acid	Eg-1 Eg-5 t	Eg-1 Eg-5 p	Eg-1 Eg-7 t	Eg-1 Eg-7 p	Eg-1 Eg-8 t	Eg-1 Eg-8 p
12 : 0	< 1.0	> 0.12	0.56	> 0.18	0.77	> 0.17
3-OH 12 : 0	0.5	> 0.12	1.15	0.18	1.25	0.17
14 : 0	1.75	0.11	1.23	0.16	3.46	0.04*
3-OH 14 : 0	0.88	> 0.12	0.79	> 0.18	0.29	> 0.17
16 : 1	0.96	> 0.12	0.0	≫ 0.18	0.59	> 0.17
16 : 0	2.4	0.07*	1.2	0.18	1.99	0.09*
18 : 1	1.62	0.12	1.54	0.12	1.85	0.1
18 : 0	0.41	> 0.12	2.92	0.05*	2.9	0.05*
Degree of Freedom	2		2		2	

* significantly different

Table 3.17 Comparison of Fatty Acids between Eg-3 and Eg-5; Eg-3 and Eg-7; Eg-3 and Eg-8

	Eg-3 Eg-5		Eg-3 Eg-7		Eg-3 Eg-8	
Fatty Acid	t	p	t	p	t	p
12 : 0	3.13	0.04*	1.52	0.13	6.58	$\ll$ 0.03*
3-OH 12 : 0	0.0	$\gg$ 0.12	1.0	> 0.13	1.0	> 0.15
14 : 0	1.51	0.12	2.31	0.07*	3.64	0.03*
3-OH 14 : 0	3.5	0.04*	3.29	0.04*	1.42	0.15
16 : 1	10.67	0.004*	9.74	0.005*	1.67	0.12
16 : 0	12.0	0.003*	0.23	> 0.42	4.65	< 0.03*
18 : 1	3.99	0.03*	0.77	> 0.13	1.16	> 0.15
18 : 0	0.78	> 0.12	2.37	0.07*	2.16	0.08*
Degree of Freedom	2		2		2	

* significantly different

case, statistically significant differences exist in four of the eight measured fatty acids. Table 3.18 shows that between EG-5 and EG-7, six of eight fatty acids have different ratios. Between EG-5 and EG-8, and EG-7 and EG-8, four of eight fatty acids have different ratios.

As shown in Table 3.19, Eg-2(op) and Eg-3, Eg-2 and Eg-5, Eg-2 and Eg-7, Eg-2 and Eg-8 have similar fatty acid ratios. The transparent variant Eg-2(Tr) is very similar to the opaque variant Eg-2(Op). Comparing Eg-2(Tr) with Eg-3, 3-hydroxytetradecanoic is different. Comparing Eg-2(Tr) with Eg-5, lauric acid, myristic acid, and hexadecanoic acid are different. Comparing Eg-2(Tr) with Eg-7, lauric acid, 3-hydroxytetradecanoic acid and stearic acid are in different ratios. Comparing Eg-2(Tr) with Eg-8, lauric acid, myristic acid, palmitic acid, and octadecanoic acid are in different ratios.

Most of the Egyptian strains showed significantly different fatty acid profiles with each other. A summary of the characteristics of fatty acid content of Egyptian strains is listed in Table 3.20. Another summary of differences among Egyptian strains is listed in Table 3.21.

3.3 Neisseria meningitidis

The comparison between each two meningococcus strains will be shown in Tables 3.22 through 3.25.

As shown in Table 3.22, oleic acid and stearic acid are different between CF 1030(Gr A) and CF 1031 (Gr B). Comparing CF 1030(Gr A) with CF 1032 (Gr C), 3-hydroxylauric acid, 3-hydroxytetradecanoic acid and

Table 3.18 Comparison of Fatty Acids between Eg-5 and Eg-7; Eg-5 and Eg-8; Eg-7 and Eg-8

	Eg-5 Eg-7		Eg-5 Eg-8		Eg-7 Eg-8	
Fatty Acid	t	p	t	p	t	p
12 : 0	1	> 0.1	4.15	0.03*	3.18	0.04*
3-OH 12 : 0	0.63	> 0.1	1.42	0.15	0.6	> 0.1
14 : 0	2.56	0.06*	0.16	> 0.15	10.54	< 0.04*
3-OH 14 : 0	4.28	0.02*	∞	≪ 0.03*	2.86	0.06*
16 : 1	2.0	0.09*	1.37	0.17	0.69	> 0.1
16 : 0	3.02	0.05*	0.13	> 0.17	2.57	0.06*
18 : 1	3.75	0.03*	4.58	< 0.03*	0.17	> 0.1
18 : 0	3.0	0.05*	3.0	0.05*	0.75	> 0.1
Degree of Freedom	2		2		2	

* significantly different

Table 3.19 Comparison of fatty acids between Eg-2 (Op & Tr) with the rest of the Egyptian strains

Fatty Acid	Eg-2(Op) Eg-3		Eg-2(Op) Eg-5		Eg-2(Op) Eg-7		Eg-2(Op) Eg-8	
	t	p	t	p	t	p	t	p
12 : 0	-1.06	0.18	-2.0	0.07*	1.40	0.13	-4.14	0.01*
3-OH 12 : 0	-1.09	0.16	2.07	0.06*	-0.23	0.42	0.74	0.26
14 : 0	-0.02	0.5	-0.34	0.38	1.56	0.11	-2.42	0.05*
3-OH 14 : 0	-0.90	0.21	3.22	0.02*	-2.56	0.04	-1.21	0.16
16 : 1	0.96	0.2	0.34	0.34	1.44	0.12	1.73	0.09
16 : 0	-2.06	0.07*	-0.01	0.5	-1.05	0.19	2.56	0.04*
18 : 1	1.06	0.18	-2.51	0.04*	1.47	0.12	1.93	0.07*
18 : 0	-0.44	0.34	0.08	0.47	1.35	0.14	1.14	0.17
Degree of Freedom	3		3		3		3	

* significantly different

Table 3.19 Cont'd

Fatty Acid	Eg-2(Tr) Eg-3		Eg-2(Tr) Eg-5		Eg-2(Tr) Eg-7		Eg-2(Tr) Eg-8	
	t	p	t	p	t	p	t	p
12 : 0	-0.55	0.3	-2.58	0.03*	-1.76	0.08*	-5.3	0.003*
3-OH 12 : 0	0.45	0.34	0.46	0.33	0.04	0.49	0.3	0.39
14 : 0	-0.57	0.3	-1.69	0.08*	0.41	0.35	-2.07	0.05*
3-OH 14 : 0	-1.94	0.06*	-0.87	0.22	-4.1	0.007*	-2.5	0.03*
16 : 1	0.12	0.46	2.34	0.04*	0.15	0.44	1.13	0.2
16 : 0	-0.71	0.26	0.99	0.19	-0.76	0.24	1.04	0.2
18 : 1	1.25	0.14	-1.25	0.14	1.55	0.1	1.68	0.08*
18 : 0	0.15	0.44	0.68	0.27	1.7	0.08*	1.53	0.1
Degree of Freedom	4		4		4		4	

