O

THE ROLE OF GUM ARABIC AS SUPPLEMENTARY THERAPY FOR CHRONIC RENAL FAILURE

BY

Amira Abdel Azeem Ahmed Behairy
B.Sc, U. K. (1984), M. Sc., U. K. (1990)

A Thesis Submitted to the University of Khartoum for the Degree of Doctor of Philosophy in Science
Department of Botany, Faculty of Science, University of Khartoum

Supervisor:
Prof. Suliman Mohammed El-Sanousi

Co-supervisor:
Prof. Karamalla Ahmed Karamalla

Co-supervisor:
Dr. Salma Mohammed Suliman

August-2003

Contents

Abstract

This work was conducted in 2000, which deals with comparative study between the commercial gum arabic samples and the authentic ones in the scope of their physico-chemical properties and bacterial load to determine the purity and safetiness of the commercial samples. The effect of ingestion of 25g/day processed gum arabic for 28-days on the levels of urea, creatinine, haemoglobin percentage and minerals (Na^{+1}, K^{+1}, Ca^{+2} and P^{+5}) in the blood of healthy and chronic renal failure subjects. The effects of this dose on the contents of protein and volatile fatty acids in their faeces and polyamines in their blood as metabolic products in their colon. The domination of *Klebsiella* sp. over *E. coli* after ingestion of gum arabic by those subjects.

The proximate analysis for each 25 samples from the commercial and authentic *Acacia senegal* gum revealed that, the moisture percentage and the pH value were more in the commercial samples than in the authentic ones. Whereas, the ash percentage, specific rotation, nitrogen and protein content were less in the commercial samples than in the authentic ones. All these values were significantly different, except the nitrogen and the protein, but inspite of this they were laid within the range specified by the JECFA.

The bacterial load in the nodules of the commercial and authentic gum arabic samples was more in the outer surface than in the cortex, which is completely sterile. With gum processing the bacterial load was decreased. The major bacterial isolates from gum arabic were Gram-positive *Bacillus* spp. *B. mycoides* and *B. licheniformis* represents the highest percentage of isolates in all forms of gum arabic samples studied.

Whereas, Gram-positive cocci; *Staphylococcus caseolyticus*, represent the minor bacterial isolates. Coliforms were not detected.

The effect of ingestion of 25g/day gum arabic for 28-days by healthy and chronic renal failure subjects revealed the following when compared with the pre-gum arabic periods. No side effects were observed except few abdominal troubles either in the first period or in the second one. The changes in blood composition for Hb%, K^{+1}, Na^{+1}, Ca^{+2} and P^{+5} levels were laid within the normal range except P^{+5} in chronic renal failure subjects, it was high . About more than 50% of chronic renal failure subjects showed an increase in the level of K^{+1}, Na^{+1} and P^{+5}, whereas healthy subjects showed that increased only for P^{+5}. The BUN was decreased in 33.3% of chronic renal failure subjects and in 20% of healthy ones. No obvious correlation was observed between the total acids, volatile fatty acids and nitrogen content in the faeces and the BUN inspite of the increased in the total acids, volatile fatty acids and nitrogen content in the faeces of some chronic renal failure and healthy subjects during the study period. Formation of polyamines was not observed in the sera of these subjects either before or after ingestion of gum arabic. The domination of *Klebsiella* sp. over the other *Enterobacteriaceae* (*E. coli, Pseud.* sp. *and Staph.* sp.) was not due to bacteriocine effect. It was suggested to be due to the lowering in pH when gum arabic was fermented in the colon or due to the release of urease enzyme from *Klebsiella* sp. and hence formation of ammonia which elevate the pH of the colon.

الـــخلاصـــة

أجريت هذه الدراسة خلال عام 2000 وتناولـت دراسـة عينـات مـن الصـمغ العربي التجارية المعروضة فـي الأسـواق المحليـة لمدينـة الخرطـوم ومقارنتهـا مـع العينات النقية من حيث الخصـائص الفيزيائيـة والكيمائيـة والمحتـوى البكتيـري للتأكـد من نقاء العينات التجارية وخلوها من البكتريـا الممرضـة. بالإضـافة إلـى أثـر تنـاول 25 جرام يوميا من الصمغ العربـي المصـنع ولمـدة 28 يـوم علـى مرضـى الفشـل الكلوي والأصحاء وذلك بقيـاس نسـبة اليوريـا، الكريـاتينين، الهيموقلـوبين والمعـادن (الصوديوم، البوتاسيوم، الكالسيوم والفسفور) في الدم. وأثـر تلـك الجرعـة مـن الصـمغ عل نسبة وجود البروتين والأحماض الدهنيـة الطيـارة فـي البـراز وعديـدات الأمينـات في الدم كنواتج استقلابية في الأمعاء الغليظـة للإنسـان، وتعليـل سـيادة *الكلبسـيلا* علـي الاشريكية القولونية بعد تناول الصمغ العربي.

أظهر التحليل التقريبي لخمس وعشرين عينة لكل من عينات الصمغ العربي التجارية و النقية أن الرطوبة النسبية والأس الهيدروجيني عاليان في عينات الصمغ العربي التجارية عن تلك التي في العينات النقية ، في حين أن نسبة الرماد،الدوران النوعي ومحتوى النيتـروجين والبروتين كانت أقل في العينات التجارية عن تلك التي في العينات النقية. كانت كل الفروقات معنوية عدا النيتروجين والبروتين، لكن مع ذلك تقع في المدى المحدد من قبل أل JECFA.

دل الفحص الميكروبيولوجى لعينات الصمغ العربي التجارية والنقية الخام أن المحتـوي البكتيري كان أعلي في القشرة الخارجية للكعاكيل الخام بينما وجد اللاب عقيم، وقد قل المحتوي البكتيري بالتصنيع. معظم البكتريا المعزولة من كل عينات الصمغ العربي المفحوصة تنتمـي إلى البكتريا العصوية موجبه الجرام، وأحـرزت *B.mycoides* ، *B. licheniformis* أعلى نسبه مقارنة مع الأنواع الأخرى. أمـا البكتريـا الكرويـة موجبـة الجـرام *Staph. caseolyticus* *عزلت* بكمية بسيطة ولم تعزل بكتريا القولون من كـل العينـات التـي تـم اختبارها.

أوضحت النتائج أن تناول الصمغ العربي بمقـدار 25 جـرام فـي اليـوم ولمـدة 28 يوم لكل من الأشخاص الأصحاء ومرضي الفشل الكلوي مقارنـة مـع الفتـرة قبـل تناول الصمغ العربي ليس هنالك آثـار جانبيـه مـن تنـاول الصـمغ عـدا اضـطرابات

معوية خفيفة في الفترة الأولي أو الثانية من تناوله. وعلي صعيد آخر فقد كانت التغيرات في مكونات الدم (نسبه الدم، البوتاسيوم، الصوديوم، الكالسيوم والفوسفور) تقع في مدي التغير الطبيعي، عدا الفوسفور الذي كان عاليا في مرضي الفشل الكلوي. أكثر من 50% من مرضي الفشل الكلوي أظهروا ارتفاعا في محتوي البوتاسيوم والصوديوم والفوسفور بينما تمثلت هذه الزيادة فقط في الفسفور عند الأصحاء. انخفضت اليوريا في 33,3%من مرضي الفشل الكلوي وفي 20% من الأصحاء، ولاتوجد علاقة واضحة بين مستويات الأحماض الكلية والطيارة والنيتروجين في البراز واليوريا في الدم بالرغم من زيادة الأحماض الكلية والطيارة والنيتروجين في براز بعض مرضي الفشل الكلوي والأصحاء أثناء فترة الدراسة. علاوة علي عدم وجود عديدات الأمينات في مصل دم الأصحاء والمرضى قبل وبعد تناول الصمغ العربي. أما سيادة *الكلبسيلا* علي *الاشريكية القولنية* بعد استعمال الصمغ العربي لدى الأشخاص الأصحاء أو مرضي الفشل الكلوي لم تعزى لأثر السم البكتيري(الكلبسين) المفروز من قبل *الكلبسيلا* لكن يعزي للانخفاض في الأس الهيدروجيني في الوسط عند تخمر الصمغ العربي أو ألي إفراز إنزيم اليوريز من قبل *الكلبسيلا*، ومن ثم تحرير الأمونيا وبالتالي ارتفاع الأس الهيدروجيني في الوسط .

Acknowledgments

My venerated praise is humbly offered to Allah, the Almighty the Gracious, the Merciful, who gave me the health, strength, aptitude and patience to complete this research.

I am really deeply grateful to express my faithful appreciation and gratitude for the interest shown and the sound advice give to me by my Supervisor Prof. Dr. Suliman M. El-Sanousi. Also, my sincere thanks and gratitude are extended to my Co-Supervisors, the late Dr. Karamalla A. Karamalla and the late Dr. Salma M. Suliman due to their co-operation and zealous leadership throughout this study.

The author is indebt to the staff at Khartoum Dialysis and Kidney Transplantation Centre - University of Khartoum for analyzing samples from the healthy and the chronic renal failure patients volunteers during the study period. My thanks are further extended to the Gum Arabic Processing Company (**GAPC**), due to their either financing the above analysis or providing the processed form of gum arabic in fine powder form.

The author gratefully convoyed a special acknowledgement to the teaching staff and the students at the Faculty of Public and Environmental Health at University of Khartoum and the chronic renal failure patients at

Khartoum Dialysis and Kidney Transplantation Centre and Ibn Sinna Hospital; who volunteered in this study.

I further hearty recognize the unlimited help and friendly attitude during this study for the all staff members of the Department of Botany - Faculty of Science and Department of Microbiology - Faculty of Veterinary Science - University of Khartoum.

My deep thanks of sincerity are also extended to my colleagues and students at the Faculty of Public and Environmental Health - University of Khartoum, and all acquaintances everywhere for their valuable help in every aspect or item that I needed to attain the goals of this research.

To conclude, I would not forget to grant my warmest greetings to Mr. Mohammad Elmoutasim for typing this thesis honestly and earnestly.

Declaration

I would like to declare that the subject and analysis of this study has not been submitted to any other university before by any Researcher. All the results were gathered by my-self, and no paper from this study has been published before.

Amira A. A. Behairy

August-2003

Dedication

To:

- all members of my family

- my aunt Fatheia

- those dealing with microbiology nutrition. and

List of Tables

List of Figures

Plates

Appendices

Introduction

1.1. The aetiology of chronic renal failure:

Renal failure means disorder of renal excretory and secretary functions, leading to retention of nitrogenous waste products of metabolism (Haslett *et al*., 1999). It could be acute or chronic. The latter is a progressive loss of irreversible deterioration in renal function due to final common pathway in many different diseases, which develops over a period of years. Ninety per cent of patients reaching end-stage renal disease had chronic Diabetes mellitus, Glomerulonephritis or Hypertension (Mahan and Escott-Stump, 1996). According to Pendreigh *et al.* (1972) the most common causes of chronic renal failure for 5000 cases in Scotland included, Pyelonephritis with relative incidence of (30%), Glomerulonephritis (28%), Hypertension (10%), Polycystic kidneys (7%), Obstruction of the urinary tract (5%), Analgesic nephropathy (5%), Diabetic nephropathy (3%) and other miscellaneous disorders (12%). The United States Renal Data System Annual Reports (1997) and the Scottish Data (1993) from the Scottish Renal Registry were revealed that the most important causes of chronic renal failure were due to Diabetes mellitus (17, 37%), Glomerular disease (19, 11%), Hypertension (4, 27%), Interstitial disease (14, 5%), Polycystic kidney disease (7, 3%), renal artery stonesis (6, 2%) respectively. Beside these, verotoxins that arise from strains of *Escherichia coli* serotype 0157:H7, is the most common cause of renal failure in children and adult in the U.K. and U.S.A., due to hemolytic uremic syndrome.

Also, in the Sudan tropical diseases such as visceral leishminiasis, malaria and shistosomiasis were reported to cause serious deterioration in

renal functions in the form of high blood urea which in turn increas prevalence of chronic renal dysfunction (Suliman, 1991).

1.2. Prevalence of chronic renal failure in Sudan:

Recently, chronic renal failure is becoming more commonly encountered disease in Sudan. It is not known whether this is due to an actual increase in incidence or due to an increase in recognition and diagnosis of the condition with investigators (Abboud, 1982). The estimated incidence is 70 - 140 new cases/million inhabitants/year (Suliman, 1995). It is so difficult to get a true picture of the prevalence of chronic renal failure disease in the Sudan; because Sudan is a very large country in Africa with many ethnic groups in its population with different cultures, social habitat, climatic conditions and food makes.

In the period 1995 - 2000 there were 2216 reported cases of chronic renal failure as well as 635 death cases due to the disease (Records of Federal Ministry of Health, 1995-1999). These cases and deaths were reported from most of the Sudan's states. However, there were no chronic renal failure cases reported from Blue Nile, North and South Kordofan and North Darfur States. Other states like Khartoum, Gezira, Kassala and Gadarif have reported the highest number of cases and deaths (Table 1). Most of the cases were male at age ranged between 15 - >45-years as reported in Khartoum Teaching Hospital and Ibn Sina Hospital during 1995 - 2000 (Table 2 and 3).

In Sudan few studies have been conducted, and most of them were focusing on the aetiology of chronic renal failure with reference to the age, sex, history of the patient, their social status and occupation.

Abboud (1982) studied 100 patients with chronic renal failure at Soba University Hospital. Most of the patients were males (79%) having an age of 50-years or less. The aetiology was found to be mainly due to Glomerulonephritis (38%), Renal calculi (12%) and Diabetes mellitus (9%).

Bashier (1996) studied 42 patients with chronic renal failure at Khartoum Dialysis and Kidney Transplantation Centre, 29 were males and 13 were females, their ages were 50 $\pm$ 4.9 years. Most of them were from North State (64%), Middle State (14.3%) Khartoum State (14.3%) and Western State (7.1%). Most of the patients were without work (73.8%), malnourished and wasted due to the economic hardship and increment of infection. Their history showed that most of them have urinary tract infection (18%), Diabetes mellitus (10%), Hypertension (12%), Renal calculi (10%), Hematourea (9%) and other diseases (18%).

Ali (2000) had studied 100 cases at Khartoum Dialysis and Kidney Transplantation Centre and revealed that 58% were males and 42% were females. Their ages ranged between 50 - 70-years. Most of the patients have a history of hereditary disease within their family, and the most common diseases were Hypertension and Diabetes mellitus (Hypertension, 81% in females and 66% in males).

Hajj-Elmahi (2001) studied cases in Khartoum Dialysis and Kidney Transplantation Centre and came to the conclusion that high incidence of chronic renal failure were among males, and the average of the male subjects were found to be younger than that of female subjects. The majority of patients were from urban areas (76.5%). Hypertension (61.8%) and Diabetes mellitus (20%) were the top causes of renal failure.

failure: 1.3. Medical treatment of chronic

Eastwood and Passmore (1975) stated that the medical treatment of chronic renal failure varied according to the stage of illness. It is either done by reduction of dietary protein in the first instances to 0.5g/kg body weight (30 - 40g/day) or by dialysis (peritoneal or haemodialysis) and transplantation. At present there are six haemodialysis centres in the Sudan distributed in three main towns namely: Khartoum (four units), Medani and Port Sudan (one each). These units received 269 regular patients with an average total cost of 933.800 Sudanese Dinars (Dr. A. Rahman Ali A. Rahman, personal communications), see Table (4) and Appendix (1).

In 1998, gum arabic was introduced as a supportive therapy for chronic renal failure in the Sudan by a group of doctors headed by Professor Salma M. Suliman a consultant medical Physician at Khartoum Dialysis and Kidney Transplantation Centre. There is a pharmacy at

Khartoum Dialysis and Kidney Transplantation Centre for selling gum arabic in a form of mechanized powder, 50g/polythene bag that costs one hundred Sudanese Dinars. The goal behind that gum arabic is easily prepared, ingested, platable and had few side effects compared to other fibres used (Bliss *et al*., 1996). Also, since it is soluble-fermentable dietary fibre it will increase the number of

bacterial mass in the human colon and hence utilization of the urea by the action of bacterial urease, and elimination of ammonia which is used for the synthesis of non-essential amino acids and protein for human subject and microorganism (Vince *et al*., 1973; Assimon and Stein, 1994). The degradation of urea and the subsequent use of ammonia result in

decreasing serum-urea nitrogen and increasing faecal nitrogen excretion in chronic renal failure patients taking low protein diet (Bliss *et al*., 1996). Also, metabolites of these microorganisms such as volatile fatty acids and polyamines play a major role in regulation of DNA, RNA, protein synthesis as well as enhancing epithelial cell proliferation (Tabor and Tabor, 1976; Jacobs and Lupton, 1984; Johnson and Gee, 1986;).

1.4. The objectives of the present study:

Dietary fibres have no role in nutrition, but play a major role in prevention form the occurrence of certain common diseases such as colon cancer, diabetes millitus, coronary thrombosis, obesity, chronic renal failure…etc. Hence, this work was aimed at clarifying the medical effect of gum arabic (*Acacia senegal* (L) Willd.) when consumed as a supplementary therapy by chronic renal failure patients. This can be achieved through the following aspects**:-**

1- Determination of the specification of the physico-chemical properties of commercial gum arabic (moisture, ash, pH, specific rotation, total nitrogen and protein).

2- Estimation of the bacterial load in raw and processed forms of commercial gum arabic to set the safety limits during consumption.

3- Study the effects of the bacteriocine released by *Klebsiella* sp. (klibcine) on *Escherichia coli*, *Staphylococcus* sp. and *Psuedomonas* sp., beside other inhibitary substances specifically on *Escherichia coli***.**

4- An *in vitro* investigation of the efficiency of the human intestine bacteria in the decomposition of gum arabic, urea and formation of polyamine.

5- Determination of polyamines, urea, creatinine, P^{+5}, Ca^{+2}, K^{+1}, and Na^{+1} content in serum, and volatile fatty acids and nitrogen content in the faecal samples of chronic renal failure patients and healthy volunteers before and after consumption of gum arabic.

Literature Review

2.1. Dietary fibres:

2.1.1. Definition and types of dietary fibres:

Several carbohydrate polymers are synthesized by plant from simple sugars. These include fibrous or viscous polysaccharides, which give plants their structure and form. According to Eastwood and Passmore (1983) the structure of the plant cell wall changes with growth, stage of differentiation, cell environment and specific functions of the cell. The macromolecular components of the wall are fibrillar polysaccharides (mainly cellulose), matrix polysaccharides (mainly pectic substances, hemicelluloses and glycoproteins) and encrusting substances, mainly lignin.

Ditery fibres are generally described as the skeletal remains of plant cells in the diet which resist the action of digestive enzymes in the mammalian gastrointestinal tract (Trowell *et al*., 1976; Kay, 1982; British Nutrition Foundation, 1990). Other names such as unavailable carbohydrate or unavailable complex carbohydrate are also used for this dietary fibres (Livesey, 1992). They are composed of a group of substances**:** cellulose, hemicelluloses, lignins, gums structurally related and pectins. Dietary fibres are frequently classified as soluble or insoluble (Chen and Anderson, 1981). Soluble fibres dissolved in water and consist of pectins, gums, and some hemicelluloses. Insoluble fibres do not dissolve in water and consist of cellulose, lignins and some hemicelluloses. The chemistry of dietary fibres varies from plant to plant and is affected by the growing conditions and age of the plant (Salvendran, 1983)**:-**

Cellulose is a polymer of glucose linked by β–1–4 glycoside bonds. Its molecules are arranged with the microfibrils in a highly ordered crystalline state in chains that are 4000 – 6000nm long and 4nm diameters.

