A COMPARATIVE STUDY OF THE EFFECT OF NEOMYCIN, METRONIDAZOLE AND LACTULOSE ON COLONIC FLORA WITH SPECIAL REFERENCE TO UREA SPLITTING BACTERIA IN PATIENTS WITH LIVER CIRRHOSIS

THESIS

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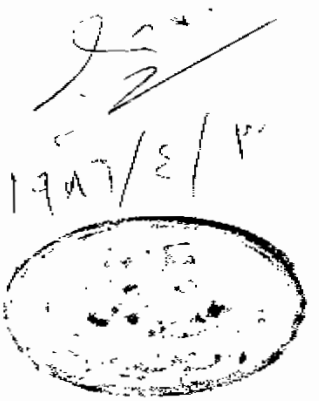

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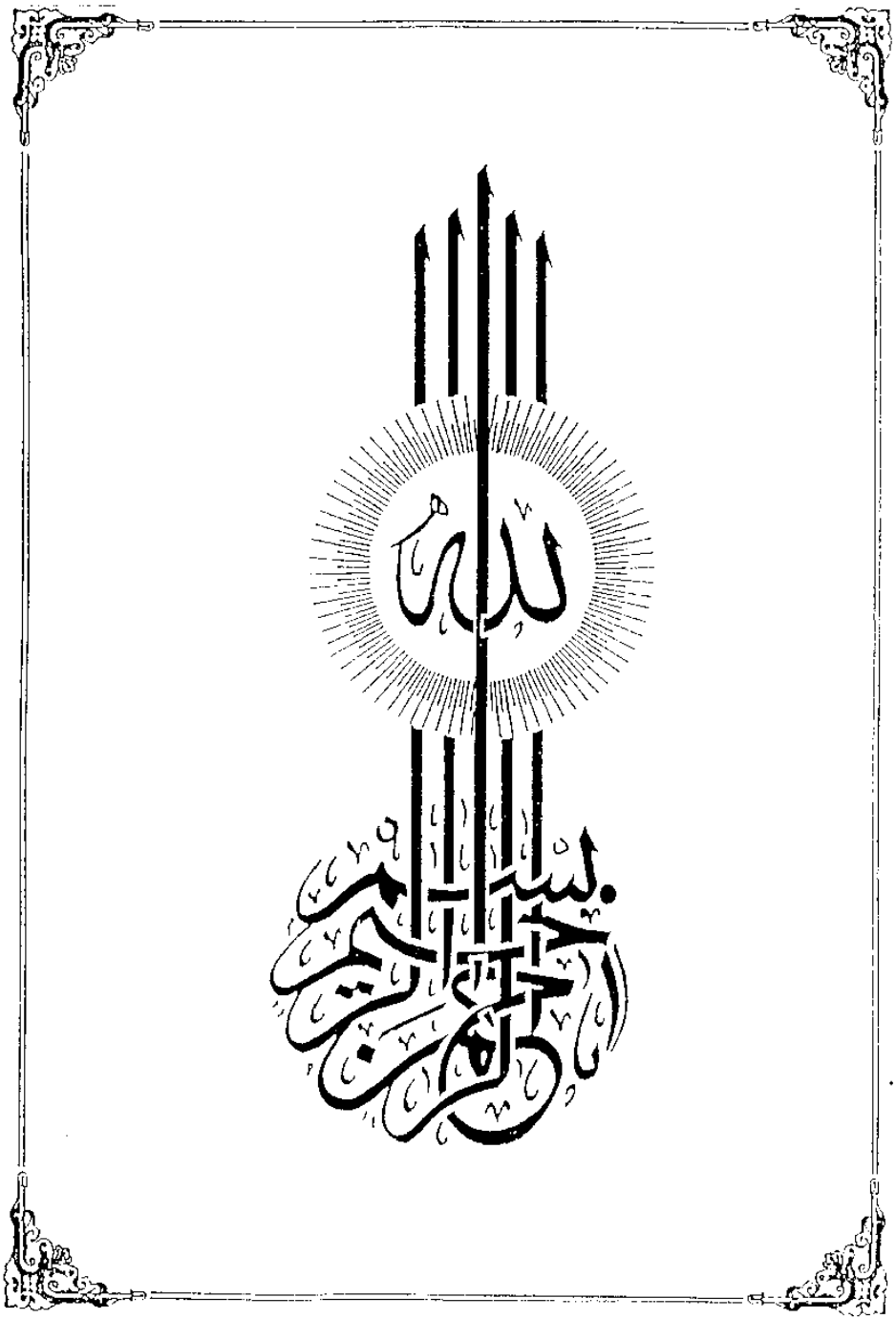