* significantly different

Table 3.20 Characteristics of Fatty Acid Content of Egyptians Strains

Eg-1	Intermediate levels of all eight fatty acids
Eg-2	Intermediate levels of all eight fatty acids
Eg-3	Low in C 12 : 0 (lauric acid)
Eg-5	Low in C 16 : 0 (palmitic acid) and C 16 : 1 (palmitoleic acid) High in C 18 : 1(oleic acid)
Eg-7	Low in C 16 : 1(palmitoleic acid)
Eg-8	Low in C 16 : 0(palmitic acid) High in C 12 : 0(lauric acid) and C 14 : 0 (myristic acid)

Table 3.21 Summary of Differences among Egyptian Strains

Strains Compared	$p < 0.1$
Eg-1 vs. Eg-2(Op)	16 : 1
Eg-1 vs. Eg-3(Tr)	12 : 0, 16 : 1
Eg-1 vs. Eg-3	16 : 1
Eg-1 vs. Eg-5	16 : 0
Eg-1 vs. Eg-7	18 : 0
Eg-1 vs. Eg-8	14 : 0, 16 : 0, 18 : 0
Eg-3 vs. Eg-5	12 : 0, 3-OH 14 : 0, 16 : 1, 16 : 0 18 : 1
Eg-3 vs. Eg-7	14 : 0, 3-OH 14 : 0, 16 : 1, 18 : 0
Eg-3 vs. Eg-8	12 : 0, 14 : 0, 16 : 0, 18 : 0
Eg-5 vs. Eg-7	3-OH 14 : 0, 16 : 0, 18 : 1, 18 : 0
Eg-5 vs. Eg-8	12 : 0, 3-OH 14 : 0, 18 : 1, 18 : 0
Eg-7 vs. Eg-8	12 : 0, 14 : 0

Table 3.22 Comparison of Fatty Acids between CF1030 (Gr A) and the Rest of Meningococcus Groups

Fatty Acid	CF1030 (Gr A) CF1031 (Gr B)		CF1030 (Gr A) CF1032 (Gr C)		CF1030 (Gr A) CF1033 (Gr D)		CF1030 (Gr A) CF1034 (Gr X)		CF1030 (Gr A) CF1035 (Gr Y)	
	t	p	t	p	t	p	t	p	t	p
12 : 0	1.45	0.14	0.91	0.23	1.48	0.14	0.87	0.24	4.0	0.03*
3-OH 12 : 0	1.5	0.14	4.29	0.025*	10.5	0.004*	0.17	0.44	1.8	0.1
14 : 0	1.45	0.14	1.33	0.16	10.7	0.004*	3.1	0.045*	4.65	0.02*
3-OH 14 : 0	0.0	0.14	4.0	0.029*	13.25	0.002*	3.85	0.031*	5.45	0.02*
16 : 1	0.89	0.23	1.18	0.18	2.68	0.058*	0.28	0.4	0.21	0.43
16 : 0	1.16	0.18	0.95	0.22	2.96	0.05*	1.67	0.12	1.32	0.16
18 : 1	2.68	0.058*	1.62	0.12	8.63	0.007*	1.8	0.1	6.58	0.001*
18 : 0	2.08	0.087*	2.5	0.065*	3.82	0.031*	1.14	0.19	0.23	0.42
Degree of Freedom	2		2		2		2		2	

* significantly different

stearic acid are found in different ratios. Comparing CF 1030 with CF 1034 (Gr X), myristic acid and 3-hydroxytetradecanoic acid are in different ratios. In CF 1030 and CF 1035 (Gr Y), lauric acid, myristic acid, 3-hydroxytetradecanoic acid, and octadecenoic acid are in different ratios.

Table 3.23 shows that five of eight fatty acids are found in different ratios between CF 1031 and CF 1032. Seven of eight measured fatty acids are in different ratios between CF 1031 and CF 1033. Only myristic acid and palmitic acid are found different between CF 1031 and CF 1034. Myristic acid, 3-hydroxytetradecanoic acid, and octadecanoic acid are different between CF 1031 and CF 1035.

Table 3.24 shows that comparing CF 1032 with CF 1033, five fatty acids are found in different ratios. Comparing CF 1032 with CF 1034, five fatty acids have similar ratios. Also, CF 1032 and CF 1035 have five fatty acids in similar ratios. Between CF 1033 and CF 1034,only lauric acid and stearic acid are in similar ratios. Comparing CF 1033 with CF 1035, six fatty acids are in different ratios. In last column, we found CF 1034 and CF 1035 have six fatty acids in similar ratios.

As shown in Table 3.25, between M1797 (Gr B) and 7 mg (Gr C), lauric acid, 3-hydroxytetradecanoic acid and stearic acid are different in composition. The content of 3-hydroxydodecanoic acid, myristic acid, 3-hydroxytetradecanoic acid and octadecenoic acid are similar in 7 mg (Gr C) and M 137 (Gr C). Comparing M 137 with M 139 (Gr A), lauric acid, myristic acid, 3-hydroxytetradecanoic acid and palmitic acid are different in fatty acid ratios. Between M 1797 (Gr A) and M139 (Gr B), lauric acid and hexadecanoic acid are different in percentages. All the fatty acid compositions are found similar between CF 1030 and

Table 3.23 Comparison of Fatty Acids between CF1031 (Gr B) and the rest of the meningococcus Groups

Fatty Acid	CF1031 (Gr B) CF1032 (Gr C)		CF1031 (Gr B) CF1033 (Gr D)		CF1031 (Gr B) CF1034 (Gr X)		CF1031 (Gr B) CF1035 (Gr Y)	
	t	p	t	p	t	p	t	p
12 : 0	∞	$\ll$ o.35*	0.43	0.35	1.68	0.12	1.42	0.15
3- OH 12 : 0	4.5	0.02*	∞	$\ll$ 0.1*	0.35	0.38	1.42	0.15
14 : 0	1.5	0.14	92.72	0.00006*	10.36	0.004*	8.75	0.006*
3-OH 14 : 0	4.25	0.03*	13.46	0.002*	1.0	0.2	6.0	0.01*
16 : 1	3.16	0.04*	8.0	0.008*	1.0	0.2	1.95	0.1
16 : 0	0.43	0.35	2.9	0.05*	3.48	0.04*	1.0	0.2
18 : 1	1.0	0.2	8.82	0.006*	1.0	0.2	8.13	0.007*
18 : 0	3.94	0.03*	5.63	0.02*	1.57	0.13	0.91	0.23
Degree of Freedom	2		2		2		2	