Hemicelluloses are branched polymers of pentose and hexose sugars (e.g. xylose, arabinose, mannose, galactose and their uronic acid derivatives). Xyloglucans are the predominant hemicellulose in parenchymal fruit and vegetable tissues.

Lignins are polymers of aromatic alcohols. They encrust the cellulose and hemicellulose during secondary thickening.

Pectins are complex mixture of colloidal polysaccharides; they are partially esterfied rhamnogalacturonans with an ∝–1–4 linked D-galacturonan chain interspersed with L-rhamnopyranosyl residues with side-chains which include D-glucuronic and galacturonic acid. Some acidic groups are methylated.

Gums are water-soluble viscous polysaccharides of 10,000 – 30,000 units, mainly glucose, galactose, mannose, arabinose, rhamnose and their uronic acids, which may be methoxylated and acetylated. Gums commonly used in the food industry are**:** gum arabic, tragacanth, karaya gum, carob gum and guar gum which are obtained as exudates from the stems or seeds of tropical and semitropical trees and shrubs.

Mucilages are polysaccharides from seed and seaweeds used in small amounts in the food industry as thickening and stabilizing agents due to their water holding and viscous properties. The mucilages of some seeds (e.g. *Ispaghula husks*) are bulk laxatives made up of highly branched arabinoxylans. Alginic acid from seaweed is a polymer of 1-4 linked β-D-mannuronic acid or of 1-4 linked ∝-L-glucuronic acid or a mixture. There are also polysaccharides containing fucose in seaweeds mucilages. The mucilages may be sulphated to a variable degree.

Table (5) shows most of the characterization of the dietary fibres (Phillips, 1998).

2.1.2. Sources of dietary fibres:

All food types of vegetable origin are useful sources of fibres. Wholemeal, brown and white bread provide 8.5, 5.1 and 2.7g/100g respectively. Potatoes and most other root vegetables provide 1 – 2.5g/100g. Leafy vegetables contain 2.5 – 3.5g/100g and fruits up to 3g/100g (Paul *et al*., 1978).

Eastwood *et al*. (1974) suggested that, sources of vegetable fibres vary from country to country and the total dietary fibres may be more related to the aetiology of colonic disorders than cereal fibres intake alone.

duration time: 2.13. Dietary fibres intake and the effective

The amount of fibres added must be adequate to produce measurable changes in the parameters studied. Addition of small amounts of fibres had no effect on subjects already have been consuming sufficient fibres, while a definite effect could be shown in subjects who habitually had low fibres intakes (Kelsay, 1978). Fibres intake was recommended by CowGill and Anderson (1932). Ninety to hundred microgram of fibres per kilogram of body weight are required

Sources, solubility and major groups of (5): Table dietary fibres polysaccharides in foods:

Main sources	Soluble in water	Major groups	Components	Polysaccharides – types	Food
Storage materials	Partially	***Starch**	Amylose , amylopectin	∝-1-4- glucan ∝-1-4, 1-6-glucan	Fruits, seeds, tubers

Structural materials	No	**Cellulose**		β-1-4-glucan	All cell walls
	Yes	**Non-cellulosic**	Pectic substances	Galacturonans arabino-galactans	Mainly fruits and vegetables
	Slightly		Hemicellulose	Arabino-xylans	Cereals
				Glucurono-arabino-xylans	Cereals
				β-glucans	Fruits, vegetables and cereals
Non-structural materials	Yes	**Mucilages**		Diverse and complex heteropolysa-ccharides	Algal seaweeds exudates, seeds and fruits
	Yes	**Gums**			

*Previously starch was regarded to be completely hydrolyzed and absorbed, now it has been shown to have a component which resists glycosidic action (Anderson *et al*., 1981). Up to 50% of starch in foods of vegetables origin may not be utilized in the small intestine (Englyst and Cummings, 1985 and 1986).

daily for satisfactory laxation. This represents an intake of 6.3 to 7.0g for 70kg person and might be amount to an intake of 13 to 35g of total fibres/day.

Williams and Olmsted (1936$_{\text{a and b}}$) fed to a man a total of 12 to 24g fibres/day. Ten grams were in the form of cellulose plus lignin, and the amount of hemicellulose varied from 2 to 14g/day. They observed that, the level of fibres they had fed approached the maximum tolerable

amount of indigestible residue, and large amounts of fibres would have resulted in diarrhea. Whereas, gum arabic when ingested in a relatively large dose 25g/day equal to 350mg/kg/day has no toxic effect on the test subjects (Ross *et al*., 1983).

Little information is available on the amount of dietary fibres ingested by chronic renal failure patients consuming low protein diet (LPD). Bliss *et al*. (1996) in their studies revealed that 50g gum arabic/day was well tolerated by the subjects. The gradual increase of gum arabic dose to the desired amounts over a period of one to two weeks may decrease flatulence by allowing adaptation. People who increase their fibres intake up to 50g/day have no risk of any serious adverse effects on their health. But enough is enough since no benefits gain from the excess, and expose themselves to known and unknown hazards (Eastwood and Passmore, 1983).

The study period or extension of time might have produced changes that were not noted in a short time, while age and sex had a little influence on the effect of fibres intake (Kelsay, 1978). Adaptation to the guar gum and gum arabic diets was relatively long, at least ten days so as to be completely fermented in the large bowl (Nyman and Asp, 1982; Wyatt *et al.,* 1986). Whereas, the adaptation to the gum arabic diet appeared longer than to the guar gum diet and short-term administration of this diet was rather diarrheic (Tulung *et al*., 1987).

Elsenhans *et al*. (1981) considered a period of 52 days to be optimum when they studied the effect of carbohydrate-gelling agent on the anatomy of the rat gastrointestinal tract. Ross *et al*. (1984) found that, diet and the addition of fibres was more important than the duration of feeding during period zero, 14 and 28 day with either low or high diet

fibres. Walter *et al*. (1986) suggested that, four weeks period for the stool weight collection and related analysis were necessary while a longer time interval, eight to twelve weeks period were needed for metabolic events.

fibres and their physiological changes: 2.1.4. Dietary

Different amounts and composition of fibres from various plant sources were included in the diets of human subjects. Therefore, various physiological changes were measured as indices of the effects of fibres (Chen and Anderson, 1981). These physiological changes were related to the physico-chemical properties of the fibres, which relied in its capacity to absorb water and other organic substances to act as a cation exchange and susceptibility to bacterial fermentation (Kritchevsky and Story, 1975; Kay, 1982). All these properties were reported to be affected by processing and cooking and hence their digestion and absorption (Wyman *et al*., 1976; Eastwood and Passmore, 1983).

The physico-chemical properties of dietry fibres play a significant role in certain disorders of colonic functions, glucose and lipid metabolism, stool bulk and transit time, and increasing faecal bacterial volume (Anderson and Chen, 1979; Kay, 1982). The published data on these studies provide the following tentative conclusions**:-**

2.1.4.1. Faecal weight and nature of the faeces:

The inclusion of fibres in the diet increases stool weight in control or self selected diet. An increase in stool bulk was reported when such types of fibres were given**:** wheat fibres (CowGill and Anderson, 1932; Eastwood *et al*., 1974; Findlay *et al*., 1974; PiePmeyer, 1974; Jenkins *et al*., 1975$_{a}$ and Walter *et a*l., 1986), cellulose (Eastwood *et al*., 1973; Stanley *et al*., 1973), wheat and vegetables (Antonis and Bersohn, 1962) and wheat bran and gum tragacanth (Eastwood *et al*., 1986)

Small increase in faecal weight was found by Durrington *et al*. (1976) of 24% with 12g pectin/day, and 20% by Kay and Truswell (1977) with 15g/day. The large dose of pectin 36g/day studied by Cummings *et al*. (1979) produced only 33% increase in stool weight. Whereas, no change or insignificant increase in stool weight was observed when gum arabic 100g/kg dry weight diet was fed to rats for twelve weeks (Walter *et al*., 1986) neither when 25g/day for weeks (Ross *et al*., 1983) nor when gum arabic and pectin were fed (Eastwood *et al*., 1986).

The nature of the faeces was about 70 – 80% water, a proportion that varies little and is independent of the daily output (Wyman *et al*., 1978). Of the solid contents about half is bacterial mass and half dietary fibres with a little undigested food. A greater intake of dietary fibres leads to a larger faecal mass in one of the two ways (Stephen and Cummings, 1980). If the extra fibres comes from wheat bran, the predictable increase in stool weight is a function of the water–holding capacity of the bran (Smith *et al*., 1981; Eastwood *et al*., 1983) which was digested to a lesser extent (Southgate *et al*., 1976). If the extra fibres come from fruit, vegetables and pectin, the effect is less predictable. However, there is a slight rise in stool weight as a result of increased bacterial proliferation during fermentation of fibres (Cummings *et al*., 1979; Stephen and Cummings, 1980; Cummings, 1981 and Kay, 1982).

Large soft stool was observed in diet containing natural amount of fibres (i.e. traditional unrefined carbohydrates eaten by villagers) than refined low fibres in economically developed countries (i.e. citizens at western world) who produce small firm stools (Burkitt *et al*., 1972). Also, the addition of bran to the diet increases the frequency of defaecation and lessened the need to strain at stool (Painter *et al*., 1972).

2.1.4.2. Intestinal transit time:

Fibres may slow down the passage of food in the mouth and stomach (Holt *et al*., 1979) and so help to promote a sensation of satiety, and by delaying entry of food into the small intestine modifying the rate of absorption of nutrients. However, results of studies on the effect of fibres on stool transit time are not conclusive. Decreased transit times were reported after unprocessed bran 20g/day was given to boys 15 to 19-year-old (Payler *et al*., 1975) and to normal subjects with slow time, 30g/day (Harvey *et al*., 1973). Decreased transit times were also reported for subjects given bagass (Walters *et a*l., 1975), and for children given oranges, 350g/day (Walker, 1975). African Ugandan villagers eating unrefined diet had shorter transit times and larger stools than did British navy personnel eating refined diet (Burkitt *et al*., 1972; 1974). Overall transit time in people on low–fibre diets are between 60 and 90 hours and is reduced by adding fibres to the diet. It is only about 30 hours in rural Africans.

In studies on the fibres: wheat bran (CowGill and Anderson, 1932; Eastwood *et al*., 1973), Cellulose (Eastwood *et al*., 1973), pectin (Durrington *et al*., 1976) and gum arabic (Ross *et al*., 1983) no effect was observed on transit time. In these studies the amount of fibres added to the diet may have not been sufficient to result in decreased transit time, or the subjects may already have been receiving sufficient fibres in diet (Kelsay, 1978). Moreover, Kay and Truswell (1977) attributed the lack effect of pectin on transit time, to its dehydration action on the faeces and hence affect defaecation frequency. Or since pectin is extensively degraded by colonic bacteria it is probable that the metabolic consequences of pectin-ingestion are attributable to its physical properties in the upper intestine. In addition, psychological and emotional factors

may affect defaecation, or previous habits of elimination may not be easily changed in short periods of time (Kelsay, 1978).

2.1.4.3. Faecal bile acids:

Bile acids excretion appears to be increased by fibres. This was noted with a diet containing maize, wheat and vegetables (Antonis and Bersohn, 1962), bagasse (Walters *et al.*, 1975) or high cholesterol diet with added cellulose (Shurpalekar *et al*., 1971). Insignificant increase in faecal bile acid was observed when gum arabic was fed for three weeks (Eastwood *et al.*, 1983; Ross *et al*., 1983). In other studies bile acid/gram dry weight of faeces decreased when wheat bran or cellulose was added to the diet (Eastwood *et al*., 1973; Walters *et al*., 1975).

Aries *et al*. (1969) and Hill *et al*. (1971) showed that the bacteria (mainly *Bifidobacteria* and *Bacteroides*) in the colon of people eating a Western diet degrade bile acids to form the precursors carcinogens. These noxious compounds would remain in contact with the colonic mucosa for a longer time and in a more concentrated form if fibres deficient diet were eaten. Therefore, it was suggested that dietary fibres may protect the colon from bile acids carcinogens by absorbing them and promoting rapid elimination (Eastwood, 1975).

2.1.4.4. Serum cholesterol levels:

Serum cholesterol levels were lowered by cellulose (Shurpalekar *et al*., 1971), pectin (Palmer and Dixon, 1966; Jenkins *et al*., 1975_{b}; Durrington *et al*., 1976; Kay and Truswell, 1977), gum arabic (Eastwood *et al*., 1983; Ross 1983), guar gum (Jenkins *et al*., 1975_{b}), and mixed diet containing fruits, vegetables and legumes (Keys *et al*., 1960). In other studies serum cholesterol levels were not lowered by wheat fibres (Eastwood, 1969; Eastwood *et al*., 1973; Heaton and Pomare, 1974;

Jenkins *et al*., 1975$_{a;}$ Heaton *et al*., 1976), baggas (Walters *et al*., 1975) or cellulose (Grande *et al*., 1965; Eastwood *et al*., 1973). However, pectin is more effective in lowering serum cholesterol levels than was the fibres in wheat bran (Kelsay, 1978). This was attributed to the fact that, pectin is highly methylated and esterified, or its gel forming property (Kay and Truswell, 1977).

2.1.4.5. Serum triglycerides and other lipid levels:

There is scanty evidence that fibres are effective in lowering level of serum triglyceride or other blood lipids (Kelsay, 1978). Serum triglyceride levels were decreased when 18 to 100g of unprocessed bran was added to the diet (Heaton and Pomare, 1974). Changes in triglyceride levels did not generally occur when wheat fibre was added (Durrington *et al*., 1975; Jenkins *et al*., 1975$_{a}$; Truswell and Kay, 1975 and Heaton *et al*., 1976), nor when bagass (Walters *et al*., 1975) cellulose (Huth and Fettel, 1975) or pectin (Durrington *et a*l., 1976) were given.

Plasma phospholipids and free fatty acids levels were not altered by wheat fibres (Persson *et al*., 1975). Serum total lipids and phospholipid were not affected by adding 13g of cellulose to the diet (Prather, 1964), but they were decreased when 100g of cellulose were added to a diet supplemented with 4g of cholesterol (Shurpalekar *et al*., 1971). Whereas faecal fat increased in children given orange 350g/day (Walker, 1975) and in young normal men fed 36g pectin/day (Cummings *et al*., 1979).

2.1.4.6. Serum glucose levels:

Glucose level and glucose tolerance curve and serum insulin levels were significantly lowered in African cleaner than in European. This was

postulated to higher consumption of unrefined carbohydrates by the African (Wapnick *et al*., 1972). Glucose tolerance is not significantly altered after ingestion of 25g of gum arabic (Ross *et al*, 1983). They attributed this to the fact, that gum arabic is highly water soluble and has a low viscosity and hence is unlikely to modify glucose absorption. While other gums such as guar have been shown to significantly alter glucose tolerance in both normal and diabetics (Jenkins *et al.,* 1976; Jenkins *et al*., 1978).

Guar gum, pectin and gum tragacanth are used clinically to treat Diabetes mellitus and obesity (Jenkins *et al*., 1977; Leeds *et al*., 1981; Kortkiewski, 1984). They are not broken down by normal human digestive enzymes and form viscous gels in the stomach and small intestine which delay the absorption of glucose and other nutrients (Holt *et al.,* 1979). Therefore, high carbohydrate and high fibres diets are now used widely in the treatment of diabetes (Editorial, 1983; Parillo *et al.* 1988).

2.1.4.7. Mineral absorption:

Higher fibres diets have been reported to adversely affect the absorption of minerals. Potassium, calcium, magnesium and phosphorus were less absorbed when 92% extraction flour was fed than 69% extraction (McCance and Widdowson, 1948). Whereas, Demigne and Remesy (1985) and Tulung *et al*. (1987) observed the absorption of potassium, magnesium and calcium in a large amount from the caecum of rats fed 15% gum arabic diet and 50% pectin.

Also, the addition of dietary fibres in the form of wheat bran biscuits to the diet cause an increased excretion of most inorganic constituents, particularly sodium, phosphorus, iron and magnesium

(Southgate *et al*., 1976). In Iran and elsewhere in the Middle East, a clinical syndrome of small stature, delayed puberty and anemia has been attributed to zinc deficiency, arising from poor absorption of the mineral from a diet containing a large amount of coarse unleavened bread (Sandstead *et al*., 1967).

2.1.4.8. Blood mineral levels:

An increase in fibres intake leads to higher faecal excretion of minerals followed by mineral deficiency. Rickets and osteomalcia in the Middle East, Pakistan and India continue to be associated with diet in which 70% or more of the energy comes from wholemeal bread or chapattis (Rab and Baseer, 1976).

Some reports indicate that fibres may decreased blood level of some minerals. Fall of serum calcium level was observed in the studies of Heaton and Pomare (1974), when subjects received 18 to 100g of unprocessed bran per day, but not in subjects who received 20g (Persson *et al*., 1975). Also, fall of serum iron levels was observed when 20g of bran were given (Persson *et al*., 1975). Haemoglobin level also fell when 36g of wheat fibres were added to the diet (Jenkins *et al*., $1975_{\mathbf{a}}$).

2.1.4.9. Energy absorption:

Some reports indicate that fibres decrease energy absorption when the fibres were increased in the diet. Southgate and Durnin (1970) reported that, increasing the fibres intake; fruits and vegetable and whole meal bread, resulted in a greater loss of energy. Small amount of fibres has minor therapeutic role in the obese patient (Walters *et al*., 1975; Southgate *et al*., 1976).

The loss of energy in the faeces ranged between 40 – 80kcal/day when wheat bran biscuits 13g/100g were added to the diet (Southgate *et*

al., 1976). Therefore, a large supplemental intake of dietary fibres had only minor effects on energy metabolism, which is unlikely to induce a useful loss of calories in the management of obesity. But if weight loss occurs concomitantly with the administration of high fibres to obese patients, this loss is likely to be due to the displacement in the diet of more readily available carbohydrates or even more rapid satiety due to raised dietary bulk (Eastwood and Passmore, 1983). Hence, the lack of fibres in the diet of Western people contributes to obesity (Heaton, 1973).

2.1.4.10. Fermentation in the large intestine:

Feeding omnivores with diets containing high proportion of fibres or related carbohydrate, the major part of carbohydrates reaching the caecum were potato starch which escapes hydrolysis in small intestine, non-cellulosic polysaccharides, cellulose, ∝-galactosides and lignin (Demigne and Remesy, 1985). Non-cellulosis dietary fibres such as pectin, gum arabic, and to some extent hemicellulose were reported to be easily fermented by colonic bacteria (Yang *et al*., 1969; Cummings *et al*., 1979; Heller *et a*l., 1980; Cummings, 1981; Nyman and Asp, 1982; Wyatt *et al*., 1986). Whereas, dietary fibres in wheat bran was rather resistant to fermentation and 63% was recovered in the faeces (Nyman and Asp, 1982).