TO MY PARENTS

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Introduction

INTRODUCTION

Although our knowledge of the gut flora both in items of its composition and of its function has increased dramatically in recent years, the unanswered questions still greatly outnumber those answered. Studies in this field will undoubtedly lead to a fuller understanding of the enormously complex and still mysterious microbial populations which live within our gastrointestinal tract, and will make a contribution to the understanding not only of gastro-enterological but also of general medical and pharmacological problems.

The human intestinal flora is one of the most complex populations of bacteria known to exist. The caecum and colon provide suitable conditions for the establishment of the relatively stable microbial complex that characterizes the flora of the large intestine and faeces. Such large intestine's luxuriant microllora performs different functions and carries out various metabolic activities that play a significant role in the overall economy of the human body.

There is evidece of alteration of faecal flora in certain gastrointestinal disease. Also, the faecal flora is involved in the pathogenesis of a number of complications of liver cirrhosis such as portal-systemic encephalopathy and spontaneous enteric bacteraemia.

Colonic bacterial metabolism is one of the major sources of blood ammonia. Although ammonia is unlikely to be the only neurotoxin, it still has to be considered an important possible cause of portal-systemic encephalopathy in man. Other substances such as amines, mercaptans and short chain fatty

Review of Literature

THE NORMAL FLORA OF THE COLON

The normal colonic flora is usually inferred from the composition of the normal faecal flora, since suitable techniques for sampling various levels of the colon have yet to be developed. Data from animal studies would support the assumption that the flora does not alter during defaecation, indicating that the faecal flora adequately represents that of the recto-sigmoid (Hill and Drasar, 1975).

Moore et al (1978) have tried to validate the use of faeces as an indicator of colonic microflora by obtaining samples of colonic contents from six subjects who had died sudden violent deaths. They demonstrated that whilst there is a rapid increase in the number of bacteria between terminal ileum and colon, after this the composition of the flora and its total numbers remain constant. Therefore, the bacteria in faeces do reflect the flora of the large colon. Total cultural counts (Log_{10} per gram dry material) were ileum 6.4-10.1; ascending colon 11.0-11.3; transverse colon 11.1-11.9; descending colon 10.9-11.7 and rectum 11.1-11.7. However, it must be noted that the faecal flora represents only the luminal flora of the recto-sigmoid region and that the mucosal flora (the bacteria growing in close association with the mucosal surface and in the villous crypts) may differ markedly from this. It is suspected that these mucosal flora is more important than the luminal flora in, for example, urea metabolism (Hill et al., 1975).

Banwell et al (1981) concluded that the microbial mass is the major component in all regions of the large intestine. A figure of around 45 gram of bacterial solids has been suggested as the normal mass.

TYPES AND DESCRIPTION OF NORMAL COLONIC FLORA

Human colonic microflora is a highly diverse community. It comprises up to 500 taxonomically distinct species whose rank order of abundance vaires among individuals (Holdeman et al., 1976). Anaerobes constitute the predominant normal flora and outnumber aerobic bacteria by at least 1000:1.

Table (1): Summarizes the different types of normal colonic flora

Group, Genus or Species	Description	Remarks
Anaerobes		
- Bacteroides	Gran-negative rods,	Constitute the majority of the normal
	Obligate anaerobes	faecal flora (Jawetz et al., 1982).
. B. fragilis group		The dominant species (Duerden, 1980).
Subspecies vulgatus		The commonest faecal subspecies
Subspecies thetaiotaomicron		
.B.asaccharolyticus		Has been isolated regularly from faeces but in much smaller numbers than the B. fragilis group (Duerden, 1980).
Other Bacteroides,		"found inconsistently
and Fusobacterium	Gram-negative rods, obligate anaerobes	in small numbers
		in faeces".
-Bifidobacterium		Variable
-Eubacterium		Variable