* significantly different

Table 3.24 Comparisons of Fatty Acids Between CF1032 and CF1033; CF1032 and CF1034; CF1032 and CF1035; CF1033 and CF1034; CF1033 and CF1035;CF1034 and CF1035

	CF1032(Gr.C) CF1033(Gr.D)		CF1032(Gr.C) CF1034(Gr.X)		CF1032(Gr.C) CF1035(Gr.Y)	
Fatty acid	t	p	t	p	t	p
12 : 0	1.41	0.15	1.41	0.15	1.42	0.15
3-OH 12 : 0	4.5	0.02*	1.8	0.11	4.24	0.02*
14 : 0	295.7	0.0006*	3.21	0.04*	9.0	0.006*
3-OH 14 : 0	15.8	0.002*	3.33	0.04*	1.92	0.1
16 : 1	1.89	0.1	1.27	0.17	1.42	0.15
16 : 0	2.93	0.05*	14.28	0.002*	0.77	0.26
18 : 1	4.34	0.02*	1.32	0.16	9.65	0.005*
18 : 0	0.68	0.28	0.25	0.41	2.03	0.1
Degree of Freedom	2		2		2	

* significantly different

Table 3.24 Cont'd

Fatty acid	CF1033(Gr.D) CF1034(Gr.X) t	p	CF1033(Gr.D) CF1035(Gr.Y) t	p	CF1034(Gr.X) CF1035(Gr.Y) t	p
12 : 0	1.73	0.11	1.0	0.2	1.53	0.11
3-OH 12 : 0	3.57	0.03*	1.42	1.5	0.52	0.33
14 : 0	7.68	0.008*	14.77	0.002*	1.0	0.1
3-OH 14 : 0	10.0	0.004*	16.88	0.002*	4.38	0.02*
16 : 1	2.4	0.07*	4.29	0.03*	1.0	0.2
16 : 0	4.55	0.02*	2.3	0.07*	3.51	0.04*
18 : 1	3.15	0.04*	4.64	0.02*	1.0	0.2
18 : 0	0.0	0.1	2.8	0.05*	1.1	0.2
Degree of Freedom	2		2		2	

* significantly different

Table 3.25 Comparison of Fatty Acid amoung different Strains of meningococcus

Fatty acid	M1797(Gr.B) 7mg(Gr.C)		7mg(Gr.C) M137(Gr.C)		M137(Gr.C) M139(Gr.A)		M1797(Gr.B) M139(Gr.A)	
	t	p	t	p	t	p	t	p
12 : 0	5.43	0.02*	3.0	0.05*	-1.94	0.09*	2.68	0.06*
3-OH 12 : 0	1.43	0.14	-0.83	0.25	-0.59	0.3	-0.97	0.2
14 : 0	1.03	0.21	2.65	0.06*	-6.0	0.01*	-1.0	0.2
3-OH 14 : 0	5.66	0.01*	-0.63	0.3	2.83	0.05*	1.9	0.1
16 : 1	0.98	0.2	4.14	0.03*	0.9	0.24	-2.78	0.05*
16 : 0	0.61	0.3	-5.1	0.02*	3.11	0.05*	0.56	0.31
18 : 1	-0.36	0.38	0.0	0.1	1.84	0.1	0.87	0.24
18 : 0	-6.4	0.01*	-9.8	0.005*	-0.8	0.25	0.36	0.38
Degree of Freedom	2		2		2		2	

* significantly different

M 139 (Gr A). In the last column , we found six fatty acids in similar ratios between M 1797 (Gr B) and CF 1031 (Gr B).

3.4 Comparisons among Egyptian strains, Neisseria gonorrhoeae and Neisseria meningitidis

As shown in Table 3.26, Eg-2 and CF 1030 (Gr A) have seven fatty acid contents in similar ratio. Six fatty acids are found in similar ratios in Eg-1 and CF 1031 (Gr B). There are also six fatty acids found in similar ratios between Eg-1 and CF 1032 (Gr C). Myristic acid is the common "different" fatty acid of the above four groups. This fatty acid is more abundant in Group A, B, and C Neisseria meningitidis strains (mean=12.7%), than in Neisseria gonorrhoeae strains (mean=9.6%). Between Eg-1 and CF 1033 (Gr D), five fatty acids are found in similar ratios.

Table 3.27 lists a comparison of (1) strain Eg-2(Op) with CF 1030 (Gr A): there is a statistically significant difference in the content of four of the eight fatty acids measured; (2) strain Eg-2 (Op) with CF 1031 (Gr B): four of the eight fatty acids are found in different ratios; (3) Eg-2 (Op) with CF 1032 (Gr C): four of the eight fatty acids are in different ratios; (4) Eg-2 (Op) with CF 1033 (Gr D): five of the eight fatty acids are in different ratios; (5) Eg-2 (Op) with CF 1034 (Gr X): only palmitic acid is found in different ratio; (6) Eg-2 (Op) with CF 1035 (Gr Y): palmitoleic acid is the only fatty acid found different in ratio. According to the results, EG-2 (Op) is very similar to Gr X and Gr Y meningicoccus.

Also in Table 3.27 are the following comparisons: (1) Eg-3 (Tr) with CF 1030 (Gr A): five of the eight fatty acids are found in

Table 3.26 Comparison between Eg-1 and meningococcus; Eg-1 and Gonococcus Groups

Fatty Acid	Eg-1 CF1030(Gr.A) t	p	Eg-1 CF1031(Gr.B) t	p	Eg-1 CF1032(Gr.C) t	p	Eg-1 CF1033(Gr.D) t	p
12 : 0	0.006	>0.1	0.55	>0.14	0.34	>0.11	0.61	>0.1
3-OH 12 : 0	-2.83	0.005*	-3.0	0.048*	0.0	≫0.11	3.0	0.05*
14 : 0	-3.96	0.029*	-6.26	0.012*	-6.52	0.01*	5.64	0.02*
3-OH 14 : 0	0.0	≫0.1	0.0	≫0.1	0.78	>0.1	-3.9	0.004*
16 : 1	0.0	≫0.1	-0.63	>0.1	0.83	>0.1	1.9	0.1
16 : 0	0.55	>0.1	-0.24	>0.1	-0.06	>0.1	-1.9	0.1
18 : 1	2.06	0.08*	1.45	0.14	1.75	0.1	-0.58	>0.1
18 : 0	0.47	>0.1	1.94	0.1	0.65	>0.1	-2.2	0.08*
Degree of Fredom	2		2		2		2	