The extent of fermentation is influenced by a number of factors such as changing structure of the fibres (Nyman and Asp, 1982), estrification (Dekker and Richards, 1973), particles size (Heller *et al.*, 1980), processing (Bjorck *et al*., 1984) and other components of the diets and the amount ingested (Keys *et al.*, 1970). It is the chemical structure

rather than the physical appearance that determines the resistance to bacterial break down (Nyman and Asp, 1982). Whereas,

Eastwood *et al*. (1986) studies on gum tragacanth, wheat bran, gum arabic and pectin revealed that, there were no correlations between the chemical composition and structure of the fibres and their physiological effects.

The bulk of the bacteria in the gastrointestinal tract of mammals reside in the rumen, caecum or large intestines, depending on animal species (Smith and Bryanyt, 1979). Their main metabolic functions involve generation of energy for growth and maintenance by catabolism, which results in anaerobic degradation of organic matter (Smith and Bryanyt, 1979).

The population of bacteria colonizing the colon soon after birth and after some weeks is similar to that in adults although there is convincing evidence that the bacterial flora of the colon differ in people fed on African, European, or North American diet (Eastwood and Passmore, 1983). All human colon bacteria are obligate anaerobes and most of them

require a fermentable carbohydrate (Moore and Holdeman, 1974**a and b;** Finegold, 1977). Strong proteolysis is rare. The five major genera in the colon are *Bacteroides, Eubacterium, Bifidobacterium Peptostreptococcus* and *Fusobacterium*. One genus, *Fusobacterium*, contains organisms that are predominantly non-saccharolytic. The bacterial species which degrade different types of dietary fibres and mucines fall into two genera, *Bacteroides* and *Bifidobacterium* and the ability to utilize mucines and plant polysaccharides varies considerably among the *Bacteroides* species tested (Salyers *et al*., 1977**a and b;** Betain *et al.*, 1977).

Diets have little effect on the species composition of the human colonic flora (Finegold, 1977), inspite of this diet may still produce considerable changes in the metabolic activities of the flora, because diet will provide new type of substrate which will lead to changes in the levels of different catabolic enzymes (Salyers, 1979). This to some extent was similar to the results obtained by Wyatt *et al*. (1986). They observed that the addition of 10g/day of gum arabic to normal Western diet was accompanied by a rapid adaptation of faecal flora, and proportion of the fermenting bacteria increased from 6% at the beginning of the study to 50% after several days of adaptation. When gum arabic was withdrawn from the diet, the proportion of the gum arabic fermenters (*Bacteroides* and *Bifidobacterium*) returned to the level present before ingestion. These confirm that the response was due to dietary change.

Generally, the main end products of microbial metabolism of carbohydrate are non-utilizable gases**:** hydrogen, methane, and carbon dioxide and organic acids; particularly short-chain fatty acids; butyrate, propionate and acetate (Cummings, 1981; Nyman and Asp, 1982; Ross *et al*., 1983 and 1984). Complete anaerobic degradation does not occur because materials are retained in the system for too short time period for

bacteria to be active. Titgemeyer *et al*. (1991) observed that the lag in production of short chain fatty acids (SCFAs) *in vitro* relates to various factors including**:** 1) the time required for substrates to hydrate, 2) the need for the bacteria in the inoculum to proliferate to levels capable of significant fermentation, and 3) the time required for bacteria to degrade polysaccharides once they are present in sufficient numbers. Within an *in vivo* system, lag presumably would be dependent on the third factor because microbial populations would be present in large numbers, and substrates, after passage through the stomach and small intestine, would be fully hydrated (Titgemeyer *et al*., 1991).

Fermentation of substrates *in vivo* is clearly dependent on the retention time of that substrate. Retention time in the colon of humans will indicate the length of fermentation *in vivo*. In cases where retention time is great, the extent of substrate fermentability will be the factor that influences SCFA production. If retention time is short, the rate of substrate fermentation becomes more important (Titgemeyer *et al*., 1991). Also, they observed that type of fibres selected may stimulate the production of individual SCFA. Fermentation of pectins (primarily composed of uronic acids) resulted primarily in the production of acetate (McBurney and Thompson, 1989; Titgemeyer *et al*., 1991). Pectin produce a higher proportion of acetate when fermented than did tragacanth, guar, or soy fibres (McBurney and Thompson, 1989). The fermentation of gums (both arabic and the arabic-guar mixture) resulted in greater proportions of both propionate and butyrate than did the fermentation of pectin (McBurney and Thompson, 1989; Titgemeyer *et al*., 1991). The relative proportions of acetate, propionate, and butyrate produced during fermentation are related to the monosaccharide composition of the fibres source (Mortensen *et al*., 1988).

Also, the selection of substrates on the basis of the profiles of SCFA produced could allow an estimate of gas-production potential (Miller and Wolin, 1979). The production of two acetate or one butyrate from a hexose would be associated with the release of two single-carbon compounds. On the other hand, production of one propionate and one acetate from a hexose would be associated with release of only one single-carbon compound. In addition, both propionate and butyrate are more reduced compounds than acetate, therefore, fermentative production of butyrate and propionate would presumably result in less hydrogen production than fermentation principally resulting in acetate formation. It is therefore likely that, fermentation of the pectins (high acetate production) led to greater gas production than that of soy fibres, gum arabic, or the arabic-guar mixture i.e. higher proportion of propionate and butyrate production (Titgemeyer *et al*., 1991).

Faecal volatile fatty acids increased with increasing gum arabic intake. The acetate concentration increased and butyrate concentration decreased with increasing gum arabic dosage (Ross *et al*., 1984). Also, another study by Sayer *et al*. (1985), showed that in humans fed with gum arabic, the principal fermenters were species of the genera *Bacteroides* and *Bifidobacterium*, and the latter strain being an acetate and lactate producer. Topping *et al*. (1985) studied the production of volatile fatty acids in rats fed with diet containing gum arabic and cellulose separately, and as a mixture. In the cellulose group, acetate comprises 66% of the total, and propionate and butyrate 19% and 12% respectively. Feeding the gum arabic diet raised the contribution of butyrate to 20% and lowered that of acetate. In the combined diet, the relative percentages were 50, 26 and 20% respectively for acetate, propionate and butyrate. Hence, fibres mixtures is more beneficial than single components taken individually.

The mucosa of the small and large bowel depends upon respiratory fuel to maintain cellular turnover and function. Respiratory fuel can either be derived from the bowel lumen or from the circulation (Hanson and Parsons, 1977). The preferred respiratory fuels of the mucosa in the small bowel are glutamine and ketone bodies rather than glucose, which is poorly oxidised and largely converted to lactic acid (Windmueller and Spaeth, 1978). While the respiratory fuels used by the colonic epithelial cells coloncytes are the fatty acid of anaerobic bacteria particularly of the distal colon (Roediger, 1980), and n-butyrate is the major respiratory fuel of the colonic mucosa.

Jacobs and Lupton (1984) described changes in caecal growth and mucosal cytokinetics in rat fed on various components of dietary fibres. They concluded that readily fermented polysaccharides give rise to greater caecal enlargement than poorly degraded material. This suggests that the fermentation products of nondigestible carbohydrates may be the trophic factor, while non-fermentable bulking agents do not stimulate mucosal growth (Dowling *et al*., 1967). Also, Rolandelli *et al*. (1986) observed that, short chain volatile fatty acids lower pH and improve healing when pectin was fed, because they stimulate colonic epithelial cell proliferation (Jacobs and Lupton, 1984), and butyrate had the strongest correlation between specific short chain volatile fatty acids and of cell proliferation (Lupton and Kurtz, 1993).

2.1.4.11. Intestinal destruction of urea:

The major human nitrogenous waste products are urea, creatinine and uric acid which they eliminate from the body only by excretion, but a sizable proportion of the daily production of each is destroyed by

intestinal bacteria (Rouf and Lomprey, 1968; Jones and Burnet, 1974; Wrong, 1978).

Urea is the main precursor of intestinal ammonia. It is continually entering the gastrointestinal tract by diffusion and hydrolysed in the colon by bacteria ureases, liberating ammonia (Wrong, 1978). It was reported that, at least one fourth of synthesized urea is continuously being hydrolyzed by intestinal bacteria in normal subjects (Mackenzie *et al*., 1950). Other ammonia sources can be obtained by the deamination of protein and other nitrogenous substances (Walser and Bodenlos, 1959; Wolpert *et al*., 1970). This ammonia is absorbed and recycled to the liver (Wrong, 1978). Some of this recycled nitrogen is reutilize for the synthesis of body protein (Richards *et al*., 1967; Gioradano *et al.*, 1968). Both in health and uraemia about 40% of the urea is metabolised by bacteria every 24 hours, thus liberating in uraemic individuals sufficient ammonia nitrogen for the synthesis of the minimum daily requirement of non-essential and some essential amino acids (Richards *et al*., 1971). Therefore, two potential pathways exist for recycled nitrogen to re-enter the body (Fig. 1). The first is via reabsorption of the ammonia from gut into the portal system and then into the liver. The second pathway involves incorporation of nitrogen from urea into amino acids by the intestinal flora, subsequent death of the bacteria, followed by the hydrolysis of the bacterial protein and absorption of some of the resultant amino acids into the portal system (Richards, 1972; Jackson, 1983; Fujita *et al.*, 1986). The increasing bacterial activity in the gut leads to increase synthesis and supply to the body of the essential amino acid lysine (Assimon and Stein, 1994). For the ability of intestinal microbes to synthesize amino acids from ammonia, Takahashi *et al*. (1980) observed

that, ammonia was preferentially utilized even when the protease peptone content was eight times greater than that of ammonia nitrogen. It is also known that monogastric animals utilize non-protein nitrogen (NPN) under low-protein diet

conditions (Yoshizawa *et al*., 1973). Hence, many viable intestine organisms, such as *Bacteroides, Bifidobacterium, Colostridium, Proteus* and *Klebseilla* spp. possess urease activity. *Escherichia coli* which is the dominant Gram-negative aerobic bacilli in the intestines of most subjects, do not have urease, but they release ammonia by deamination of substances other than urea. Some organisms produce ammonia by both mechanisms (Wolpert *et al*., 1970).

O'Grady (1966) showed that the most active of the intestine organisms in the production of ammonia were Gram-negative aerobic bacilli, *Escherichica coli, Klebciella* spp. *Proteus* spp *and Pseudomonus* spp. Whereas, the increase in the growth of acidophilic organisms such as *Lactobacillus* and *Bifidobacterium* could depress the growth of putrefactive ammonia producing organism such as *Escherichia coli* and *Bacteroides* spp. (Vince *et al*., 1973).

The faecal urease-activity of uraemic patients increased in proportion to their blood-urea concentration. The increase was the result of production of urease by a greater proportion of strains of the major genera (anaerobic bacteria) neither the number of bacteria, the species, nor the mean urease activity per active strain differed from normal (Brown *et al*., 1971).

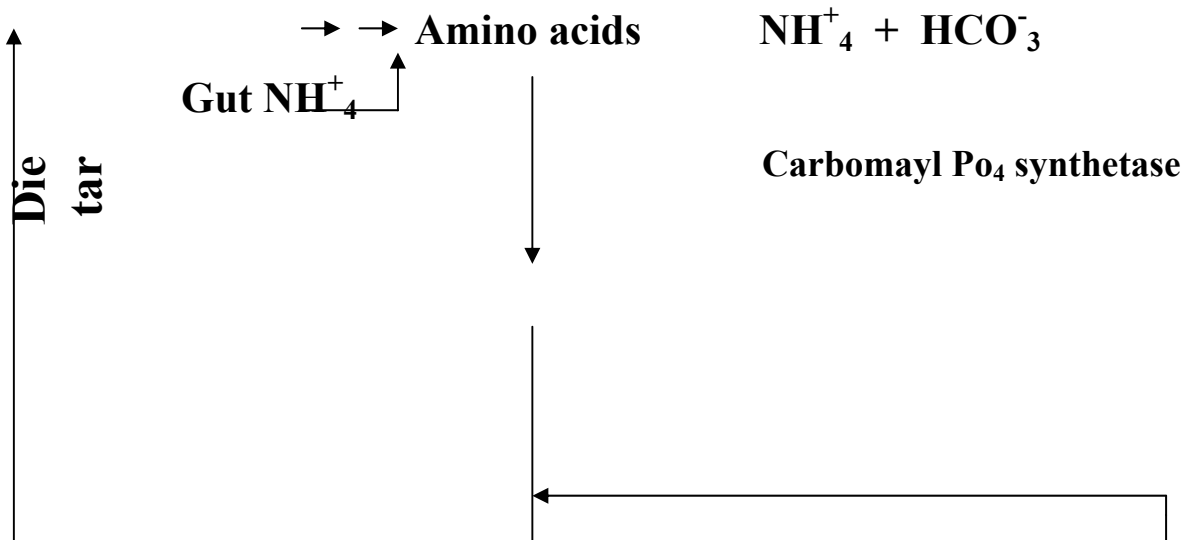
Whatever the diet, there was a net transfer of urea from blood to the caecum, but this process was greatly enhanced with the high fibres diet. Such a transfer is governed by various factors: blood flow, caecal surface of exchange, systematic concentrations of urea and urease activity in the digestive contents, which could be affected by digestive pH (Vince *et al*., 1973).

Nyman and Asp (1982) and Noack *et al.* (1998) showed that, indigestible, fermentable carbohydrate, such as guar gum or pectin, increased faecal nitrogen excretion much more than indigestible nonfermentable carbohydrates such as wheat bran. Noack *et al*. (1998) reported that, the elevated levels of total nitrogen were coincided with an increase in total microbial counts. Also, Younes *et al*. (1995) observed that, the availability of fermentable carbohydrate such as oligosaccharides (fructo-oligosaccharide or xylo-oligosaccharide) in the diet, induced a 20% to 30% decreases in blood urea and renal nitrogen excretion relative to control. This indicates the potentiality of oligosaccharides diet therapy in chronic renal failure than gum arabic and oat fibres.

Ulman and Fisher (1983) observed that, dietary guar gum but not dextrin or wheat bran, increasing faecal nitrogen loss and decreasing the need for urea synthesis partially ameliorates the effect of arginine deficiency. They attributed this to that, guar gum may decrease urea hydrolysis via inhibition of microbial urease, or normal urea hydrolysis may occur, but due to guar gum's gelling characteristics more urea or ammonia may be eleminated from the gut. Alternatively, guar gum could acidify gut contents allowing for greater ammonia concentration in faeces. Finally, guar gum could support greater gut microbial fermentation, resulting in increased protein synthesis, thus effectively removing ammonia as faecal microbial protein. Also, the decrease in the need for arginine in the process of urea synthesis may spare arginine for use in other functions including protein synthesis and growth. Therefore, guar gum may be useful in treating patients with defective urea cycle functions, such alcoholic cirrhotics livers (Weber and Fresard, 1981), or infants with inborn enzymatic defects in urea synthesis (Batshaw *et al*., 1982).

Most dietary attempts to treat chronic renal failure via reducing protein intake. Another approach would be through increased nitrogen excretion via faeces. Bliss *et al*. (1996) observed that, the supplementation of dietary fibres (50g gum arabic/day) had greater faecal bacterial masses, greater faecal nitrogen excretion, and lower serum-urea nitrogen concentration in chronic renal failure patients consuming a low-protein diet than they did when consuming low- protein diet alone or supplemented with one gram pectin/day. Therefore, this may be a beneficial adjunctive therapy for chronic renal disease, since the greater faecal nitrogen excretion during the supplementation period had no adverse effects on the nutritional status of the subjects; i.e. nitrogen balance not affected (Assimon and Stein, 1994; Bliss *et al*., 1996).

In addition to urea; one of the major nitrogen metabolites accumulating in renal failure, other intermediates are generated, such as citruline and ornithine (Swendseid *et al*., 1978); see Fig. (2). The urea cycle intermediate ornithine is believed to be an important regulator of urea cycle metabolism and to regulate interactions with the tricarboxylic acid cycle as well (Menichini *et al*., 1971). The catabolism of ornithine follows several routes, one of these is through decarboxylation by ornothine decarboxylase (ODC) to form putrescine, the obligate precursor of the polyamines spermidine and spermine. ODC activity was significantly lower in renal tissue of



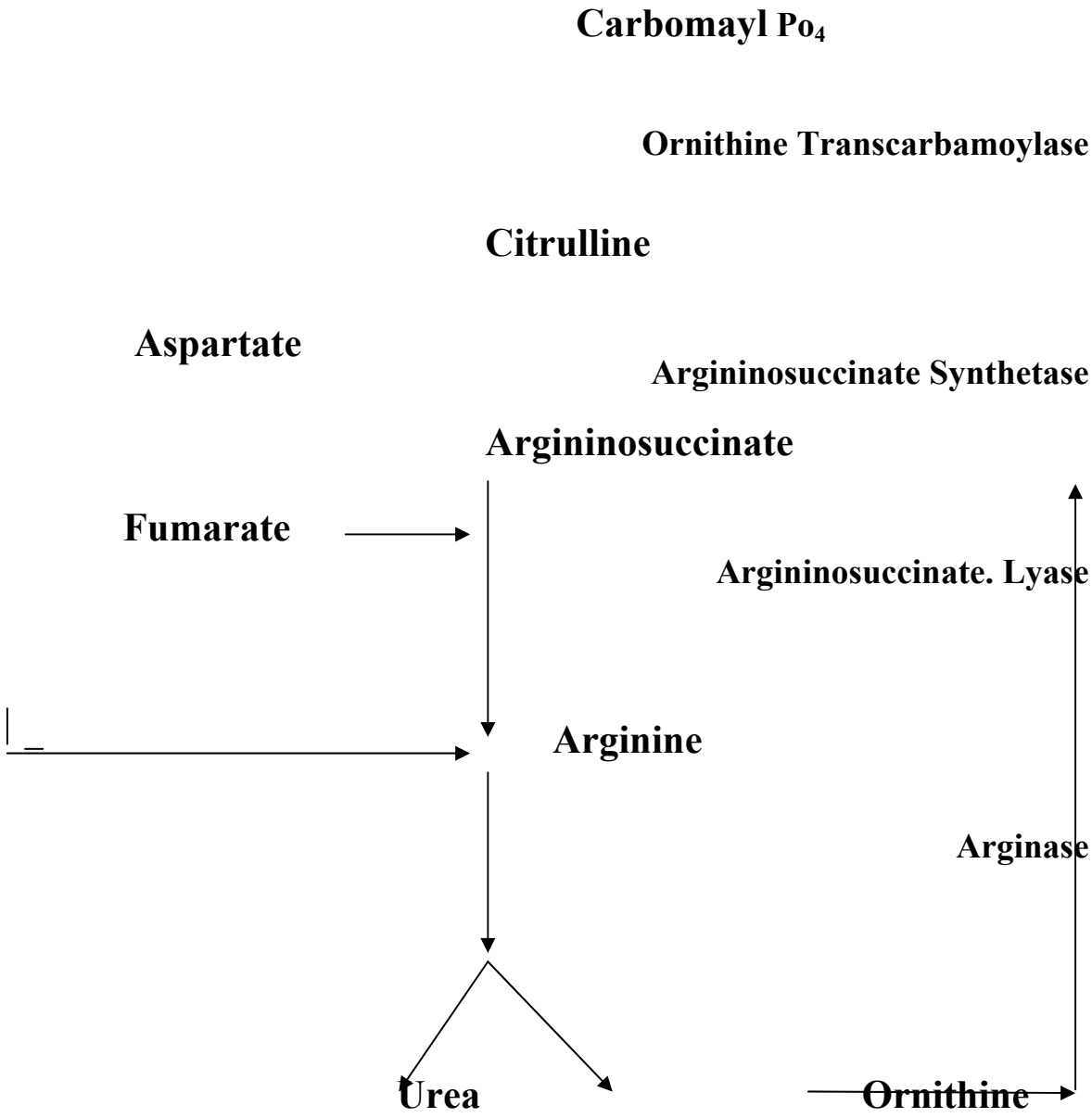


Fig. (2): The urea-ornithine cycle (Swendseid *et al*., 1978)

uremic rats than in controls and a highest plasma urea nitrogen values (Swendseid *et al*., 1978). Also, Swendseid *et al*. (1978) expect that the formation of the metabolically important polyamines spermidine and spermine might be impaired since both *in vivo* and *in vitro* evidence indicate a reduction in the catabolism of ornithine via decarboxylation in

chronically uraemic rat. Polyamines have been implicated as regulators of DNA, RNA and protein metabolism, therefore, a possible reduction in muscle protein and the wasting conditions were commonly seen in renal failure. In addition, Holtta (1977) observed that, the oxidation of polyamines was optimum at pH value ten. Therefore, the toxicity of spermine is higher at more alkaline pH; due to toxic aldehyde formation by oxidaze enzymes (Tabor *et al*., 1972).