Group, Genus or species	Description	Remarks
-Clostridium	Gram-positive rods,	Especially C. perfringens
	Obligate anaerobes	Not present in large numbers (Shaedler and Goldstein, 1976).
- Cocci		
. Peptococcus	Gram-positive	Minor components of the flora (Hill and Drasar, 1975).
.Peptostreptococcus	Gram-positive	as above
.Veillonella	Gram-negative	as above
Aerobes		
-Enterobacteria	Gram-negative rods,	faecal coliform flora may comprise a variety
	facultative anaerobes or aerobes	of serotypes (Dickinson et al., 1980).
.Escherichia coli		The commenest (Wilson and Miles, 1975)
.Other coliforms: Klebsiella		Minor components of the flora (hill and Drasar, 1975)
Proteus		
-Pseudomonas	Gram-negative rods, obligate aerobes	Absent in more than half of normal individuals (Shaedler and Goldstein, 1976).
-Streptococci S. viridans	Gram-positive, aerobes or facultative	
S. faecalis	anaerobes	Minor components (Hill and Drasar, 1975).

Group, Genus or Species	Description	Remarks
-Lactobacillus L.acidophillus	Gram-positive,micro-aerophilic or anaerobic	
-Staphylococci S. aureus S. epidermidis	Gram-positive, aerobes or facultative anaerobes	Absent in more than half of normal individuals (Shaedler and Goldstein, 1976)
Others		
-Candida		
-Corynebacterium		
-Sarcina		

MUCOSAL FLORA

Although the faecal flora of humans has been the subject of much research, little is known about the composition of the flora associated with the mucous layer between the intestinal epithelium and the lumen. The mucosal flora may be of importance in maintaining the stability of the faecal flora and may have specific functions (Croucher, 1983). Results from studies on the flora of the human colon wall conflict. However, microscopic examination of specimens obtained from colonoscopic examination of the human large bowel showed that bacteria adhere to the exposed epithelial surface and to the mucous sheet (Hartley et al., 1979). Gram negative and gram positive bacteria were observed within and on the surface of the mucous

layer and within plugs of mucus at the mouths of the crypts. Other workers have observed spirochetes associated with and apparently altering the architecture of the intestinal epithelium of the human colon (Lee et al., 1971).

FACTORS AFFECTING NORMAL COLONIC FLORA

We are confronted with the fact that a "normal" flora in one community may be abnormal in another consuming a different diet, or exposed to a different climate (Wilson and Miles, 1975), so for years there has been conjecture about the factors that control the composition of the flora in the gastrointestinal tract (Moore, 1976).

Person to Person Variation

Each individual has a rather unique colonic flora that remains constant over long periods of time, with minor variations conditioned by, for example, frequency of bowel movement (Zubrzycki and Spaulding, 1962). Holdeman et al. (1976) showed that there is a statistically significant greater difference in the composition of the faecal flora between different individuals than among different specimens from the same individual.

Age

During a man's prenatal life he existed in a reliably sterile environment, contact with microorganisms was excluded by his mother's resistance (Haenel, 1970). At birth the intestine contains at most a few bacteria, it is colonized rapidly, per os and to some extent per anum. Organisms were demonstrated in the meconium sometimes within 4 hours of birth, and usually within 10-17 hours; disinfection and protection of the anus delayed colonization until

after 20 hours, when food bacteria appeared in the stools (Wilson and Miles, 1975). With aging, aerobes, fungi and coliforms increase while bifidobacteria decrease.

Effect of Diet

Many studies have demonstrated that the faecal flora of individuals appears to be quite stable in that highly significant changes were not seen in response to diet or fiber content (Zubrzycki and Spaulding, 1962). So, it appears that the flora may depend primarily on the intestinal mucin and other secretions as its main source of nutrient (Moore et al., 1978).

However, qualitative and quantitative changes in the faecal flora can be brought about by extreme changes in the diet (Wilson and Miles, 1975).

- High carbohydrate diet favoured the growth of the obligatory anaerobic bacteria, particularly the bifidobacteria, and led to an increase in the total germ content.
- High fat diet repressed the enterococci and the bifidobacteria but encouraged proliferation of the bacteroides. In 1969 Aries et al reasoned that the bacterial species that produce carcinogens arise in response to the high-fat diet and will be absent from the low-fat, high-fiber diet.
- On a high-protein diet the flora did not differ significantly from that on a normal diet.
- On a diet rich in cellulose the total germ content of the human intestine diminished, as did likewise the frequency of the proteus, staphylococcal, clostridial and halophilic aerobic spore-bearing groups, and the yeasts (Wilson and Miles, 1975).