* significantly different

Table 3.27 Comparison between Eg-2(Op & Tr) and meningococcus groups

Fatty acid	Eg-2(Op) CF1030(Gr.A)		Eg-2(Op) CF1031(Gr.B)		Eg-2(Op) CF1032(Gr.C)		Eg-2(Op) CF1033(Gr.D)	
	t	p	t	p	t	p	t	p
12 : 0	-2.0	0.07*	-1.5	0.12	-2.1	0.06*	-1.93	0.08*
3-OH 12 : 0	-2.5	0.04*	-2.4	0.05*	1.65	0.1	7.6	0.003*
14 : 0	-4.9	0.008*	-7.0	0.003*	7.3	0.003*	-1.7	0.09*
3-OH 14 : 0	-1.6	0.1	-1.6	0.1	0.25	> 0.1	-9.7	0.001*
16 : 1	2.42	0.04*	2.2	0.05*	3.3	0.02*	1.7	0.1
16 : 0	1.0	> 0.1	-0.07	> 0.1	0.2	> 0.1	-2.6	0.04*
18 : 1	3.6	0.02*	2.6	0.04*	3.2	0.02*	-3.5	0.01*
18 : 0	0.2	> 0.1	0.4	0.13	-1.5	0.12	-2.2	0.06*
Degree of Freedom	3		3		3		3	

* significantly different

Table 3.27 Cont'd

Fatty acid	Eg-2(Op) CF1034(Gr.X) t	p	Eg-2(Op) CF1035(Gr.Y) t	p	Eg-2(Tr) CF1030(Gr.A) t	p	Eg-2(Tr) CF1031(Gr.B) t	p
12 : 0	-0.5	>0.1	0.45	>0.1	-2.5	0.03*	-1.9	0.07*
3-OH 12 : 0	-1.3	0.16	-2.5	0.1	-0.4	>0.1	-0.2	>0.1
14 : 0	-1.6	0.16	-1.5	0.14	4.8	0.004*	-4.8	0.004*
3-OH 14 : 0	-0.2	>0.1	-1.2	0.1	-2.9	0.02*	-3.0	0.02*
16 : 1	-0.2	>0.1	0.15	>0.1	2.3	0.04*	1.4	0.12
16 : 0	1.9	0.1	0.5	>0.1	0.5	>0.1	-0.14	>0.1
18 : 1	0.6	>0.1	0.02	>0.1	3.5	0.01*	1.7	0.08*
18 : 0	-0.7	>0.1	0.5	>0.1	0.8	>0.1	1.7	0.08*
Degree of Freedom	3		3		4		4	

* significantly different

Table 3.27 Cont'd

Fatty acid	Eg-2(Tr) CF1032(Gr.C)		Eg-2(Tr) CF1033(Gr.D)		Eg-2(Tr) CF1034(Gr.X)		Eg-2(Tr) CF1035(Gr.Y)	
	t	p	t	p	t	p	t	p
12 : 0	-2.7	0.03*	-1.3	0.14	-2.5	0.03*	-2.2	0.04*
3-OH 12 : 0	0.5	> 0.1	1.3	0.14	-0.33	> 0.1	-0.1	> 0.1
14 : 0	-5.0	0.004*	3.8	0.009*	2.0	0.07	-1.5	0.1
3-OH 14 : 0	-0.04	> 0.1	-14.5	0.00007*	-3.2	0.006*	1.4	0.1
16 : 1	2.5	0.035*	3.23	0.02*	1.4	0.13	1.9	0.06*
16 : 0	-0.04	> 0.1	-1.8	0.07*	1.8	0.08*	0.3	> 0.1
18 : 1	0.4	> 0.1	-0.7	> 0.1	1.1	0.17	0.6	> 0.1
18 : 0	-0.8	> 0.1	-1.3	0.13	0.7	> 0.1	0.9	> 0.1
Degree of Freedom	4		4		4		4	

* significantly different

different ratios; (2) Eg-2 (Tr) with CF 1031 (Gr B): five of the eight fatty acids ar different in ratios; (3) Eg-2 (Tr) with CF 1032 (Gr C): three of the eight fatty acids are different in ratios; (4) Eg-2 (Tr) with CF 1033 (Gr D): three of the eight fatty acids are found different in ratios; (5) Eg-2 (Tr) with CF 1034 (Gr X): four of eight measured fatty acids are different in ratios; (6) Eg-2 (Tr) with CF 1035 (Gr Y): two of the eight fatty acids are found different in ratios.

Table 3.28 shows that four of the eight fatty acids are found different in ratios between Eg-2 (Op) and F62p++Op. There are also four fatty acids found different in ratio comparing Eg-2 (Tr) with F62p++Tr.

As listed in Table 3.29, comparisons of strain Eg-3 with CF 1030 (Gr A), CF 1031 (Gr B), CF 1032 (Gr C), CF 1033 (Gr D) and CF 1035 (Gr Y) show that there are statistically significant differences in the content of 5, 4, 3, 5 and 3 respectively of the eight fatty acids measured. Comparing strain Eg-3 with CF 1034, palmitic acid is the only fatty acid showing a difference. In the comparison of strain Eg-3 with F62P++Op and F62P++Tr, each shows that three of the eight fatty acids measured are found different in ratios. From all the comparisons above, it appears that strain Eg-3 is close to Gr X meningococcus.