2.1.4.12. Dietary fibres and polyamines formation:

The diamine putrescine ($NH_2 (CH_2)_4 NH_2$) and the polyamines spermidine ($NH_2 (CH_2)_3 NH (CH_2)_4 NH_2$) and spermine ($NH_2 (CH_2)_3 NH (CH_2)_4 NH (CH_2)_3 NH_2$) and closely related derivatives are low molecular mass cationic molecules. They are widely distributed in nature. Fungi and animal tissue contain mainly spermidine and spermine (Dudley and Rosenheim, 1925; Harrison, 1931; Tabor *et al.,* 1958; Weaver and Herbst, 1958), whereas bacteria, especially Gram-negative bacteria, contain mainly putrescine and spermidine (Tabor *et al*., 1958). The amines have also been found in many plants (Herbst and Snell, 1949; Smith, 1970_a) and in bacterial viruses (Ames and Dubin, 1960).

Polyamines are of topical research interest, because of their growth activity in both normal and neoplastic cells proliferation and differentiation through regulating the synthesis of DNA, RNA and proteins. The concentration of these amines and their biosynthetic enzymes are also increased during these physiological processes (Tabor *et al*., 1961; Tabor and Tabor, 1972; Tabor and Tabor, 1976; Pegg *et al*., 1978; Pegg and McCann, 1982; Tabor and Tabor, 1984; Kumagai and Johnson, 1988; Seiler and Dezeure, 1990; McCormack and Johnson, 1991; Bardocz *et al*., 1995; Benamouzig *et al*., 1997). Also, Wiegand and

Pegg (1978) and Seyfried and Morris (1979) reported that, polyamine synthesis was needed for growth in regenerating liver and cultured cells.

2.1.4.12.1. Biosynthesis of polyamines:

The biosynthesis of putrescine, spermidine and spermine was first established in microorganisms (Tabor *et al*., 1958; 1961; Morris and Koffron, 1969; Tabor and Tabor, 1972; Tabor and Tabor, 1984). Latter, it was found to be very similar to that in animal cells (Raina and Janne, 1975).

Two primary precursors are required for the biosynthesis of polyamines. The first primary precursor is ornithine. It is a biosynthetic product of arginine; which is decarboxylated by L-ornithine decarboxylase (ODC, E.C.4.1.1.17) to produce putrescine in animal tissues, or decarboxylation of arginine in bacteria via agmatine (Morris and Pardee, 1966; Morris and Koffron, 1969), but the first precusor is the major one (Morris and Koffron, 1969). Also, this reaction is the first rate-limiting step in polyamines synthesis and is induced by a number of hormones and growth factors (Tabor and Tabor, 1984).

The second primary precursor is L-methionine with the aid of ATP to form S-adenosylmethionine which is decarboxylated by S-adenosyl L-methionine decarboxylase (SAMDC, E.C.4.1.1.50) (Wickner *et al*., 1970), and serves as a donor of the propylamine moiety for the synthesis of the other polyamines by their action of aminopropyl transferase (Pegg and Ashman, 1969; Bowman *et al*., 1973), and the supplementation of decarboxylated S-adenosylmethionine is the limiting factor in spermidine and spermine synthesis (Pegg and Hibasami, 1979). Fig. (3) shows these biochemical syntheses clearly.

The enzyme responsible for the oxidation of polyamine (spermine and spermidine) has been found in rat liver cells. This enzyme (Polyamine oxidase) is optimum at pH value close to ten. It is flavoprotein dependant and hydrogen peroxide is evolved in the reaction (Holtta, 1977). The equations below show this obviously**:-**

1- $NH_2(CH_2)_3NH(CH_2)_4NH_2 + O_2 + H_2O$

Spermidine

$NH_2(CH_2)_4NH_2 + NH_2(CH_2)_2CHO + H_2O_2$

Putrescine **3-amino propionaldehyde**

2- $NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2 + O_2 + H_2O$

Spermine

$NH_2(CH_2)_3NH(CH_2)_4NH_2 + NH_2(CH_2)_2CHO + H_2O_2$

Spermidine **3-amino propionaldehyde**

2.1.4.12.2. Sources of polyamines:

Polyamines in the intestinal lumen originate from endogenous and exogenous sources. In healthy humans and animals, the majority of protein reaching the large intestine originates from endogenous, such as pancreatic secretions and desquamated intestinal cells (Benamouzig *et al.*, 1997; Noack *et al*., 1998). Benamouzig *et al*. (1997) observed a significant polyamine concentration in the lumen of the human gut during the fasting stage. They suggested this to endogenous secretion from epithelial cells released as the cells are sloughed off and disrupted or from secretion into the gastrointestinal tract. Also, Noack *et al*. (1998) observed the predominance of putrescine in the caecal content of the germ free rats. This confirms that putrescine was the major endogenous polyamine within the gut lumen. Exogenous polyamine may originate from both food and gut bacterial microflora (Sarhan *et al*., 1989; Benamouzig *et a*l., 1997). Bardocz *et al*. (1993) reported that, typical diets for humans could supply hundreds of micromoles of polyamine per day. Fruit, cheese and non-green vegetables were the major sources of putrescine. All food stuffs especially green vegetable are rich in spermidine while meat is rich in spermine (Bardocz *et al*., 1995).

Food polyamines are rapidly absorbed in the small intestine (Brachet and Tome, 1992; Bardocz *et al*., 1993; 1995; Benamouzig *et al*., 1997). Experiments on rats established that polyamines were readily taken up from the gut lumen, probably by passive diffusion, and were partially metabolized during the process of absorption and more than 80% of the putrescine was converted to other polyamines and non-polyamine metabolites, mostly to amino acids (Bardocz *et al*., 1995).

Since food polyamines, as one of the most important exogenous sources, were rapidly absorbed in the small intestine thereby they were not available to meet the high metabolic demand for polyamines of the mucosal tissues in the large bowel. Hence, the production of polyamines by the intestinal microflora may play a major role in providing polyamines for these purposes (Noack *et al*., 1998). It has been shown that a small proportion of dietary protein, in particular after consumption of high levels of protein may result in the accumulation of undigested or partially digested proteins in the lower part of the gut. The bacterial population in the large intestine has an enormous enzymatic capacity for degradation of proteins resulting in peptides and amino acids that are safe as precursors of biosynthetic reaction or as energy substrates for the microorganisms (Macfarlane and Macfarlane, 1997).

Noack *et al*. (1998) mentioned that bacteria synthesize polyamines by decarboxylation of amino acids ornithine, arginine and lysine. Olson (1993) observed that the bacterial amino acid decarboxylase is influenced by the pH of the medium. Moreover, the production of amines by *Bacteroides fragilis* and *Clostridium perfringens* is maximal under acidic condition (Allison and Macfarlane, 1989) and this is influenced by availability of carbohydrate and growth rate of the bacteria. Noack *et al*. (1998) showed that feeding of rats with soluble indigestible polysaccharides; pectin and guar gum, lower the pH and enhance polyamine formation (putrescine, sperimidine and cadavarine) in the large intestine. The production of cadavarine was more in pectin than in guar gum-fed convential rats.

2.1.4.12.3. Types of polyamines produced by bacteria (prokaryotes):

In general, prokaryotes have a higher concentration of 1,4-diaminobutane (putrescine) than spermidine and lack spermine (Tabor and Tabor, 1976). Tabor *et al*. (1958) made an interesting observation that, the polyamines are present in highest concentration in Gram-negative bacteria. Much lower concentrations are found in Gram-positive bacteria, and many Gram-positive species have little or no detectable amounts of these amines.

Members of the genus *Bacteroides* are spermidine producers and *Fusobacteria* synthesize mainly putrescine, whereas *Bifodobacteria* neither synthesis any polyamine nor do they grow in polyamine free medium (Noack *et al*., 1998). Also, none of the above microorganisms synthesize cadavarine under the *in vitro* growth conditions used. Enterobacteria such as *Escherichia coli* and *Klebsiella* produce cadavarine (Tabor and Tabor, 1985; Noack *et al*., 1998), and the factors that induce cadavarine synthesis have not yet been identified.

The importance of these amines to the bacteria may be assumed from the observation that large amount of amines are synthesized even when bacteria are grown on minimal media, and bacteria form substantial quantities of spermidine even when the precursor of amino acid ornithine and arginine are markedly restricted by slow growth of an arginine auxotroph in a chemostat (Tabor and Tabor, 1969).

2.1.4.12.4. Types, distribution and concentration of polyamine in humans (eukaryotes):

Eukaryotes usually have little 1,4-diaminobutane and have spermine as well as spermidine (Tabor and Tabor, 1976). Considerable variations have been found in different tissues and different species, but particularly high concentrations have been reported in pancreas, prostate and human semen (Tabor *et al*., 1961).

Polyamines have been detected in human serum at a concentration of 0.013nmol/ml for putrescine, 0.49nmol/ml for spermidine and 0.057nmol/ml for spermine (Bartos *et al*., 1977). Therefore, the vasculature may supply polyamines to any given tissue. Polyamines in serum, however, are largely bound to proteins, thus reducing their effective free concentration (Seidel and Snyder, 1989).

Polyamines especially putrescine and cadaverine; derived from prokaryotic rather than eukaryotic; are found in the lumen of the gastrointestinal tract in a high concentrations that are free and available for use by the intestinal cells. These polyamines are recirculated in the gastrointestinal lumen via an enterohepatic circulation and once transported may stimulate DNA, RNA and protein synthesis (Ginty *et al*., 1989; Osborne and Seidel, 1990). In addition, alteration of luminal polyamine content results in alteration of gastrointestinal mucosal structure (Osborne and Seidel, 1989).

2.1.4.12.5. Clinical role of polyamines:

Earlier work by Rosenthal *et al*. (1952) demonstrated that, single injections of spermine (0.1 to 0.2mM/kg.) in a variety of animals resulted in acute renal insufficiency and death within four to ten days. This was due to an acute necrosis of the epithelium of the proximal convoluted tubules of the kidneys. In man, intramuscular administration of spermine

caused albuminuria, haematuria and azotomia (Risetti and Mancini, 1954).

Saito *et al*. (1983) measured the serum levels of four polyamines (putrescine, spermidine, spermine and cadavarine) in normal subjects and in patients with chronic renal failure. The results show that, compared to normal subjects, the serum levels of all four polyamines are significantly elevated either in nondialyzed patient with chronic renal failure or in dialyzed patients.

However, for tumours diagnosis polyamines were used. Spermidine and spermine are mainly transported in blood by RBC, but RBC polyamines levels did not allow discrimination between malignant and non malignant tumours, and this confirms earlier findings that RBC polyamines are markers of cell proliferation rate but not of the presence of malignant tumour. Hence, elevated RBC polyamine concentrations are an index of the intensity of hyperlastic processes, which can be clinically used for the early detection of proliferate phases of tumours, thus, allowing timely therapeutic measures (Quemener *et al*., 1995).

Feeding a polyamines free diet concomitant with oral administration of nonabsorbable antibiotic and treated with irreversible ornithine decarboxylase inhibitor (difluromethylornithine) decrease the growth rate of grafted lewis lung carcinoma or prostatic tumours in rodents by > 80%(Seiler *et al*., 1990).

Therefore, there is no reason to conclude that the increase in amines is especially related to the neoplastic process, other than as a reflection of a relatively rapid growth rate. Some of these increases may be related to destruction of tumour or normal tissue especially during therapy or weight loss, infection, variation in food intake and bacterial

contamination of urine may alter the amount of amines found in urine (Tabor and Tabor, 1976).

2.1.5. The role of fibres in nutrition and cause of disease:

While proteins, fats and carbohydrates have been studied extensively the unabsorbable fibres portion of our food has almost been ignored, probably because it is indigestible and negligible nutrient value (Burckitt *et al*., 1972).

Many diseases of Western civilization are rare in less developed countries where the diet is normally much richer in cereal and vegetable fibres; because fibres are lost when carbohydrates are refined. Clinically the addition of fibres to a refined diet reverses the effect of food refining (Burckitt *et al*., 1972).

Painter and Burkitt (1971) and Trowell (1972) claimed that, the diseases of middle and old age are due to failure by the population in Western countries to consume, throughout early life, adequate amounts of vegetable fibres, in particular wheat bran. These diseases are appendicitis, atheroma, cancer of the colon, constipation, coronary thrombosis, dental caries, deep-vein thrombosis, diabetes mellitus, diverticular disorders, gallstones, haemorrhoids, hiatus hernia, ischaemic heart diseases, peptic ulcer, polyps of the bowel (both simple and malignant) and varicose veins. They suggested that the incidence of these diseases is primarily due to excessive gastrointestinal transit times, especially through the colon. Excessive transit times permit much more extensive degradation of partially digested material by colonic bacteria (Aries *et al*., 1969; Painter, 1971), with the production of harmful products which remain in contact with the gut for undesirably long time. Moreover, the residue associated with high fibres diets may absorb some metabolites; such as bile acids;

thereby facilitating their elimination. Also, they mention that large amount of refined sugar, fat and meat now consumed provides edible plant fibres supposedly superior to vegetable and fruit fibres.

Therefore, the preventive medicine must specify measures for better life style; more physical activity, less alcohol and tobacco and a better diet containing more fibres. Hence, food producers and manufacturers must be challenged to put a greater variety of high quality breads, potatoes, vegetables and fruits in the shops and supermarkets at prices that will encourage people to buy and eat more of these good foods.

2.2. Gum arabic:

According to Glicksman and Sand (1973) gum arabic is an ancient natural product used by man since the days of that Pharaohns. Gum arabic found its way into Europe through various Arabic ports, therefore, it acquired the name gum arabic, depending on origin and port of export. It is known as Turkey gum when Turkish Empire controlled ports. When re-export trade developed in India (Bombay) gum arabic acquired the name East Indian gum or Indian gum although it completely differs from Indian gum which more commonly known as gum ghatti. Other names; pertaining to the local area where it is collected, colour and grade; have been given to it so that there is a great deal of confusion concerning the relationship between the name, the actual gum and the species of *Acacia* from which it is derived (Smith and Montgomery, 1959; Glicksman, 1969).

2.2.1. Theories of gum formation:

Various views have been put forward concerning the processes where by gum is formed in plants (Howes, 1949). This gummy exudate

may be formed under pathological conditions caused by microbial infections, or that, gum formed to prevent micro-organism infection at the injury site on the bark or fruit scar of tree (Blunt, 1926). Other thought that gum may be formed as a result of bad natural factors such as poor soil; lack of moisture and hot weather which will affect the vitality of the trees and improved gum yields, or as a normal metabolic process in the plant (Malcolm, 1936).

Till now formation of gum (gummosis) is a matter of contraversity, and there is no any universal proposal to explain this phenomenon clearly (Glicksman and Sand, 1973).

2.2.2.Sources and production of gum arabic in the Sudan:

Acacia senegal (L.) Willd., english name is gum arabic tree and vernacular name is Hashab (family Leguminaceae, Mimosidae), is the major gum arabic producer. This tree (Plate 1) is about twelve to twenty feet (4.5 – 6 m) tall with a life span of twenty five to thirty years, and production of the gum starts after they are five-year-old (Glicksman and Sand, 1973). Sudan produce 70 – 90% of 80% of the world supply (Glicksman and Sand, 1973; Gabb, 1997).

Sudan: 2.2.3. Geographical location of gum arabic in the

Acacia senegal tree found in the Sudan throughout part of the twelve states, North Kordufan, South Kordufan, West Kordufan, North Darfur, South Darfur, West Darfur, Kassala, Gadarif, Sinnar, Blue Nile, White Nile, Upper Nile forming what is known as the Gum Belt, which is lie betwwen $11^{o} – 15^{o}$ latitude North (Gabb, 1997); see Fig. (4). This belt covers a large climatic variation in the Sudan from tropical in the South to extensive savannah in the North. Rainfall and soil conditions are the most important climatic factors in the Gum Belt.

The best grade of gum in the Sudan comes from cultivated trees (garden gum or hashab genieina) or from untended wild tree (wild gum or hashab wady). One of the reasons of its excellent quality is that the limitation of botanical sources results in a uniform product (Glicksman and Sand, 1973).

2.2.4. Tapping and collection of gum arabic:

According to Glicksman and Sand (1973) tapping and collection are important activities carried out by farmers during the early part of the dry season; from October and extended to May. Tapping is carried out by a simple axe or soonky once a year. The first collection is made after four to six weeks and continues at an interval of about two weeks. About four to eight collections may be made per season. The gum is picked up by hand and stored until sufficient quantities formed. Then it was transported to marketing center e.g. Al-Obied (known as gum market). The average annual production from *Acacia* tree ranges from 0.5 to two kilogram of crude gum nodules (MNP, 1980), and the yield varies according to the water condition,

tapping, collection techniques, availability of drinking water, availability of crops (Dura) and marketing.

Commercial gum arabic is reported by Howes (1949) and Glicksman and Sand (1973) to be available in several grades**:** hand picked selected, cleaned, sifted bleached and dust. The crude gum is processed in Sudan into kibbled, granular, powder and spray-dried. Both raw and processed gum arabic is exported from Sudan. The company in charge of foreign trade is the Khartoum Gum Arabic Company Ltd. All exports are subjected to quality grading by the Sudanese Standards and Metrology Organization (Gabb, 1997).