Comparisons of Eg-5 with CF 1030 (Gr A), CF 1031 (Gr B), CF 1032 (Gr C), CF 1033 (Gr D) and CF 1035 (Gr Y) are listed in Table 3.30. Statistically significant differences in the content of 2, 3 or more fatty acids are observed. Comparing strain Eg-3 with F62P++Op and F62P++Tr, each showed differences in the content of three of the

Table 3.28 Comparison between Eg-2 and F62 Gonococcus

Fatty acid	Eg-2(Op) F62 P++Op t	p	Eg-2(Tr) F62 P++Tr t	p
12 : 0	-0.96	> 0.1	1.04	> 0.1
3-OH 12 : 0	-2.2	0.04*	-0.4	0.1
14 : 0	2.04	0.06*	-3.3	0.009*
3-OH 14 : 0	0.86	> 0.1	1.6	0.08*
16 : 1	0.43	> 0.1	-0.64	> 0.1
16 : 0	-1.66	0.08*	1.17	> 0.1
18 : 1	-0.9	> 0.1	2.1	0.04*
18 : 0	-1.6	0.09*	3.3	0.008*
Degree of Freedom	5		6	

* significantly different

Table 3.29 Compaison between Eg-3 and Meningococcus Groups;
Eg-3 and Gonococcus Groups

Fatty acid	Eg-3 CF1030(Gr.A)		Eg-3 CF1031(Gr.B)		Eg-3 CF1032(Gr.C)		Eg-3 CF1033(Gr.D)	
	t	p	t	p	t	p	t	p
12 : 0	-1.56	0.13	-1.77	0.11	-2.8	0.05*	-0.7	> 0.1
3-OH 12 : 0	3.05	0.05*	-4.0	0.03*	0.28	> 0.1	5.0	0.02*
14 : 0	-3.9	0.03*	-7.5	0.009*	-7.9	0.008*	7.99	0.008*
3-OH 14 : 0	-1.4	0.15	-1.4	0.15	2.1	0.09*	10.8	0.004*
16 : 1	2.5	0.07*	5.0	0.02*	5.4	0.02*	12.4	0.003*
16 : 0	1.48	0.14	2.8	0.05*	2.0	0.09*	-1.4	0.14
18 : 1	2.1	0.08*	1.2	0.18	1.8	0.1	-3.8	0.03*
18 : 0	0.7	0.08*	1.7	0.12	-0.9	> 0.1	-1.44	0.15
Degree of Freedom	2		2		2		2	

* significantly different

Table 3.29 Cont'd

Fatty acid	Eg-3 CF1034(Gr.X) t	p	Eg-3 CF1035(Gr.Y) t	p	Eg-3 F62 P++Op t	p	Eg-3 F62 P++Tr t	p
12 : 0	-1.5	0.15	-2.03	0.09*	1.6	0.13	-4.4	0.006*
3-OH 12 : 0	-1.2	0.16	-0.34	> 0.1	-0.5	> 0.1	0.7	> 0.1
14 : 0	-1.4	0.15	-1.3	0.16	1.8	0.1	3.2	0.017*
3-OH 14 : 0	-1.3	0.16	3.8	0.04*	0.5	> 0.1	-0.3	> 0.1
16 : 1	1.6	0.12	5.1	0.02*	0.8	> 0.1	0.5	> 0.1
16 : 0	3.1	0.04	0.7	> 0.1	-2.0	0.09*	-1.8	0.09
18 : 1	-0.09	> 0.1	-1.7	0.12	0.64	> 0.1	-0.5	> 0.1
18 : 0	-0.5	> 0.1	0.6	> 0.1	-1.8	0.1	-3.5	0.01*
Degree of Freedom	2		2		4		4	

* significantly different

Table 3.30 Comparison between Eg-5 and meningococci; Eg-5 and gonococci

Fatty acid	Eg-5 CF1030(Gr.A) t	p	Eg-5 CF1031(Gr.B) t	p	Eg-5 CF1032(Gr.C) t	p	Eg-5 CF1033(Gr.D) t	p
12 : 0	-0.57	> 0.1	1.35	0.15	0.13	> 0.1	0.93	> 0.1
3-OH 12 : 0	-3.67	0.03*	∞	≪0.1	0.33	> 0.1	-1.3	0.17
14 : 0	-2.0	0.09*	-0.43	> 0.1	-1.9	0.1	4.2	0.03*
3-OH 14 : 0	-3.0	0.05*	-3.0	0.05*	1.0	0.2	-12.0	0.003*
16 : 1	-0.7	> 0.1	-2.7	0.06*	0.34	> 0.1	2.4	0.07*
16 : 0	-0.5	> 0.1	0.2	> 0.1	-3.1	0.045*	1.2	0.18
18 : 1	4.7	0.02*	4.3	0.02*	4.8	0.02*	-1.5	0.1
18 : 0	0.3	> 0.1	2.1	0.08*	-1.7	0.12	-2.7	0.06*
Degree of Freedom	2		2		2		2	

* significantly different

Table 3.30 Cont'd

Fatty acid	Eg-5 CF1034(Gr.X) t	p	Eg-5 CF1035(Gr.Y) t	p	Eg-5 F62 P++Tr t	p	Eg-5 F62 P++Op t	p
12 : 0	-0.97	> 0.1	0.63	> 0.1	-6.4	0.002*	-3.6	0.001*
3-OH 12 : 0	-1.3	0.17	-7.0	0.009	0.74	> 0.1	-0.5	> 0.1
14 : 0	-0.07	> 0.1	-1.1	0.2	0.12	> 0.1	-0.44	> 0.1
3-OH 14 : 0	-1.6	0.13	3.0	0.05*	0.7	> 0.1	0.64	> 0.1
16 : 1	-0.8	> 0.1	-0.9	> 0.1	1.5	0.1	1.8	0.07*
16 : 0	1.2	> 0.1	-2.6	0.06*	-0.12	> 0.1	-0.1	> 0.1
18 : 1	1.8	0.1	1.99	0.09*	-4.6	0.005*	-4.0	0.008*
18 : 0	-0.74	> 0.1	0.32	> 0.1	-4.7	0.005*	-1.5	0.1
Degree of Freedom	2		2		4		4	

* significantly different

eight fatty acids measured. Strain Eg-5, however, showed perfect similarity to CF 1034 (Gr X) meningicoccus.

As shown in Table 3.31, comparisons of Eg-7 with CF 1030 (Gr A), CF 1031 (Gr B) indicate only one of the eight measured fatty acids is different in ratios. Comparisons of Eg-7 with CF 1032, CF 1033 and CF 1034 showed significant differences in 3, 5 and 2 of the 8 fatty acids respectively. In the comparison of Eg-7 with F62P++Op and F62P++Tr, three of eight fatty acids are found different in ratios.

Comparisons of Eg-8 with meningicoccus groups and gonococcus groups are listed in Table 3.32. CF 1030 (Gr A), CF 1031 (Gr B), CF 1032 (Gr C) and CF 1033 (Gr D) showed statistically significant differences in the content of 2,3,3 and 7 of the 8 fatty acids respectively. Comparing Eg-8 with F62P++Op, lauric acid is found different in ratio. Comparing Eg-8 with F62P++Tr, lauric acid and stearic acid are found different in ratios. According to this table, strain Eg-8 has the same fatty acid profile as CF 1034 (Gr X) meningococcus.

Table 3.31 Comparison between Eg-7 and meningococcus serogroups; Eg-7 and gonococci

Fatty acid	Eg-7 CF1030(Gr.A) t	p	Eg-7 CF1031(Gr.B) t	p	Eg-7 CF1032(Gr.C) t	p	Eg-7 CF1033(Gr.D) t	p
12 : 0	-0.7	> 0.1	0.32	> 0.1	0.2	> 0.1	0.4	> 0.1
3-OH 12 : 0	-0.8	> 0.1	-0.4	> 0.1	0.8	> 0.1	2.1	0.08*
14 : 0	-4.9	0.02*	-16.3	0.002*	-18.1	0.002*	11.3	0.004*
3-OH 14 : 0	1.3	0.16	1.5	0.14	3.1	0.04*	-6.3	0.01*
16 : 1	0.0	≫ 0.1	-0.2	> 0.1	0.3	> 0.1	0.7	> 0.1
16 : 0	1.2	0.18	0.8	> 0.1	0.9	> 0.1	-0.96	> 0.1
18 : 1	0.9	> 0.1	0.05	> 0.1	0.4	> 0.1	-3.3	0.04*
18 : 0	-1.4	0.14	0.0	≫ 0.1	-2.7	0.05*	-4.0	0.03*
Degree of Freedom	2		2		2		2	

* significantly different

Table 3.31
Cont'd

Fatty acid	Eg-7 CF1034(Gr.X) t	p	Eg-7 CF1035(Gr.Y) t	p	Eg-7 F62 P++Tr t	p	Eg-7 F62 P++Op t	p
12 : 0 .	-1.02	0.2	-0.6	> 0.1	-3.2	0.02*	-2.6	0.03*
3-OH 12 : 0	-0.5	> 0.1	-0.3	> 0.1	0.35	> 0.1	-1.3	0.14
14 : 0	-2.3	0.07*	-3.4	0.04*	5.8	0.002*	3.74	0.01*
3-OH 14 : 0	0.7	> 0.1	4.0	0.03*	-2.6	0.03*	0.16	> 0.1
16 : 1	-0.1	> 0.1	0.05	> 0.1	1.1	0.17	1.35	> 0.1
16 : 0	2.4	0.07*	0.4	> 0.1	-1.7	0.08*	-1.9	0.07*
18 : 1	-0.5	> 0.1	-1.6	0.12	0.46	> 0.1	1.33	1.3
18 : 0	-1.1	0.2	-0.6	> 0.1	-1.6	0.09*	-0.2	> 0.1
Degree of Freedom	2		2		4		4	

* significantly different

Table 3.32 Comparison between Eg-8 and Meningococcus Groups; Eg-8 and Gonococcus Groups

Fatty acid	Eg-8 CF1030(Gr.A) t	p	Eg-8 CF1031(Gr.B) t	p	Eg-8 CF1032(Gr.C) t	p	Eg-8 CF1033(Gr.D) t	p
12 : 0	0.92	> 0.2	4.5	0.02*	-0.88	> 0.2	3.1	0.05*
3-OH 12 : 0	-2.5	0.06*	-3.0	0.05*	0.83	> 0.2	6.0	0.01*
14 : 0	-3.1	0.05*	-13.0	0.003*	-17.0	0.002*	33.7	0.0004*
3-OH 14 : 0	-1.0	> 0.2	-1.0	0.2	3.0	0.05*	-11.0	0.004*
16 : 1	0.41	0.04*	1.08	> 0.2	1.82	0.1	0.2	0.2
16 : 0	-0.53	> 0.2	-2.3	0.08*	-1.9	0.12	-2.66	0.06*
18 : 1	0.94	> 0.2	-0.13	> 0.2	0.4	> 0.2	-4.1	0.03*
18 : 0	-1.2	0.6	0.6	> 0.2	-2.7	0.06*	-4.1	0.03*
Degree of Freedom	2		2		2		2	

* significantly different

Table 3.32 Cont'd

Fatty Acid	Eg-8 CF1034(Gr.X) t	p	Eg-8 CF1035(Gr.Y) t	p	Eg-8 F62P++Op t	p	Eg-8 F62P++Tr t	p
12 : 0	-0.2	> 0.2	3.8	0.03*	-9,9	0.0003*	-6.2	0.002*
3-OH 12 : 0	1.0	> 0.2	-2.2	0.08*	0.6	> 0.1	-1.2	0.15
14 : 0	-0.3	> 0.2	1.0	> 0.2	0.6	> 0.1	-0.5	> 0.15
3-OH 14 : 0	-1.0	0.2	5.0	0.02*	-0.7	> 0.1	0.5	> 0.15
16 : 1	0.2	> 0.2	0.6	> 0.2	1.0	> 0.1	-0.03	> 0.15
16 : 0	0.9	> 0.2	-2.0	0.09*	0.02	> 0.1	1.61	0.12
18 : 1	-0.62	> 0.21	-2.3	0.07*	0.8	> 0.1	1.87	0.12
18 : 0	-1.05	0.2	0.46	> 0.2	-2.66	0.03*	-0.5	> 0.12
degree of Freedom	2		2		4		4	

* significantly different

Table 3.33 Summary of Differences in Fatty Acid Content between CF1034(Gr.X) and Other Meningococci

	Difference in Content
CF1030(Gr.A)	C 14 : 0, 3-OH C 14 : 0
CF1031(Gr.B)	C 14 : 0, C 16 : 0
CF1032(Gr.C)	C 14 : 0, C 16 : 0, 3-OH C 14 : 0
CF1033(Gr.D)	3-OH C12 : 0, C 14 : 0, 3-OH C 14 : 0 C 16 : 1, C 16 : 0, C 18 : 0
CF1034(Gr.Y)	3-OH C 14 : 0, C 16 : 0

Table 3.34 Summary of Differences between CF 1034 (Group X) Meningococcus and Egyptian Strains

	Difference in Content
Eg-2(Op)	None
Eg-2(Tr)	C 12 : 0, 3-OH C 14 : 0, C 16 : 0
Eg-3	C 16 : 0
Eg-5	None
Eg-7	C 14 : 0, C 16 : 0
Eg-8	None

CHAPTER IV

DISCUSSION AND SUMMARY

The objectives of this study were two-fold: (1) To determine the differences or similarities of fatty acid composition among gonococcal variants which differ in piliation or opacity; and (2) To examine Egyptian strains whose biochemical characteristics are intermediate between those of typical N. gonorrhoeae and N. meningitidis. In order to differentiate closely related organisms, gas liquid chromatography with a hydrogen flame detector was used which can detect the presence of a fatty acid in quantities as low as 10^{-11} gram [39].