2.2.5. The physico-chemical properties of gum arabic:

The important physical parameters in determining the use and commercial values of gums are solubility, colour, odour, viscosity, taste and shape (Howes, 1949). *Acacia senegal* gums have a considerable degree of variation in physico-chemical, function and toxicological properties according to different locations, soil type and age of the tree (Anderson and Karamalla, 1966). Hence, *Acacia senegal* gum has various colours ranging from colourless to light brown, almost odourless soluble in water and form transparent viscous solution, and insoluble in ethanol

and other organic solvents and it is acidic with specific rotation levorotatory.

Chemically gum arabic is described by Anderson and Stoddart (1966), Street and Anderson (1983) and Phillips and Williams (1993) as a large molecule with a high molecular weight. It consists of the carbohydrate moieties L-rhamnose (12.8 – 14.0%), L-arabinose (26 – 28%), D-galactose (40 – 44%), and D-glucuronic acid (15.5 – 16.0%) and its 4-O-methyl (1.5%) derivative which form calcium, magnesium and potassium salts. The carbohydrate moieties covalently attached to the protein backbone (~2%) which formed from ala, arg, asp-acid, cys, glu-acid, gly, his, hyd-pro, iso-leu, lys, met, phe, pro, ser, thre, tyr and val (Anderson *et al*., 1985).

2.2.6. The Joint Expert Committee of Food Additives (JECFA) specifications of gum arabic:

Gum arabic as commercial products may occur in a mixture with various materials quite often with other gums that have properties similar to those of gum arabic. Therefore, the JECFA proposed certain specification for gum arabic, which is reviewed every four years. The last specifications JECFA, 1999 defines gum arabic as a dried exudate obtained from the stems and branches of *Acacia senegal* (L.) Willd. or *Acacia seyal* (family leguminosae). Gum arabic consists mainly of high molecular weight polysaccharides and their calcium, magnesium and potassium salts, which on hydrolysis yields arabinose, galactose, rhamnose and glucuronic acid. Gum arabic from *Acacia senegal* is a pale white to orange-brown solid, which breaks with a glassy fracture. The best grades are in the form of whole spherical tears of varying size with a matt surface texture. When ground, the pieces are paler and have a glassy appearance. Gum from *Acacia seyal* is more brittle than the hard tears of

Acacia senegal. Gum arabic is soluble in water forming a solution which flows readily and it is acidic, insoluble in ethanol. Optical rotation of *Acacia senegal* solution are levorotatory and those of *Acacia seyal* are dextrorotatory. Loss on drying ($105^{o}C$/5h) not more than 15% for granular and not more than 10% ($105^{o}C$/4h) for spray-dried material. The total ash is not more than 4%. The acid insoluble matter is not more than 1%. Lead is not more than 2mg/kg. No starch and tannin were found. Microbiologically no *Escherichia coli* or *Salmonella* spp**.** could be detected. As food additive gum arabic is used as emulsifier, stabilizer and thickener.

2.2.7. The microbial load of gum arabic:

Microbial examination of authentic and commercial *Acacia senegal* gum samples from Sudan; seasons 1995/96, 1996/97, 1997/98 collected from different parts of Sudan (Al-Obied, Al-Gadarif and Port Sudan) at different conditions (Trees, markets and stores) was carried out. All samples tested showed no signs of definite microbial spoilage, and the maximum microbial load of these samples were about $9.1x10^{6}$CFU/g bacteria and about $1.5x10^{6}$CFU/g mould. The microbial loads of processed gum samples were much lower than the loads of raw samples. These limits are suggested to be adopted by (JECFA) specification in the microbial criteria (Osman, 1998).

Bokhary *et al*. (1983) isolated certain fungi of genera *Aspergillus, Alternaria, Curvularia*, and *Helminthosporium* as well as bacteria of genera: *Bacillus, Serratia,* and *Micrococcus* from gum arabic samples and no algal growth was observed.

Idris (1989) isolated five genera of moulds, namely *Aspergillus, Penicilium, Rhizopus, Glaicaladium* and *Cladosporium. Aspergillus* was

the most dominant one. No yeasts were observed. He also isolated Gram-positive spore-forming bacilli, non-form-sporing Gram-negative bacilli, and Gram-positive cocci. No *Staphylococcus aureus* and other pathogenic organisms were detected.

Mohammed (2000) studied the microbial load of gum arabic obtained from West and East of the Sudan. The bacterial load in both samples do not exceed $8.2x10^3$CFU/g, and 84.3% of isolates were Gram-positive rods, 7.1% Gram-negative rods and 7.1% were Gram-positive cocci, while the fungal load was comparatively low and most of it belongs to the genus *Aspergillus* and *Penicillium*. No yeast could be isolated from samples.

Mohammed (2000) observed that the microbial flora in gum arabic were completely eradicated by autoclaving for 10 minutes at 100^{o}C for three successive days. Bokhary *et al*. (1983) and Osman (1998) reported that the exposure of gum arabic to sunlight, UV-light, and gamma irradiation results in a very clear decrease in the bacterial and mould count. They also observed that such treatment does not affect the quality and the quantity of the carbohydrate content significantly. These treatments were recommended as a simple procedure to reduce the microbial contamination of gum arabic (JECFA, 1997).

2.2.8. Uses of gum arabic:

Gum arabic is a very important hydrocolloid in food and nutrition (Glicksman and Sand, 1973). As food, gum arabic is used as food additive to improve desirable properties through its influence on the viscosity, body and texture of food. It is odourless, colourless, tasteless and completely soluble in water therefore, it does not affect the flavor, odour or colour of the food to which it is added. It is also nontoxic; no

upper limits have been recommended by the FAO/WHO Committees. It is used as emulsifier and stabilizer for frozen dietary products to prevents crystallization of sugar. It is also act as a source of soluble fibre in low calorie and dietetic beverages (Phillips, 1998); hence in such a manner it replaces calorific foods.

Medically gum arabic is used as demulcent to smooth irritation, especially of the mucous membranes and has been shown to lower the cholesterol levels in the blood of the laboratory animals (Gabb, 1977). Gum arabic has been used for the treatment of low blood pressure caused by haemorrhage or surgical shock (Maytum and Magath, 1932). Intravenous saline injections alone were not successful because the salt escaped too rapidly from the blood vessels. The addition of a 7% gum arabic solution reduced the dissipation rate of the sodium chloride solution, and this treatment was successfully used in the 1920's. In plastic surgery, a 5% gum arabic adhesive has been used successfully in grafting destroyed nerves (Osol and Farrar, 1955). In 1933, intravenous injections of gum arabic solutions were recommended for the treatment of nephritic oedema. Some reports cited consequent liver and kidney damage, whereas other reports; in which as much as 330g of gum arabic were administered; presented no evidence of hepatic or renal damage but stated only that the treatment was successful in alleviating or eliminating the oedema under treatment (Anderson *et. al*., 1947; Osol and Farrar, 1955). Also, some allergic reactions, manifested by asthmatic conditions, were caused by exposure to gum arabic used in offset printing (Osol and Farrar, 1955) and most cases seem to have arisen as a result of inhaling the gum as dust (Sprague, 1942). In addition to the above, it is used in the treatment of chronic renal failure diseases since it decreased serum

nitrogn concentration and increased feaecal nitrogen excretion (Bliss,*et al*. 1996).

Gum arabic also used in cosmetics, inks, lithography, paper, paint, adhesives, textiles industry and inhibit metal corrosion. Table (7) summarizes these industrial uses clearly.

Table(7): Industrial uses and properties of gum arabic (Glicksman and Sand, 1973) :

Industries	Uses	Properties
FOODS	Confectionery	Prevent sugar crystallization and as emulsifier
	Diary products	Stabilizse frozen products (fine texture).
	Bakery products	For topping smoothness.
	Flavour fixation	Fixative for flavour.
	Beverages	Foam stabilizer and clouding agent.
	Dietic and diabetics products	Low calorific value.
Pharmaceutical	Demulcent syrup	Smoothing character.
	Emulsifying agent	Stabilizer (stable over pH 2-10).

	Antiseptic properties	**Reduce the bactericidal action.**
	Suspending agent	**Suspend insoluble drug and prevent precipitation and facilitate absorption from the intestine.**

Table (7) continued:

Cosmetics	**Face mask and cream**	**As a binder.**
	Hair cream	**Fixatives.**
	Protective cream	**Stabilizer.**
Other Industries	**Adhesives**	**As a glue for general purposes.**
	Inks	**Protective and suspending agent.**
	Lithography	**Sensitizer for lithographic plates.**
	Papers	**Coating.**
	Paints	**Protective and control flocculent and setting.**
	Textiles	**Sizing finishing agent for cloth.**
	Metals	**Inhibit metal corrosion.**
	Liquid glue	**Transparent films and adhesion.**

	Ceramics	**Binding of glazing.**
	Pesticides and insecticides spray	**Spraying and suspending agent**

Materials and Methods

3.1. Materials:

3.1.1. Source of sample:

3.1.1.1. Gum arabic samples:

3.1.1.1.1. Raw form of gum arabic (gum nodules):

Twenty-five commercial samples were collected randomly from local markets of Khartoum State to study their physico-chemical properties and microbial load (Plates 2 and 4:A). Another five authentic ones; hand picked and stored in paper envelopes samples, were provided by Dr. K. A. Karamalla of the Faculty of Agriculture – University of Khartoum to study their microbial load.

3.1.1.1.2. Processed form of gum arabic:

a) Twenty-five samples were provided by Dr. K. A. Karamalla of the Faculty of Agriculture – University of Khartoum as a ready crushed form (crushed by electrical grinder), see Plate (4:B). Appendix (3) shows their origin and code number, and were randomly selected.

b) Twenty-five samples (twenty as commercial and five as authentic) were represented the crushed form of the raw material above; crushing by electric grinder.

c) Twenty-five samples were represented the fine powder form were provided by Dr. Salma M.

Sulieman at Khartoum Dialysis and Kidney Transplantation Centre and Dr. Karamalla of the Faculty of Agriculture – University of Khartoum (Plates 3 and 4:C).

All these 75-processed forms were studied to detect their natural microbial load, mainly bacterial load.

3.1.1.1.3. Source of gum arabic sample used for the preparation of M-70-inorganic medium and as dose for chronic renal failure patients:

Fifteen kilograms of gum arabic was provided by the Gum Arabic Processing Company (GAPC). This sample was used as a dose (25g/day) for chronic renal failure patients, and for the preparation of M-70-inorganic medium containing 2% gum arabic as the only sole of carbon source.

3.1.1.2. Faecal samples:

Sixty faecal samples were collected from each ten healthy and chronic renal failure patients at three intervals; one before and two after the consumption of gum arabic, to measure the nitrogen and volatile fatty acids content in their faeces.

3.1.1.3. Blood samples:

Sixty blood samples were collected from each ten healthy and chronic renal failure patients at three intervals; one before and two after the consumption of gum arabic, to measure changes in polyamines formation, haemoglobin percentage, creatinine, urea and minerals content.

3.2. Methods:

3.2.1.1. Microbiological methods:

3.2.1.1. Sterilization and disinfectant:

3.2.1.1.1. Sterilization:

3.2.1.1.1.1. Media:

Media, normal saline and distilled water were sterilized by autoclaving at 121^{o}C for 15 minutes, while peptone water sugar and gum solutions were sterilized by autoclaving at 110^{o}C for 10 minutes.

3.2.1.1.1.2. Glassware and other equipments:

Petri dishes, test tubes, flasks, pipettes were sterilized in hot air oven at temperature 150 – 170^{o}C for one hour, while bottles; MacCarteny and Bijou were sterilized by autoclaving at 121^{o}C for 20 minutes (Merchant and Packer, 1977).

Platinum loops and forceps were sterilized by direct flame, and UV lamb radiation for the UV-room for 20 minutes.

3.2.1.1.2. Disinfectant:

Alcohol was used for disinfecting floors and benches.

3.2.1.2. Examination of culture:

Examination of turbidity, change in colour in liquid media and type of colony in solid media were examined physically by naked eye.

3.2.1.3. Isolation and identification of isolates:

3.2.1.3.1. Primary isolation and identification:

Samples of gum arabic and faeces were cultured on blood and MacConkey's agar for primary isolation.

The primary identification of the isolates was done by examination with light microscope and with the help of oil immersion lens, after staining with Gram-stain.

Gram-stain was done according to the method described by Cruickshank *et al.* (1975). The smears were prepared by emulsifying a small inoculum in a drop of normal saline and

spreaded on a clean slide, allowed to dry in air and fixed by gentle flaming. Then the slide was placed on the rack and flooded with crystal violet-stain for one minute, washed with distilled water, covered with iodine for one minute, washed again with distilled water and acetone for no time and washed again with water. Then the carbofuchsin was added for one minute, washed with water and allowed to dry by blotting the slide gently with filter paper.

3.2.1.3.2. Secondary isolation and identification:

Primary isolates were cultured on nutrient agar as pure isolates for secondary identification and preservation of the isolates. Secondary identifications were achieved by chemical test.

3.2.1.4. Buffers:

3.2.1.4.1. Normal saline:

The normal saline solution was prepared according to Cruichshank *et al*. (1975) by dissolving 8.5g of sodium chloride in 1000ml distilled water. The mixture was then dispensed into test tubes (9ml each), autoclaved at 121^{o}C for 15 minutes and stored at 4^{o}C. This solution was used as a diluent medium.

3.2.1.4.2. Distilled water:

Distilled water was prepared by distillation of tap water using a distillator (Elga, England) Cowan and Steel (1977).

3.2.1.5. Media used for cultivation of isolates:

3.2.1.5.1. Nutrient broth:

This medium was prepared according to Oxoid (1973). It consists of:

1.0 g	**Lab-lemco powder**
2.0 g	**Yeast extract**
5.0 g	**Peptone**
5.0 g	**Sodium chloride**

All these constituents (13.0g) were dissolved in 1000ml distilled water by heating in steamer. The pH was adjusted to 7.2, then distributed in test tubes 5ml each, sterilized by autoclaving at 121^{o}C for 15 minutes and stored at 4^{o}C.

A sterile loop was used for inoculation of pure colony into the medium for sub-culture.

3.2.1.5.2. Nutrient agar:

Two percent (20.0g) agar (Oxoid) was added to 1000ml nutrient broth constituents dissolved by heating in steamer, sterilized by autoclaving at 121^{o}C for 15 minutes, cooled (45 – 50^{o}C), poured into sterile Petri dishes (about 15ml each) and stored in sterile cans at 4^{o}C.

Using sterile loop a single colony was cultured on the surface of the media, incubated at 37^{o}C for 24 hours and then preserved in the refrigerator for biochemical test.

3.2.1.5.3. Blood agar:

Blood was taken from the jugular vein of sheep aseptically by bleeding needle, which was connected to flat bottom flask with glass beads to prevent fibrination of the blood during continuous shaking for 15 minutes. The defibrinated blood was aseptically distributed into bottles in portions 10ml each. To cooled (45 – 50^{o}C) sterilized nutrient agar, 10ml of the defibrinated sheep blood was added aseptically to each 90ml of the medium. Then gently mixed, poured into sterile Petri dishes (15ml each) and stored in sterile cans at 4^{o}C after they were solidified.

Two grams of processed gum arabic and one gram of faecal sample were dissolved in 9ml normal saline solution. Then serial dilutions were prepared, 0.025ml from each dilution were spread on the medium. For raw form of gum arabic, a loop full of normal saline solution was placed on the surface of gum arabic nodule and rubbed for few seconds then streaked on the surface of the medium. The same procedure was repeated for the cortex of the nodule after it has been broken by a sterile pincer. Then the plates were incubated at 37^{o}C for 48 hour.

The isolates were counted according to Miles and Misra method (Miles and Misra, 1938), and identified by primary and secondary methods.

3.2.1.5.4. MacConkey's agar:

MacConkey's agar was prepared according to Oxoid (1973). It consists of**:**

Peptone	**20.000**	**g**
Lactose	**10.000**	**g**
Bile salts	**5.000**	**g**
Neutral red	**0.075**	**g**
Agar	**12.000**	**g**

All these constituents were suspended in 1000ml distilled water and dissolved by heating in an Arnold's steamer. The pH was adjusted to 7.4, sterilized by autoclaving at 121^{o}C for 15 minutes, distributed into Petri dishes in portions of 15ml each and stored at 4^{o}C.

This medium was used for the detection of coliform (*Escherichia coli*, *Salmonella* spp. and *Shigella* spp.) occurring in gum arabic. The procedure for preparation of samples and the inoculation were similar to those described in the blood agar medium.

3.2.1.5.5. The M-70-inorganic mixture medium containing two per cent gum arabic:

This medium was used as a carbon source utilization test.

According to Veron (1975) it was composed of the following**:**

a) Trace elements solution:

H_3PO_4	**1.960**	**g**
$FeSO_4.7H_2O$	**0.056**	**g**
$ZnSO_4.7H_2O$	**0.029**	**g**
$MnSO_4.4H_2O$	**0.022**	**g**
$CuSO_4.5H_2O$	**0.002**	**g**
$Co(NO_3)_2.6H_2O$	**0.003**	**g**
H_3BO_3	**0.007**	**g**

All these salts were dissolved in 1000ml distilled water and kept unsterilized at 4^oC.

b) Solution (A):

0.015 g	**$CaCl_2.2H_2O$**
0.123 g	**$MgSO_4.7H_2O$**
0.680 g	**KH_2PO_4**
2.610 g	**K_2HPO_4**
Trace elements solution	**10.000 ml**

All these salts were dissolved in distilled water up to 500 ml. The pH was adjusted to 7.2 using a pH meter and autoclaved at 121^oC for 20 minutes.

c) Solution (B):

NaCl	**7.0 g**
1.0 g	**$(NH_4)_2SO_4$**

All these constituents were dissolved in 500ml distilled water, the pH was adjusted to 7.2 and autoclaved at 121^{o}C for 20 minutes.

d) The carbon source solution:

In this study, the carbon source was considered to be gum arabic. Twenty grams were completely dissolved in 100ml distilled water, the pH was adjusted to 7.2 and autoclaved at 110^{o}C for 10 minutes.

450ml of solution (A) were mixed aseptically to 450ml of solution (B) and 100ml of carbon source. This represents the M-70-inorganic medium with 2% carbon sources.

The faecal isolates *Klebsiella* sp. and *Escherichia* coli, were each inoculated to some extend with equal quantities into the M-70-inorganic medium with 2% carbon source at a time, and incubated at 37^{o}C. Plate (5) shows the flasks that represent an *in vitro* incubation system; *Klebsiella* sp. (A) and *Escherichia coli* (B) cultures three liters each.

Samples from these media were taken after zero time (as base line) then every day for fifteen days to detect the biodegradation of gum arabic by these microorganisms *in vitro* through changes in pH, viscosity, specific rotation, total acid, volatile fatty acids, reducing and non-reducing sugars within the incubation period mention above.

The same media mentioned above was used and instead of $(NH_4)_2SO_4$ 2% urea was added to detect the formation of polyamines *in vitro*. Each *Escherichia coli, Klebsiella* sp. and a mixture of them were inoculated into 200ml of the media and incubated for 10-days at room temperature. A portion from each culture was taken regularly every day to assess the metabolic changes in pH, ammonia, polyamines formation and viability of these microorganisms under these conditions.

:3.2.1.6.Media and reagents used for identification of isolates

3.2.1.6.1. Motility medium:

This medium was prepared according to Cowan and Steel (1977).

It is composed of 100ml of nutrient broth to which 0.27 g of

agar were added. Then distributed into test tubes 9ml each, sterilized by autoclaving at 115^{o}C for 10 minutes and stored at 4^{o}C.

Using straight wire, a young culture was inoculated to a depth of 5mm into the glass tube under aseptic condition. Then, incubated for 24 hour at 37^{o}C. If the growth was confined to the stab-inoculum, the organism was considered as non-motile, while if is spreaded in a radiating manner off the tract of needle it is considered as motile.

3.2.1.6.2. Sugar media (peptone water sugars):

The sugar media were prepared as described by Holdman and Moore (1972) from the following**:**

a) Ten grams of Bacto peptone and 5g of sodium chloride were dissolved in 1000ml distilled water by heating in steamer and the pH was adjusted to 7.6. Then dispensed in bottles (190ml each), autoclaved at 121^{o}C for 15 minutes. This represents peptone water.

b) Anderson's indicator was prepared by dissolving 5g of acid Fuchsin in 1000ml distilled water. To this 150–180ml of alkali solution (0.52% N-NaOH) were added till the colour changed from red to brown. After 24 hour 1% (10ml) of the indicator was added to solution (a); peptone water.

c) To the peptone water (190ml), plus the indicator (10ml) 2g of the appropriate sugars (Glucose, Xylose, Mannitol, Raffinose and Mannose) were added to each bottle to give a final concentration of 1%. Then the medium was

distributed into test tubes (5ml each), autoclaved at 110^{o}C for 10 minutes and stored at 4^{o}C.

The test was achieved by inoculation of the test organism into the peptone water sugar medium, incubated at 37^{o}C and examined daily for seven days. Acid production was indicated by the appearance of reddish colour.

3.2.1.6.3. Starch agar:

This medium was prepared according to Barrow and Feltham (1992) as follows**:** potato starch 10g/50ml distilled water were added to 1000ml nutrient agar, sterilized by autoclaving at 115^{o}C for 10 minutes, dispensed into Petri dishes (15ml each) and stored at 4^{o}C.

The test organism was cultured on the medium, incubated at 37^{o}C for 48 hour. Then iodine solution (2g/10ml N-NaOH and 90ml distilled water) was poured over the medium; a blue colour indicates the presence of starch.

3.2.1.6.4. Casein agar (Milk agar):

This medium was prepared according to Oxoid (1973) as follows**:** 10ml of freshly boiled skimmed milk were added to 90ml of cooled (45–50^{o}C) sterilized nutrient agar. The medium was distributed into sterile Petri dishes and stored in sterile cans at 4^{o}C.

The test organism was cultured and incubated at 37^{o}C and examined daily to test the ability of the organism to coagulate casein.

3.2.1.6.5. Nutrient gelatin agar:

The medium was prepared according to Oxoid (1973). The medium consists of**:**

Lab-lemco powder	**3**	**g**
Peptone	**5**	**g**
Gelatin	**120**	**g**

These ingredients (128g) were dissolved in 1000ml distilled water, the pH was adjusted to 6.8, sterilized by autoclaving at 121^{o}C for 15 minutes, poured into sterile Bijou bottles in portions 2ml each and stored at 4^{o}C.

The isolate to be tested was stabbed into nutrient gelatin and incubated at 37^{o}C for 14 days and every three days was placed in the refrigerator to determine the gelatin liquefaction, by either the gelatin retained its gelling property or liquefied.

3.2.1.6.6. Citrate medium:

This medium was prepared according to Difco (1972). It composed from**:**

0.20	**g**	**Magnesium sulphate**
1.00	**g**	**Monoammonium phosphate**
1.00	**g**	**Dipotassium phosphate**
2.00	**g**	**Sodium citrate**
5.00	**g**	**Sodium chloride**
15.00	**g**	**Bacto agar**
0.08	**g**	**Bacto–brom–thymol blue**

All these constituents of Simmons citrate agar (24.28g) were dissolved in 1000ml distilled water by heating in a steamer, the pH was adjusted to 6.8 and then dispensed into 5ml capacity bottles, slanted and stored at 4^{o}C.

The test organism was cultured on the surface of slope Simmon's citrate medium and incubated at 37^{o}C and examined daily for 14 days. Positive tests were indicated by changing of colour from green to blue, i.e. the test organism utilize citrate as a sole source of carbon and inorganic ammonium salt as a source of nitrogen.

3.2.1.6.7. Nitrate broth:

The medium was prepared according to Barrow and Feltham (1992) as follows**:** 1g of potassium nitrate was added to 1000ml nutrient broth. Then distributed into test tubes (5ml each), autoclaved at 121^{o}C for 20 minutes and stored at 4^{o}C.

Two reagent solutions were prepared according to Cowan and Steel (1977) as follows**:**

Solution A: Composed of 0.8% sulphanilic acid in 5N-acetic acid, dissolved by gentle heating and kept at 4^{o}C.

Solution B: Composed of 0.5% ∝-naphthylamine in 5N-acetic-acid, dissolved by gentle heating and kept at 4^{o}C.

Before use, equal volumes of solution A and B were mixed to form the test reagent. 1ml of the mixture was added to the culture medium.

The test organism was cultured into the medium by sterile loop and incubated at 37^{o}C for two days. Then 1ml of the test reagent was added, a red colour indicates the presence of nitrite (positive test). If the colour was not developed, 20mg of zinc powder were added, a red colour indicates the presence of nitrate in the medium (negative test).

3.2.1.6.8. Indole medium:

The indole medium was prepared according to Cowan and Steel (1977) as follows**:**

10.0 g	**Peptone**
5.0 g	**Sodium chloride**

All these (15g) were dissolved in 1000ml distilled water by heating in steamer and the pH was adjusted to 7.4 and dispensed into 5ml capacity bottles, autoclaved at 121^{o}C for 15 minutes and stored at 4^{o}C.

Kovac's reagent was prepared as described in Cowan and Steel (1977) as follows**:**

P–dimethylamine benzaldehyde	**5.0 g**
75.0 ml	**Amyl alcohol**
25.0 ml	**Conc. HCl**

The aldehyde was dissolved in the alcohol by gentle warming in water bath (50 – 55^{o}C), cooled and then the acid was added then stored in brown bottle at 4^{o}C.

The test organism was inoculated in the medium and incubated at 37^{o}C for two days. 0.5ml of Kovac's reagent was added, a red colour will develop after one minute in the reagent layer, indicates the presence of indole in the medium.

3.2.1.6.9. Urea agar base:

The medium was prepared as described in Oxoid (1973) as follows**:**

1.000 g	**Peptone**
1.000 g	**Dextrose**
5.000 g	**Sodium chloride**
1.200 g	**Disodium phosphate**
0.800 g	**Potassium dihydrogen phosphate**
0.012 g	**Phenol red**
15.000 g	**Agar**

All these constituents were dissolved in 95ml distilled water by heating in steamer adjusted to pH 6.8, autoclaved at 115^{o}C for 20 minutes, cooled to 45 – 50^{o}C. Aseptically 5ml of sterile 40% urea

solution was added, mixed well and then dispensed into 5ml capacity sterile bottles, slanted and stored at 4^{o}C.

The test organism was inoculated on the surface of urea agar slope, incubated at 37^{o}C and examined daily for seven days. Positive test was indicated by the presence of purple-red colour in the medium.

3.2.1.6.10. Voges–Proskauer test medium (V.P. test) and the methyl red test:

The Voges–Proskauer medium and the methyl red were prepared as described by Cowan and Steel (1977) as follows**:**

10 g	**Peptone**
5 g	**Dipotassium phosphate**
5 g	**Glucose**

All these compounds were dissolved in 1000ml distilled water by heating in steamer filtered and pH was adjusted to 7.6. Then the medium was distributed into test tubes, autoclaved at 115^{o}C for 10 minutes and stored at 4^{o}C.

The test organism was inoculated into the medium and incubated at 37^{o}C for two days. For V. P. test, 0.2ml of 40 % KOH and 0.6ml of 5% alcoholic solution of ∝-naphthol were added to 1ml of the test medium with culture, and the tube was put in slope position with the cap loose for up to 15 minutes. Positive reaction was indicated by the development of red colour in the medium.

For methyl red test, to 5ml of culture five drops of methyl red (0.1g methyl red dissolved in 300ml of 95% alcohol diluted to 500ml with distilled water) were added. Positive test indicated by the development of red colour.

3.2.1.6.11. Oxidase test:

The oxidase test reagent was prepared as 1% tetramethyl–P–Phenylene diamine dihydrochloride aqueous solution, and stored at $4^{o}C$ in a stoppered glass bottles away from light. Strips of Whatman No. (1) filter paper were soaked in this solution for 30 seconds then oven dried.

The test organism was cultured onto blood agar medium. Then a piece of filter paper prepared above was placed in sterile slide, and with sterile bent glass rod the test organism was added and rubbed over the filter paper. Dark purple colour will be developed after 5 to 10 seconds for positive result.

3.2.1.6.12. Catalase test:

A drop of 3% aqueous solution of hydrogen peroxide (H_2O_2) was placed on a clean glass slide. A small portion of bacterial culture under test was then placed in the hydrogen peroxide drop using a glass rod. Production of gas bubbles indicated positive results for the catalase test.

3.2.1.6.13. Novobiocin sensitivity test:

Standard disc diffusion method was used to carry out the sensitivity of the test organism to the antibiotic 5mg of novobiocin sensitivity disc. The diluted suspension of the organism was spreaded over the surface of the nutrient agar medium. Then the antibiotic disc was gently applied on the plate using sterile forceps and incubated at $37^{o}C$ for 24 hour. The zone of inhibition was recorded.

3.2.1.7. Preparation of *Klebsiella* sp. metabolites (klibcine):

Twenty-four hours metabolites of *Klebsiella* sp; toxins (Klebcine), were prepared as described by Sterne and Batty (1975). The

Klebsiella sp. was cultured in nutrient broth enriched with 0.5% glucose. Then centrifuged at 3000g for 20 minutes, the supernatant was aseptically removed and placed in sterile bottle, concentrated to a certain volume (75%) by using dialysis (against polyethylene powder), filtered through membrane filter 0.45µ (Oxoid) and the filtrate was collected in sterile bottle and stored at 4^{o}C.

3.2.1.7.1. Methods used to detect the inhibitory effect of *Klebsiella* sp. metabolites over *Escherichia coli*, *Staphylococcus* sp.: *caseolyticus* and *Pseudomonas*

3.2.1.7.1.1. Immersion of the bacterial species in the metabolites of *Klebsiella* sp.:

Eschericha coli, Staphylococcus caseolyticus, Klebsiella sp. and *Pseudomonas* sp. each cultured in nutrient broth and incubated for 24 hour. Three fold dilutions for each were done in normal saline solution. From the last dilution 1ml was taken and added to 9ml of the *Klebsiella* sp. metabolite. This was left at room temperature for two hours, then incubated in the refrigerator for 24 hour. For control, 9ml of normal saline were added instead of metabolite. After that seven serial dilutions were done for the metabolite and the control. Also, seven Petri dishes for each MacConkey's agar and blood agar were divided into two halves, on one half one drop (0.025ml) from the metabolite plus the test organism was spreaded. On the other half one drop (0.025ml) from the control plus the test organism was spreaded in the same way. Then they were incubated at 37^{o}C for an overnight (Plates 6, 7, 8, and 9).

3.2.1.7.1.2. Scraping method:

Fresh subculture of *Klebsiella* sp. was made in blood agar plate and incubated for 24 hour at 37^{o}C. Colonies were removed by scraping and

few drops of chloroform were added and left to dry. Then *Escherichia coli, Staphylococcus caseolyticus* and *Pseudomonas* sp. and *Klebsiella* sp. were cultured by spreading each on a quarter of a plate which has been already divided into four quarters, then incubated at 37°C for an overnight (Plate 10:B).

3.2.2. Analytical methods:

3.2.2.1. Proximate analysis for gum arabic (*Acacia senegal*):

3.2.2.1.1. Moisture content:

One gram of air dry sample was heated in an oven (Heraeus oven) set at 105°C for 5 hours to a constant weight. The procedure was carried out in duplicate for each sample and the average was taken to calculate the percentage of moisture (AOAC, 1984).

$$M\% = \left(\frac{M_2 - M_3}{M_2 - M_1} \right) x100$$

Where:

M_1 : weight of the empty crucible.

M_2 : weight of the crucible + sample.

M_3 : weight of the crucible + sample after drying.

3.2.2.1.2. Total ash content:

Total ash content was prepared according to FAO (1990). The crucible was heated at 55°C, cooled in desiccator and weighed (W_1). One gram of gum sample was weighed accurately in a crucible (W_2) and ignited at 550°C in a Heraeus electronic muffle furnace until free from carbon for 5 hours. Cooled in a desiccator and weighed (W_3). Then the total ash percentage was calculated as follows:

$$Ash\% = \left(\frac{W_3 - W_1}{W_2 - W_1} \right) x100$$

Where:

W_1 : weight of the empty crucible.

W_2 : weight of crucible + sample.

W_3 : weight of crucible + ash.

3.2.2.1.3. Nitrogen and crude protein content:

Nitrogen content was determined by a semi-micro Kjeldahl method (AOAC, 1984). Accurately, weighed 0.2g of gum sample was transferred to the digestion flask of a semi-micro kjeldahl apparatus. One gram of powdered mixture of potassium sulfate and cupric sulfate (10 : 1) was added, 3.5ml of concentrated nitrogen free sulfuric acid was added. The flask and contents were then heated over an electric heater until the solution attained a clear blue colour and the walls of the flask were free from carbonized materials. The contents of the flask were then transferred to a steam distillation unit, and 20ml of 40% sodium hydroxide solution were added, and distillation was carried out with steam. The distillation was collected in 10ml of boric acid solution (2%) to which has been added three drops of methyl red/methyl blue indicator, and titrated against 0.01N-HCl. The same procedure was carried out for the blank.

$$\mathbf{N\% = \left(\frac{(M_1 - M_2) \times N \times 14}{S \times 1000} \right) X\ 100}$$

Where:

M_1 : ml of HCl that neutralized the sample distillate.

M_2 : ml of HCl that neutralized the blank distillate.

N : normality of HCl titrant (0.01).

14 : each ml of HCl is equivalent to 14mg nitrogen.

S : sample weight (0.2g).

1000 : conversion factor from gram to mg.

The nitrogen contents were multiplied by the factor 6.25 to obtain percentage of crude protein.

Crude protein % = N% x 6.25

3.2.2.1.4. Specific optical rotation:

The specific rotation was determined for 1% solution of the commercial gum arabic sample and for the biodegradation production of M-70-inorganic media containing 2% gum arabic using an Optical Activity Bellingham and Stanley Ltd. polarimeter fitted with a sodium lamp and with a cell path length of 20cm. The solutions were passed through a No. (42) filter paper before carrying out the measurements at room temperature. Readings were taken twice and averaged. The specific rotation for gum solution was calculated according to:

$$\text{Specific rotation} = \frac{\propto \times 100}{C \times L}$$

Where:

$\propto$: observed optical rotation

C : concentration of gum solution

L : length of the polarimeter tube in dm.

3.2.2.1.5. pH value:

The pH was determined for the 1% aqueous solutions of commercial gum arabic samples, and for the biodegradation product of M-70-inorganic media containing 2% gum arabic, using a Beckman Zeromatic:1V pH meter at room temperature..

3.2.2.1.6. Viscosity measurements

The viscosity (flow time) was determined by using capillary viscometer (Shott Great Type 50/20/II) with the flow time for 1% aqueous solutions of commercial gum arabic samples and for the biodegradation product of M-70-inorganic medium containing 2% gum arabic at room temperature.

$$\text{Relative viscosity} = \frac{T - T_0}{T_0}$$

Where:

T : The flow time of solution.

T_0 : The flow time of distilled water.

3.2.2.2. Quantitative analysis of sugars:

This was carried out according to the method of Somogi (1937). In which the sugar solution is heated in excess cupric ions in the form of cupric tartarate complex. Some of the cupric ions proportional to the sugar in the solution would be reduced to cuprous oxide. A certain amount of iodine is then liberated in the medium, some of this free iodine oxidizes the cuprous oxide. The excess of iodine not consumed in the reaction is then titrated with standard sodium thiosulphate. A blank with no sugar is run simultaneously to determine the total amount of iodine in the medium. The iodine equivalent to cuprous oxide formed could be calculated as a result of the reducing action of the sugar. The process could be summarized as follows:

N Sugar + Y Copper tartarate complex cupric → N cuprous oxide

N cuprous oxide + iodine → cupric oxide + (X - N) iodine

X iodine (blank) - (X - N) → N

a) Reagents:

1) Solution (A) was made by dissolving 50g anhydrous $NaCO_3$, 25g Rochelle salt, 75g $CuSO_4.5H_2O$, 160g anhydrous sodium sulphate and 1.5g KI in the given order in 750ml boiling water. On cooling KIO_3 was added and the value made up to one litre. This was stored at 37^oC.

2) Solution (B) was a mixture of equal volumes of freshly prepared 5% KI and 5% neutral potassium oxalate.

3) $2NH_2SO_4$

4) $0.01MNa_2S_2O_3.5H_2O$

5) 1% starch in saturated NaCl solution.

b) Procedure:

In a Pyrex boiling tube 5ml of the copper reagent and 5ml of the sugar solution were taken and heated in a boiling water bath for 15 minutes. The tubes were then rapidly cooled at room temperature. To each tube, 2ml of solution (B) followed by 3ml of $2N\text{-}H_2SO_4$ were added. The solutions were allowed to stand for three minutes before titrating with 0.01M thiosulphate using starch as indicator. The titration value of the blank was recorded in ml 0.01M thiosulphate (1ml 0.01M thiosulphate equal 0.82mg reducing sugar).

For detecting the non-reducing sugars, the gum solution was hydrolyzed by the acid. 5ml of gum solution were pipetted into a test tube. Few drops of concentrated HCl were added. The mixture was boiled

for five minutes. After cooling, two drops of N-NaOH were added to neutralize the medium.

3.2.2.3. Detection of volatile fatty acids:

Volatile fatty acids in samples were determined by two ways**:**

3.2.2.3.1. As total acid by titration (titerable acidity):

Ten millimeters of the sample were placed in a flask, then phenolphthaline indicator was added. This mixed well and titrated with 0.1N-NaOH till pink colour appears. The result was expressed as percentage of lactic acid. The acidity was calculated from the following equation according to AOAC (1990)**:**

Titerable acidity % = No. of ml of 0.1 N-N0H X 0.09

3.2.2.3.2. As volatile acid by titration:

Ten millimeter of sample were placed in semi-micro kjeldal distillation unit, then 2ml of syrupy phosphoric acid were added, distilled for 15 minutes, titrate the distillate with 0.1N-NaOH solution and the total volatile fatty acids (VFA) of sample were calculated as percentage acetic acid**:**

No. of ml of 0.1N-NaOH x 0.06 VFA% =

(Since 1ml of 0.1 N-acetic acid contain 6mg of the acid).