The fatty acid content of cells can be influenced by several factors [31], which include:

(a) media used: Fatty acid content can be affected by the composition of the growth medium [32]. In this study, PPT(GC modified agar medium) was the only clear solid medium used for all the analysis. This medium stablizes the gonococcal colony variants [37].

(b) CO_2 tension: CO_2 supplies part of the initial growth energy for Neisseria gonorrhoeae and Neisseria meningitidis [33,38]. CO_2 can stablize the colonial variants of the gonococcus [40]. In this study, all the tested organisms were grown in 5% CO_2 incubator.

(c) Analytical techniques: For example, 3-hydroxydodecanoic acid (3-OH 12 : 0) is firmly attached to the outer cellular components through a strong chemical linkage, e.g., a covalent bond. Therefore a long saponification time is needed to release the

3-OH 12 : 0 fatty acid from the bacterial cell. Also, the derivatizing agent will affect elution profiles [35]. According to Sulpelco Bulletin 765A [29], 10% boron trichloride-methanol reagent (w/vol) gives the best resolution and most reproducible elution profiles.

(d) Column: The column used can affect the result of the chromatogram. Only a polar column can detect hydroxy acids. The presence of hydroxy acids is very critical to the identification of pathogenic Neisseria [36].

According to the data shown in chapter III, all the gonococcus, meningococcus and Egyptian strains have the same eight fatty acids: lauric acid (C12 : 0), 3-hydroxydodecanoic acid (3-OH C12 : 0), myristic acid (C14 : 0), 3-hydroxytetradecanoic acid (3-OH C14 : 0), 9-hexadecenoic acid (C16 : 1), palmitic acid (C16 : 0), oleic acid (C18 : 1), and stearic acid (C18 : 0). The hexadecenoic acid and palmitic acid are the two major components of the whole cellulat fatty acid, comprising about 60-70% of the total fatty acid composition of the cell.

The cellular fatty elution profiles were obtained for each colonial type of different strains of gonococci, each serotype of meningococcus and each of the Egyptian strains. The statistical analysis showed the following results:

(1) Among six colonial types for a given strain(F62), chromatographic patterns for fatty acid were not distintive for individual colonial types. Transparent variants were found more resistant to killing by pooled human serum whereas the isogenic organisms from opaque variants were sensitive to killing, suggesting that

transparent variants are more virulent than opaque ones [41]. However, these two variants can not be differenciated by chromatograms. Heavily piliated gonococci, which possess greater virulence than non-piliated gonococci showed very similar cellular fatty acid profiles.

(2) Non-distinctive fatty acid profiles were obtained from the comparisons with each other among F62 gonococcus, 1436 gonococcus and 1446 gonococcus.

(3) Fatty acid profiles of seven meningococcus serogroups were obtained and compared. Comparisons between serogroups and between strains of a given serogroup show that statically significant differences (at p 0.1 level) exist in the content of two or more of the eight fatty acids measured.

(4) Eg-1, Eg-2(opaque), Eg-2(transparent), Eg-3, Eg-5, Eg-7, and Eg-8 were separately compared with each serogroup of meningococcus and two variants of F62 gonococcus. Fatty acid profiles of meningococcus group X (CF 1034) showed statistically significant similarity (p 0.1) to all of the Egyptian strains tested, except for Eg-7 strain. The ratios between C12 : 0 and C14 : 0 (Table 3.9) among the Egyptian strains are greater than 1.0, and have a range of 1.66 to 2.50, which is very close to CF 1034 (GroupX) meningococcus. Those two findings suggest that the unidentified Egyptian strains could be related to Group X meningococcus. More studies need to be done to determine if they are genetically related

(5) Pyruvate and acetate are the only two short chain fatty acids

found in all six colonial types of F62. The organisms were grown in a defined medium (modified John James' Catlin broth) overnight. No other medium was used for this study of metabolites since other media may give different results. Pyruvate and acetate are the only two acids detected. These are the normal metabolites from glucose and can be obtained from any organism tested. No other peak was present in the chromatogram,suggesting that no detectable metabolite was being produced by F62 which may related to the pathogenicity of gonococcus. More tests have to be done on fresh clinical isolates to confirm that no toxic metabolites are produce by pathogenic gonococcus.

(6) The cellular fatty acid composition of Neisseria gonorrhoeae and Neisseria menigitidis can be distinquished from that of the non-pathogenic bacteria [34, 41, 42] . This distinction is manifested by the relatively larger amount of 3-OH C12 : 0 existing in these pathogenic organisms [43, 44]. The non-pathogenic bacteria, the ratio between 3-OH C12 : 0 to3-OH C14 : 0 should be less than or equal to 0.05. In gonococcus or meningococcus, accordinf to the results, the ratios are much greater than 0.05.

In summary, fatty acid profile comparisons made by GLC among six colonial types of F62 gonococcus showed no significant differences between each two types (Table 3.12-3.15). Among meningococcus serogroups, GLC study showed significantly different ratios for two or more of the eight fatty acids measured (Table 3.22-3.24). Both gonococcus and meningococcus have the same eight fatty acids, and C16 : 0 and C 16 : 1 are the major fatty acids comprising about

60-70% of the total fatty acids. All the Egyptian strains (Table 3.26-3.32) were found to be significantly different in two or more of the eight fatty acids measured compared to most of the meningococcus serogroups and fonococci. Group X meningococcus(Table 3.34) showed the great test similarity to the Egyptian strains. The one strain of Group X meningococcus studied had more similarity to the Egyptian isolates than the other strains of meningococcus.