3.2.2.4. Determination of polyamines:

An increased interest in the biochemistry of polyamines has created a demand for sensitive and specific methods for their analysis in biological samples. The studies on polyamines have been hindered by inadequated and sophisticated analytical tools, which might not be available in all laboratories for routine analysis (Tabor *et al*., 1961; Kneib

et al., 1979). Most methods used were**:** thin–layer chromatography (Seiler, 1971) gas–chromatography (Smith, 1970_b); ion–exchange chromatography with fluoroscamine (Veening, *et al*., 1974; Alder *et al*., 1977; Kneib *et al*., 1979) high speed liquid chromatography (Samejima *et al*., 1976); electrophoresis radioimmunoassay, gas chromatography–mass spectrometry and high pressure cation-exchange chromatography (Bartose *et al.,*1977). Most of these methods were time consuming, inadequate to the accurate estimation of small quantity, with limited sensitivity, consumed large quantity of expensive reagent and no individual procedure can be suitable as a general method unless combined with other techniques.

In the present study, polyamine was detected by thin layer chromatography technique as follows**:**

a) The samples:

1) Samples of biodegradation products of gum arabic in M-70-inorganic media containing 2% gum arabic and 2% urea at the different incubation periods in the *in vitro* experiments.

2) Serum samples of healthy and chronic renal failure patients before and after consumption of gum arabic *in vivo* experiments.

d) Extraction of polyamines:

Extraction of polyamines was carried out with trichloroacetic acid (Noack *et al*., 1998). Ten millimeters of sample was extracted in 20ml of 5% trichloroacetic acid for half an hours at 5^0C. The mixture was centrifuged at for 30 minutes. Then the supernatant was taken and adjusted to pH 7.0 with 0.1N-NaOH and the extract was used for chromatography.

c) Separation and identification of polyamines:

Thin layer chromatography (TLC) was carried out using silica gel G. The solvent system used was n-butanol : acetic acid : water (4:1:1v/v) for 4 hours at room temperature (Harborne, 1973). The plate was removed and dried before treatment with ninhydrin reagent (0.2g/100 ethanol), then dried in an oven at 110^{o}C until colour development was reached (Stahl, 1969). The Rf values were recorded for each type of polyamine in each sample.

3.2.2.5. Detection of ammonia:

Total ammonia concentration; due to the hydrolysis of urea by bacterial unease in liquid media, was detected according to (Mobley *et al*., 1988). Two millimeters from the liquid medium was taken after filtration through millipore size filter. This was mixed well with 1ml of phenol red indicator (0.05mg/ml). Then this mixture was read spectrophotometry at 560nm directly.

Standard curve; (Fig. 5), for ammonia concentration was prepared by a series of dilutions between 0.450 to 7.20mmol/ml from a stock solution of 270mmol/ml. Results were expressed as mmol/ml.

3.2.2.6. Analysis of faecal and blood samples in subjects after ingestion of gum arabic:

Twenty volunteers; ten healthy and ten with chronic renal failure, were participated in this study. The subjects were randomly chosen and questioned; (Appendix 6). The healthy subjects had taken their usual diet with the addition of 25g/day gum arabic. Whereas, the renal failure ones were under conservative management i.e.; taking a diet with low protein as shown in the program in Appendix (7) with the addition of 25g/day gum arabic. The 25g gum arabic were supplemented in water or juice

once daily for 28 days. The study period was divided into three intervals for collection of samples; one as a base line and two after 14 and 28 days.

Faecal samples on each period for each subject were collected in plastic containers, rapidly frozen and stored at -20 ^{0}C until analyzed

for**:** pH, total acid, volatile fatty acids and nitrogen content by methods previously described in this chapter.

Blood samples (10ml) were drawn on each period from each subject. Plasma was separated by centrifugation, then divided into two portions, 5ml each. The first portion was subjected for the detection of changes in Hb%, urea, creatinine, P^{+5}, Ca^{+2}, Na^{+1}, K^{+1} content. The analysis of these parameters was carried out at the laboratory of Khartoum Dialysis and Kidney Transplantation Centre - University of Khartoum. The second portion was frozen at $-20^{0}C$ until used for the detection of polyamines.

Na^{+1} and K^{+1} were analyzed by flame-photometer (Croning 410). Whereas, P^{+5}, Ca^{+2}, creatine and urea were analyzed by Autoanalyzer (Technicon RA–XT) after preparation of serum samples as follows for each**:-**

Inorganic phosphorus (P^{+5}) in the sample reacts with molybdate in - acid medium forming a phosphomolybdate complex which measured by Autoanalyzer (Gamst and Try, 1980). The samples were prepared as follows**:**

In four test tubes the following were pipetted**:-**

	Reagentblank	Sample blank	Sample	Standard
Distilled water	10µl	-	-	-
Sample (serum)	-	10µl	10µl	-
Phosphorus standard (phosphorus 5mg/dl)	-	-	-	10µl

working regent: 7ml regent (A); sulphuric acid 0.36mol/L and sodium chloride 154mmol/L, plus 3mL reagent (B); sulphuric acid 0.36mmol/L, sodium chloride 154mmol/L and ammonium molybdate 3.5mmol/L	1.0ml	-	1.0ml	1.0ml
Reagent (A)	-	1.0ml	-	-

Then mixed thoroughly and let the tube to stand for 5 minutes at read at room temperature. The absorbance (A) of the Sample Blanks was 340nm against distilled water whereas, the absorbance (A) of the Samples 340nm against the Reagent Blank. Then read at and of the Standard was the phosphorus concentration in mg/dl in the sample was calculated using the following formula:

$$\frac{\text{A Sample Blank} \times \text{Conc.Standard} \times \text{Sample dilution factor}}{\text{A Standard}} = \text{Conc. of Sample}$$

Calcium (Ca^{+2}) in serum reacts with O-Cresolphtaleine at alkaline - pH. The coloured complex formed is proportional to the amount of calcium present in the serum (Stern *et al*., 1957). The samples were prepared as follows:

In three test tubes the following were pipetted:

	Blank	Unknown	Standard
Sample (serum)	-	20µl	-
Standard	-	-	20µl
R1 (Ethanolamine 500mmol/L)	1.0ml	1.0ml	1.0ml
0.62mmol/L (O-cresolphtalein R2 and 8-hydroxyquinoline 69mmol/l)	1.0ml	1.0ml	1.0ml

minutes at Then each was mixed well and allows to stand for 5 room temperature. The absorbance of the Standard and Unknown was read against the Blank Reagent at 570nm and the calcium concentration in the sample was calculated using the following formula**:**

$$\frac{\text{Abs.Unknown}}{\text{Abs. Standard}} \times \text{Conc. Standard} = \textbf{Serum calcium in mg/dl}$$

- Creatinine in the sample reacts with picrate in alkaline medium forming a coloured complex (Bartels and Bohmer, 1971; Fabiny and Ertingshausen, 1971). The samples were prepared as follows**:**

In three cuvettes the following solutions were pipetted**:**

Standard (2mg/dl) or Sample (serum)	0.Iml
Working reagent**:** (Equal volume of reagent A (Picric acid 25mmol/L) and reagent B (Sodium hydroxide 0.2mmol /L)	1.0ml

Then the cuvettes were mixed well and inserted into the after photometer. Stopwatch was used to record the absorbance at 500nm 30 seconds (A_1) and after 90 seconds (A_2). The creatinine concentration using the following formula**:** in mg/dl in the sample was calculated

$$\frac{(A_2 - A_1)\ \text{Sample} \times \text{Conc. Of Standard} \times \text{Sample dilution factor}}{(A_2 - A_1)\ \text{Standard}} = \text{Conc. of creatinine in sample}$$

- Urea in the sample originate by means of a coloured complex which measured by Autoanalyzer (Chaney and Marbach, 1962; Searcy *et al*., 1967; Tabacco *et al*., 1979). The samples were prepared as follows**:**

In three test tubes the following solutions were prepared**:**

	Blank	Standard	Sample
Standard S urea (50mg/dl)	-	10µl	-
Sample	-	-	10µl
Reagent A (Sodium Salicylate nitroprusside 62mmol/L Sodium 3.4mmol/L, Phosphate buffer 20mmol/L pH 6.9)	1.0 ml	1.0 ml	1.0 ml
Wait ten minutes plus mixing			
Reagent B (Sodium hydrochloride 7mmol/L, Sodium hydroxide 150mmol/L)	1.0 ml	1.0 ml	1.0 ml

Then the absorbance A was read for the Standard S and Sample at 600nm against the blank, and the following equation was used to calculate urea concentration in mg/dl:

$$\frac{\text{A Sample}}{\text{A Standard}} \times \text{Conc. Standard} \times \text{Sample dilution factor} = \text{Serum urea Conc.}$$

Results

and commercial of analysis 4.1. Proximate gum samples: *senegal* authentic *Acacia*

Appendix (2) shows the analytical results for 25 commercial *Acacia senegal* gum samples collected randomly from local markets at Khartoum state in 2000. Whereas, Appendix (3) shows the analytical results for another 25 authentic *Acacia senegal* gum obtained from Siddig (1996). The comparative analytical results between the means values of commercial and authentic gum samples were shown in Table (8) and Fig. (6).

4.1.1. Moisture content:

From Table (8) and Fig. (6), the mean value of the moisture percentage of 25 commercial samples of gum arabic was 12.62 with the minimum value of 10.80 and maximum value of 14.15. Whereas, the mean value of the 25 authentic samples was 10.40 with minimum value 8.10 and maximum value 13.11. In general, the moisture percentage of the commercial sample was more than that of the authentic samples using the t-test, the increase was highly significant.

4.1.2. Ash percentage:

As shown in Table (8) and Fig. (6), the mean value of ash percentage for the 25 commercial gum arabic samples was 3.49 with the range of 2.10 and 4.53. Whereas, the authentic samples have shown a mean value of 3.85 with the range of 3.20 and 5.02. Using the

t-test, the ash percentage was significantly lower in value for the commercial samples when compared to the authentic ones.

4.1.3. The pH value:

From Table (8) and Fig. (6), the mean value of the pH for the commercial samples was 4.82 with range of 4.60 and 5.10 and that one for the authentic samples was 4.74 with the range of 4.42 and 5.56. Using the t-test the commercial samples were significantly lower in value when compared to the authentic ones.

4.1.4. The specific rotation:

From Table (8) and Fig. (6), the mean value of the specific rotation for the commercial samples was –24.45 with minimum value –10.00 and maximum value –31.00, whereas that for the authentic samples was –32.90 with minimum value –24.84 and maximum value –44.14. Using the

t-test the commercial samples were significantly much more in value than the authentic ones.

4.1.5. The nitrogen content:

As shown in Table (8) and Fig. (6), the commercial samples of gum arabic have a mean of 0.29 for nitrogen percentage with a range of 0.22 and 0.36, and it was 0.31 with the range of 0.19 and 0.46 for the authentic samples. Using the t-test, commercial samples were insignificantly lower than the authentic ones.

4.1.6. The protein content:

From Table (8) and Fig. (6), the mean value of protein percentage was 1.91 with the range of 1.45 to 2.38 for the commercial gum arabic samples, and it was 2.02 with a range of 1.25 to 3.04 for the authentic ones. Using the t-test, the protien content of commercial samples were insignificantly lower than that of the authentic samples.

4.2. The microflora of *Acacia senegal* gum:

4.2.1. The bacterial load of gum arabic:

Table (9) shows the bacterial load in the outer surface and cortex of the 25 gum arabic nodules (20 commercial samples and other 5 authentic ones) when the scratching technique was used. The bacterial isolates were more in the commercial samples than the authentic ones, when culturing on blood agar. Also, the outer surface of the nodules contains more bacterial isolates than their cortex. The latter was completely sterile. Pathogenic bacteria mainly *Enterobacteriaceae* were not detected when the samples were cultured on MacConkey's medium.

Also, Table (10) shows the bacterial load in the processed nodules of commercial and authentic gum arabic samples when the serial

dilution technique was used. The results revealed that the bacterial load ranged from zero to $3.6x10^3$CFU/g for commercial processed nodules, and zero to $0.2x10^3$CFU/g for authentic processed ones. For the other processed forms, the ready crushed form samples have a less range from zero to $1.0x10^3$CFU/g when compared with the commercial processed ones. It was highly reduced in the fine powder form, which had a range from zero to $0.2x10^3$CFU/g. The statistical analysis showed that, the commercial processed nodules, were sinificantly different when compared with the ready crushed nodules and the fine powder

forms. Pathogenic bacteria, especially *Enterobacteriaceae* were not detected when all these processed forms were cultured on MacConkey's medium.

4.2.2. Types and percentage of isolates in gum arabic:

The major isolates were of the Gram-positive bacilli, whereas Gram-positive cocci represented the minor isolates. The Gram-positive bacilli isolates were identified according to Sneath (1986) and Barrow and Feltham (1993), Appendix (4) shows their characterization. Gram-positive cocci were identified according to El-Sanosi and Saeed (1996) who presented a scheme for identification of *Staphylococcus* spp. (Appendix 5).

Table (11) shows the type and percentage of all bacterial isolates in all forms of gum arabic samples used in this study. *Bacillus mycoides* and *Bacillus licheniformis* represent the highest percentage of isolates in all forms.

4.3. The domination of *Klebsiella* sp. over other *Enterobacteriacea* during ingestion of gum arabic:

4.3.1. The inhibitory effect of *Klebsiella* sp. bacteriocine over *Escherichia coli*, *Staphylococcus* sp. and *Pseudomonas* sp.:

Plates (6, 7, 8, and 9) show the inhibitory effect of bacteriocine released by *Klebsiella* sp. on *Escherichia coli, Pseudomonas* sp. and *Staphylococcus caseolyticus* and *Klebsiella* sp. respectively; when the immertion technique was used. Each plate shows the viable count of the

tested organism at dilution two. The left half of each Petri dish represents the control whereas, the right half shows the effect of

Klebsiella sp. bacteriocine on each tested organism, except *Staphylococcus caseolyticus* the pattern was reversed.

Plates (10:A and 10:B) show the inhibitory effect of *Klebsiella* sp. bacteriocine on the test organisms when the scraping technique was used. The left Petri dish represents the control while the right one represents the growth of the tested organisms on the medium containing *Klebsiella* sp. bacteriocine.

4.3.2 Biodegradation of gum arabic by *Klebsiella* sp. and *Escherichia coli in vitro*:

During the preparation of the medium M-70-inorganic mixture with the 2% gum arabic, the physico-chemical properties of gum arabic was changed in comparison with non-autoclaving one due to sterilization at 110^{o}C for 10 minutes. In addition, for this media the processed gum arabic in a fine powder form was used, and when this was compared with raw form its physico-chemical properties was also changed due to processing (Table 12). The main changes measured were the viscosity, specific rotation, reducing and non-reducing sugars. Autoclaving and processing increases specific rotation, reducing and non-reducing sugars, and decreases viscosity. Therefore, due to these changes which were either caused by processing or autoclaving, the author cannot detect the exact biological changes due to biodegradation by *Klebsiella* sp. or *Escherichia coli in vitro*. Hence, from this experiment the only change in pH, specific rotation and formation of volatile fatty acids may reflect the biological activity of these two organisms under these conditions (Tables 13 and 14). Both organisms grew and it seems that *Escherichia coli* is a very weak acid fermenter, since a very small change in pH was observed in

comparison to *Klebsiella* sp. Viscocity did not change whereas, specific rotation once decreased and once increased and no formation of volatile fatty acids.

4.4. An *in vitro* formation of polyamines

An *in vitro* incubation system was used to study the metabolism of urea and gum arabic in a basal medium M-70-inorganic mixture due to metabolic activity of *Escherichia coli, Klebsiella* sp. and a mixture of both. Table (15) shows these metabolic changes. For *Escherichia coli* the pH was not clearly changed and a very little amount of ammonia was released in the medium, which was observed through the incubation periods. Also, their growth continually increased up to day-ten with maximum growth at day-three. For *Klebsiella* sp., the pH of the medium was changed from 7.23 to 9.10 at day-ten with concomitant release of ammonia. Their growth was continually increased; maximum growth was obtained at day-five then decreased at day-nine and increased at day-ten. The same pattern was observed for the mixture, but the growth of *Escherichia coli* has started to diminish after day-five (Plate 11), then completely disappeared at day–eight, nine and ten. Whereas, *Klebsiella* sp. reached maximum growth at day-three then gradually decreased up to day-nine and increased at day-ten. In addition, although the main aim of this experiment was to detect the formation of polyamines, they were not found in all types of cultures through the ten–days as shown in Plates (12), (13) and (14) for *Escherichia coli*, *Klebsiella* sp. and their mixture respectively.

4.5. The effect of feeding gum arabic on human blood and faecal composition:

The characterization of healthy and chronic renal failure subjects are shown in Tables (16 and 17) respectively. Most subjects were males; 60% for healthy subjects and 88.8% for chronic renal failure ones. Their ages range between 21 - 45 years for healthy subjects and 25 - 61 for chronic renal failure ones. Healthy subjects were not suffering from any type of disease. Whereas, most of the chronic renal failure patients have hypertension (70%). One subject from the patients was excluded from the final analysis due to severe anemia. Tables (18 and 19) and Appendices (8 and 9) show the main biochemical changes in blood and faeces composition during the study periods; pre-gum and post-gum arabic ingestion, for both healthy and chronic renal failure subjects respectively. No troubles were observed during ingestion of gum arabic in both subjects, except constipation, diarrhea and flatulence either during the first or last period of study in some subjects. For blood and faecal biochemical composition in both subjects the following changes were observed with reference to the base line after ingestion of gum arabic for 14 and 28-days**:-**

The haemoglobin (HB%) level was low in chronic renal failure subjects than in healthy ones (Fig. 7). A reduction in HB% was observed in 70% (Fig. 8) and in 33.3% (Fig. 9) of healthy and chronic renal failure subjects respectively.

Potassium (K^{+1}) level was low in healthy subjects than in chronic renal failure ones (Fig. 10). An increase in K^{+1} content was observed in 30% of healthy subjects and a reduction in 20% (Fig. 11),

whereas chronic renal failure ones showed an increase in 55.5% and a decrease in 33.3% (Fig. 12).

Sodium (Na^{+1}) level was low in chronic renal failure subjects than in healthy ones (Fig. 13). It was increased in 30% and decreased in 40% of healthy subjects (Fig. 14). For chronic renal failure subjects it was increased in 66.6% and decreased in 33.3% (Fig. 15).

Calcium (Ca^{+2}) level was low in chronic renal failure subjects than in healthy ones (Fig. 16). Ca^{+2} content was increased in 70% of healthy subjects and decreased in 20% (Fig. 17). Whereas, chronic renal failure ones showed an increase in 44.4% and a decrease in 33.3% (Fig. 18).

Phosphorus (P^{+5}) level was low in healthy subjects than in chronic renal failure ones (Fig. 19). P^{+5} content was increased in 100% of healthy subjects (Fig. 20) and 66.6% in chronic renal failure ones (Fig. 21).

Creatinine level was high in chronic renal failure subjects than in healthy ones (Fig. 22). It was decreased in 10% and increased in 60% for healthy subjects (Fig. 23). Whereas, 44.4% reduction and 33.3% increase was observed in chronic renal failure patients (Fig. 24).

Blood urea nitrogen (BUN) was low in healthy subjects than in chronic renal failure ones (Fig.25). BUN was decreased in 20% and increased in 40% for healthy subjects (Fig. 26). Chronic renal failures ones showed a decrease in 33.3 % and an increase in 22.2% (Fig. 27).