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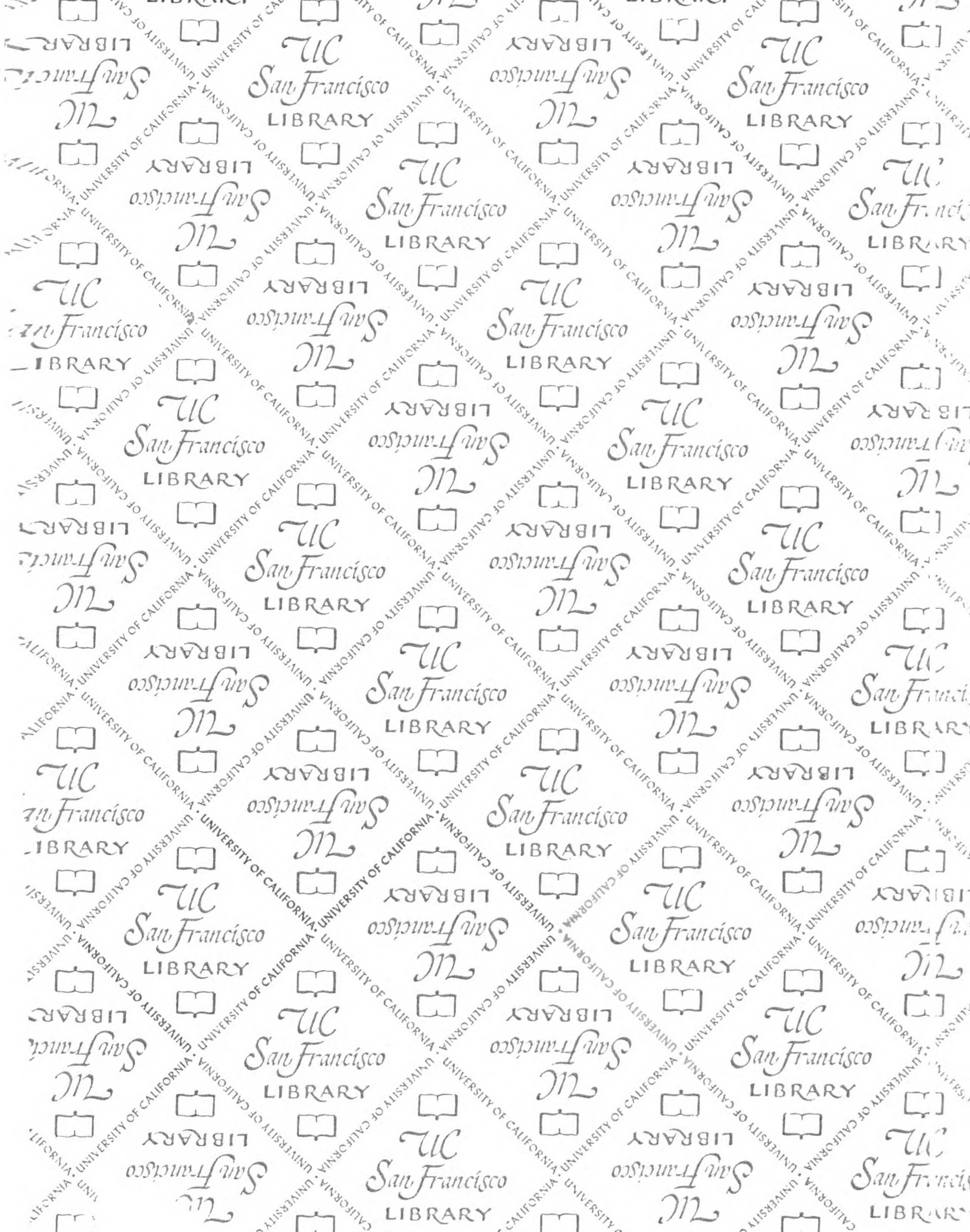
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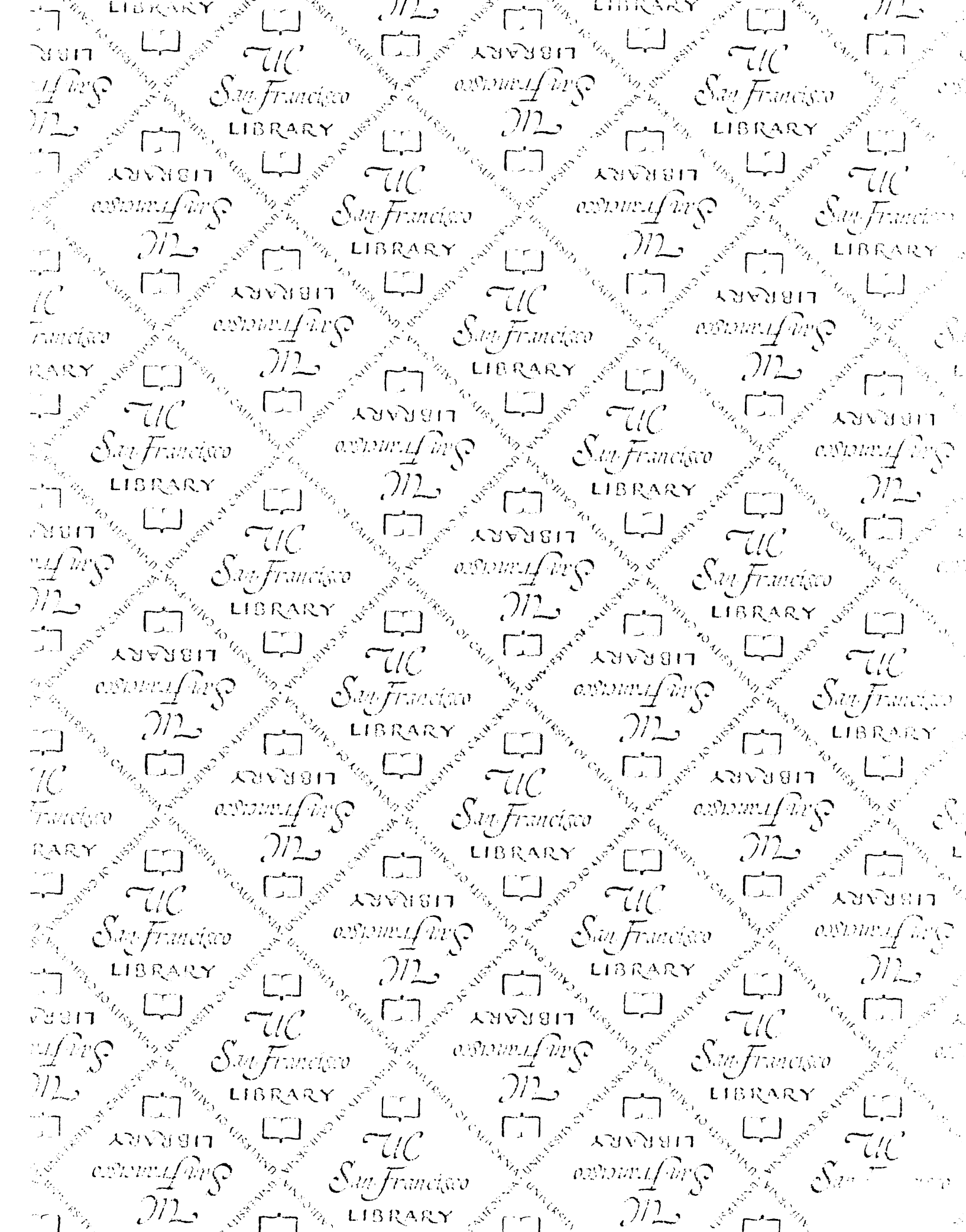
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