Faecal total acids (TA) were low in chronic renal failure subjects than in healthy ones (Fig. 28). TA increased in 66.6% and decreased in

22.2% of healthy subjects (Fig. 29), and increased in 44.4% and decreased in 33.3% of chronic renal failure patients (Fig. 30).

Faecal volatile fatty acids (VFA) were low in chronic renal failure subjects than in healthy ones (Fig. 31). VFA increased in 44.4% and decreased in 11.1% of healthy subjects (Fig. 32), and a 22.2% increase and 44.4% decrease were observed for chronic renal failure patients (Fig. 33).

Faecal nitrogen level was low in chronic renal failure subjects than in healthy ones (Fig. 34). Faecal nitrogen was increased in 33.3% and decreased in 11.1% of healthy subjects (Fig. 35), and increased in 55.5 and decreased in 11.1% for chronic renal failure ones (Fig. 36).

In general, it seems that all the mean values for the biochemical measurements in the pre-gum and post-gum arabic examination were within the limits of variation for a normal population, and insignificantly changed between healthy and chronic renal failure subjects under study (Table 18).

Free diamine (putrescince) and polyamines (spermine and spermidine) were not detected in the sera of both subjects during the study period (Plates 15 and 16) as examples.

Discussion

5.1. Proximate analysis of the commercial and authentic *Acacia senegal* gum samples:

Since gum arabic is extensively used in many industrial application and due to increase in competition from other producing countries, it is essential that certain physical and chemical data test must have been devised for identification and conformation of commercial gums to trace any adulteration processes. Specification for identity and purity of gum arabic was based on the American Food Chemical Codex, published in 1969 and the Joint Expert Committee of Food Additives (JECFA) of the FAO and WHO published in 1978. It has been reviewed every four years; JECFA 1982, 1986, 1990, 1995 (Karamalla *et al.,* 1998). Therefore, in the present study the commercial gum arabic samples; obtained from local markets of Khartoum State, were analyzed in order to set and to compare their specification with the authentic ones as will be discussed below**:-**

Moisture content of the gum indicates that the hardness of the gum, and good quality gum should have a value between 13 - 15% moisture (FAO, 1986) and not more than 15% (JECFA, 1999). In the present study the mean value of the commercial gum arabic samples was

12.62%. Therefore, it falls within the range mention above by FAO (1986) and the JECFA (1999).

However, variations in the moisture content of the commercial gum arabic samples was observed by many investigator's studies. The quality control records of the Khartoum Gum Arabic Processed Company (GAPC) and the Gum Arabic Company Ltd. show that the mean value of the moisture content was found to be 10.87% for 40 sample, 14.16% for 100 samples and 13.22% for 140 samples season 1992 – 1996**;** Awad El-Karim (1994) reported a range between 9.23 – 12.70% of moisture content for 5 samples and Osman (1998) reported a mean value of 10.36%. The author suggest that, these variations in moisture content of the commercial gum arabic samples may be attributed to the variations in the size of gum nodules, storage conditions and seasons.

Siddig (1996) and Karamalla *et al*.(1998) reported that there was no significant differences in the mean values of moisture content when commercial and authentic gum arabic samples were compared**;** 10.87 and 10.99% respectively. Hence, our results in the present study were not substantiate with their findings since the difference between the commercial and authentic samples was highly significant.

The ash percentage indicates the presence of inorganic elements. Anderson and Dea (1968) and Siddig (1996) showed that the type of the soil affects the ash content significantly. Heavy soil (eastern Sudan) gives ash content of 3.5% while sand soil (western Sudan) gives 3.3%. Also, Awad El-Karim (1994) reported that the value of ash content increase as proportion of inert matter increase because purified gum has a lower ash content than crude gum, and she reported a wide range for ash content; 3.33 - 10.00%. Also, aging increase ash content (Ali, 1996). In the

present study, the mean value of the ash percentage was 3.49% which complies with the last requirements of JECFA specification (1999), which is less than 4%. However, other studies on commercial samples of gum arabic revealed different values as shown by Anderson *et al*. (1968) to be 3.62%; Siddig (1996) 3.62%; Ali (1996) 3,62% and Osman (1998) 3.37%.

For the commercial and authentic gum arabic samples Siddig *et al.* (1998) reported that, no significant difference (1996) and Karamalla was observed between them when their mean values of ash content were compared**;** 3.62 and 3.77% respectively. But a reverse pattern was observed in the present study, they were significantly different.

The hydrogen ion concentration plays an important role in the chemistry and industry of gum. It affects the solubility of gum and precipitation of protein and therefore changes the properties of the gum as a whole. In general, *Acacia senegal* gum is slightly acidic due to the presence of free carboxylic groups of the D-glucouronic acid and its 4–0–methyl derivatives (Karamalla *et al.,* 1998). In the present work, the mean value of the commercial gum arabic samples was 4.82. The JECFA (1999) had not proposed a pH value for gum arabic, but to some extend different pH values were reported by many workers for commercial gum arabic samples. It is 4.64 with an average between 4.38 - 4.89 (Siddig, 1996)**;** 4.64 with a range of 4.38 - 4.89 for 40 samples season 1993/1994 and 4.48 with a range 4.04 - 4.84 season 1994/1995 for 100 samples (Karamalla *et al*., 1998); 4.8 with a range of 4.7 - 4.9 (Awad El-Karim, 1994) and 4.7 with a range of 4.2 - 5.2 (Osman, 1998).

reported that the mean *et al.* (1998) Siddig (1996) and Karamalla pH values of the commercial and authentic gum arabic samples were

insignificantly different**;** 4.64 and 4.65 respectively. These finding were not substantiated with the results in the present work.

The optical activity of organic molecules is related to their chemical structure, therefore, it is considered as the most important criterion of purity of any type of gum. Karamalla (1965) mentioned that autohydrolysis and mild acid hydrolysis has a significant effect on specific rotation, resulting from release of monosaccharides and oligosaccharides. Ishag (1977) reported negative optical rotation for gum sample having a high rahmnose and low arabinose content (e.g. gum arabic), and a reverse pattern was observed for gum samples having a high arabinose and low rahmnose content; they have positive optical rotation (e.g. gum talha).

In the present investigation, the mean value of specific rotation for commercial gum arabic samples was -24.45 with a range of -10.00 to -31.00. About 50% of these samples lied within the range -26.1 to -31.00 and this to some extend comply the requirement of JECFA (1990) which is ranges between -26 and -34, although the last report by JECFA (1999) excluded the criteria of specific rotation for gum arabic. In general, different optical rotation values were reported for commercial gum arabic samples. Osman *et al*. (1993) reported a range -23 to -31, Awad El-Karim (1994) -23.30 to -28.30 and Osman (1996) -29.99 to -23.31. The author suggests that, this wide variation in values of specific rotation in the present work may indicate mixing of gum arabic with other related gums such as *Acacia seyal* gum; which has a positive specific rotation. Or may be due to variations in moisture content which affects weight of the samples and hence values of sugars content and specific rotation in gum arabic samples.

et al. (1998) revealed that, the mean Siddig (1996) and Karamalla values of specific rotation for commercial and authentic gum arabic samples showed insignificant difference; - 30.54 and -31.30 respectively. This observation is conversed to the results in the present study.

The presence of nitrogen content may affect viscosity (Anderson *et al*., 1968). In the present work, the commercial samples have a mean value 0.29% for nitrogen content and 1.91% for protein content. About 72% of these samples ranged between 1.85 to 2.38 contrasts with the requirement of JECFA (1990) for protein, which ranges between 1.26 to 2.44%; and these values were equal to the range 0.26 - 0.39% for nitrogen content. This is similar to the observation of**:** Siddig (1996), she reported that 60% of the commercial samples have a nitrogen content 0.30% (equal to 1.98% protein content) for season 1995/1996 and Karamalla *et al*. (1998), who observed that 80% of the 40 commercial samples have a nitrogen content fall within the range of 0.26 to 0.4% (equal to 1.63 to 2.50% for protein content). Whereas, Idris (1989) reported that the average protein of fresh samples was 2% which increased by storage. He attributed this to the enzymatic action of microorganism. But it was constant with different ages.

et al. (1998) observed that there is Siddig (1996) and Karamalla insignificant difference in the mean values of nitrogen and protein when commercial and authentic gum arabic samples are compared. These findings are similar to the present results.

:The microflora of gum arabic 5.2.

Gum arabic is an important plant product, used extensively in the food and medical industries. Since one of its formation could be due to (Gayed and Hassan, 1964; Bokhary *et al*., the action of microorganisms

1983), therefore, the study of its normal microflora is considered important to determine the limit of safety when consumed directly or indirectly by human.

In the present work the bacterial load was more in the outer surface of the gum arabic nodules then gradually decreased in the processing samples (crushed nodules, ready crushed form and fine powder form). Also, the outer surface of nodules contains a large number of bacterial isolates than the cortex, which is completely sterile. This may explain why with processing gum arabic bacterial load decreased in addition to the heat generated during processing. Also, the author observed that the commercial gum arabic nodules contain a very small number of isolates. This was attributed to the long exposure of these nodules to the sun light during selling in the markets. More reduction in bacterial isolates was also observed in the nodules of the authentic samples; which is attributed to the way by which these samples were collected i.e. hand picked and directly stored in paper envelope. Therefore, all these observations may confirm that gummosis is not a phenomena of bacterial infection.

The study of Osman (1998) on the bacterial load of gum arabic, showed a range of $7.1x10^4$ to $8.6x10^5$CFU/g and $5.9x10^3$ to $1.3x10^5$CFU/g for the authentic picked form for Algadarif and Alobied area respectively, and with transportation insignificant increased was observed; $3.1x10^5$ to $9.1x10^6$ and $2.9x10^4$ to $7.7x\ 10^5$CFU/g for Algadarif and Alobied area respectively. Whereas, the commercial samples revealed a range between $9.5x10^4$ to $2.9x\ 10^6$, and with processing the number decreased from undetected to $2.9x10^4$CFU/g. These observations to some extend were similar to the results obtained in the present work.

For the types and percentage of isolates in samples of gum arabic studied in the present work, showed that the major bacterial flora were *B. licheniformis, B. mycoides, B.* spp.; mainly *Bacilli* Gram-positive *stearothermophilus II, B. cereus, B. laterosporus, B. stearothermophilus III, B. firmus* and *B. thuringiensis*. *B. mycoides and B. licheniformis* scored the highest percentage when compared to the other *Bacillus* spp. isolated in this study. These results were coincident with those obtained by Bokhary *et al.* (1983). They isolated four *Bacillus* spp. from gum arabic, specifically *B. cereus, B. pulvifaciens, B. brevis* and *B. licheninformis* with relative frequency 19.23%, 11.54%, 15.38% and 19.23% respectively. Also, Idris (1989) reported the presence of Gram-positive spore-forming bacteria in gum arabic samples namely *B. licheniformis, B. mycoides, B. cereus, B. subtilis, B. amyloliquefaciens, B. marcerans, B. brevis, B. stearothermophilus* and *Pasteurella ureae. B. licheniformis and B. subtilis* were the most dominant species. Whereas, Mohammad (2000) reported that, Gram-positive spore-forming bacteria were more in the studied samples of gum arabic. They constituted 83% of the isolated bacteria.

The studies of Anderson and MacDonalds (1987) revealed that natural gum arabic contains the thermophilic spore-forming *Bacilli* sp. which is tolerant to heat treatment. Therefore, since both *B. stearothermphilus II and B. stearothermphilus III* were isolated in this study, thermal sterilization to some extend must be applied to eradicate such types of contaminant.

Bacillus is an aerobic Gram-positive spore-forming The genus bacilli, therefore, they are ubiquitous, and due to spore formation they can survive in the environment as a saprophytic organisms (in dry earth, soil, water, air and on vegetation) for many years utilizing simple sources of

nitrogen and carbon for growth and energy. Most of the *Bacillus* spp. do not cause diseases and are not well characterized in medical microbiology, but few such as *B. anthracis* which cause anthrax *and Bacillus cereus* which cause food poisoning and eye or other localized infection, were the only pathogens of this group to man. In this study the pathogenic bacilli *B. anthracis* was not isolated at all but *B. cereus* was isolated in a very low incident.

The minor bacterial isolates in the present study were represented *Staphylococcus caseolytcus* in a very low percentage. This similar to by Mohammad (2000) who reported a low incident of Gram-positive cocci (7% of the bacterial isolates) as well as to Idris (1989) and Osman (1998), they reported the presence of Gram-positive cocci; but not *Staphalycoccus aureus,* in a very small amount.

Staphylococcus is Gram-positive cocci non-spore The genus forming but resist drying and heat of 50ºC. It is isolated from animal products, soil, dust air and natural water, cause diseases such as abscess suppuration and septicemia due to the enzymes action such as staphykinase, proteinase, lipase, coagulase and pencilinase. Also, it causes food poisoning due to their toxins; enterotoxin and exotoxine. *Staphlococcus caseolyticus* is a facultative anaerobe and growth is much better under aerobic conditions. It has also been found in milk and diary products, but it has not been associated with human or animal infection. Therefore, the presences of *Staphylococcus caseolyticus* within the normal flora of gum arabic do not cause big problem.

The author also observed that, no coliform bacteria were detected spp*, Klebsiella* spp*., Salmonella* spp. *and Shigella Escherichia* namely spp. Absence of *Enterobacteriaceae* were also reported in all the studies

carried out on gum arabic samples by Idris (1989); Osman (1998) and Mohammad (2000). This was prooved by the JECFA (1999) specification which states that gum arabic is negative for the presence of *Salmonella* spp. and *Escherichia coli* per gram.

Enterobacteriaceae (coliform) are large heterogeneous group The of Gram-negative short rods whose natural habitat in the intestinal tract of human and animal. The family includes many genera such as *Escherichia, Shigella, Salmonella, Enterobacteria, Klebsiella, Serratia, Proteus* and others. They are facultative anaerobic fermenter ferment large group of carbohydrates and produce variety of toxins and other virulent factors.

other over.sp *Klebsiella* of domination 5.3. The *Enterobacteriacea* during ingestion of gum arabic:

Samia (2000) (personal communication) showed that The studies of the number of *Klebsiella* sp. was increased over that of *Escherichia coli* when gum arabic was ingested by healthy and chronic renal failure patients; i.e. the pattern of domination was changed from *Escherichia coli* before ingestion of gum arabic to *Klebsiella* sp. after consumption of gum arabic.

in vitro experiments were conducted to In the present study an know whether the changes are due to bacteriocine excretion by *Klebsiella* sp. or other factors. Bacteriocine are proteinous bactericidal substances that produced by certain strains of bacteria and exhibit a narrow range of effectiveness against other strains of the same closely related sp., and bacteriocine producing strains are resistant to their own bacteriocine (Malk *et al*., 1974). Also, bacteria may produce a variety of other

inhibitory substances including metabolic products for example acids, H_2O_2, ammonia, low molcular wieght enzymes and defective bacteriophage i.e. antagonistic substance which called bacteriocine-like (Tagg and Bannister, 1978).

The results in the present study (Plate 6, 7, 8 and 10:B) may confirm the inhibitory effect of *Klebsiella* sp. bacteriocine on the tested organisms, but the same result was obtained with *Klebsiella* sp. as shown in Plate (9 and 10:A). Hence, all these findings confirm the non-inhibitory effect of *Klebsiella* sp**.** bacteriocine on the tested organisms. Therefore, the reduction in the number of colonies was not due to the inhibitory effect of bacteriocine.

in vitro experiment results in the In addition to the above the present work, in which both *Klebsiella* sp. and *Escherichia coli* were inoculated in the M-70-inorganic medium containing 2% gum arabic, revealed that *Escherichia coli* is very weak acidic fermenter in comparison to *Klebsiella* sp. This may confirm the domination of *Klebsiella* sp. over *Escherichia coli* when gum arabic was ingested. i.e., acids may act as inhibitory substances (Tagg and Bannister, 1978) which in-turn reduced the growth of *Escherichia coli*. No formation of volatile fatty acids from both organisms through all the incubation periods of study was recorded. This may confirm that production of volatile fatty acids during the fermentation of gum arabic was mainly due to anaerobic bacteria such as *Bifidobacterium* or *Bacteroides* since they were the main dominant fermenters when gum arabic was fermented *in vivo* or *in vitro* (Ross *et al*., 1983; Wyatt *et al*., 1986).

These findings were similar to those of Titgemeyer *et al*. (1991), how studied the fermentabilty of various fibrous substrates by human

faecal bacteria *in vitro*. These substrates were gum arabic, gaur gum, citrus pectin, sugarbeet fibres, soy fibres, oat fibres and pea fibres. For all substrates very few short chain volatile fatty acids were produced at either three or six hours of the total short chain volatile fatty acids products. They also observed that insoluble fibres decreased whereas soluble fibers decreased and increased at the different incubation hours for each fibre substrate. The same result was observed by the author in the present experiment, where specific rotation increased and then decreased irregularly through the incubation period; indicating hydrolysis of insoluble polysaccharides and release of soluble monosaccharides which were consumed for the growth of the bacteria during the incubation period.

Escherichia coli and *Klebsiella* sp. Also, in the present work the ability of to degrade gum arabic *in vitro* was not measured since gum arabic has been already mild hydrolyzed by autoclaving and processing. This was coincident with finding of Buffo *et al.* (2001). They observed that pasteurization in a water bath at 62.5^{o}c for 30 minutes and addition of electrolytes are known to decrease viscosity of hydorcolloids due to compaction of the electrical double layer and reduction in the effective molecular volume of the gum. They attributed all these observations to that, a single cleavage of a glycosidic bond at the centre of the chain by hydrolysis, oxidation or mechanical energy, produces two polymer molecules of one-half the original molecular weight and much lower viscosity-producing potential. In addition, Karamalla (1965) mentioned that autohydrolysis and mild acid hydrolysis significantly affect specific rotation due to release of monosaccharides and oligosaccharide.

Hence, in a general conclusion, the domination of *Klebsiella* sp. was mainly due to release of inhibitory substance; acids, and not to

klibcine. These findings were coincided with the fact that, fibres may determine the bacterial flora of the colon (Eastwood and Passmore, 1983), or diet have a little effect on the species composition of the human colonic flora (Finegold, 1977), but inspite of this diet may still produce a considerable change in the metabolic activities of the flora, because diet will produce new type of substrate which will lead to change in the level of different catabolic enzymes (Salyers, 1979). Also, Wyatt *et al.* (1986) observed that anaerobic fermenter; *Bacteriodes* and *Bifidobacterium*, increase to more than 50% when gum arabic 10g/day was added to the diet for 18-days, then the number return to the initial level; 6.5% before ingestion. Therefore, this confirms that the response was due to the dietary change.

5.4. An *in vitro* formation of polyamines

Ammonia in the large gut is thought to be derived from either hydrolysis of urea by urease or deamination of protein and other nitrogen substrate (Vince *et al*., 1973). *Klebsiella* sp. possesses urease activity whereas *Escherichia coli* does not. The purpose of the present experiment was to determine whether production of ammonia coupled with fermentation of gum arabic lead to the formation of polyamines or not. The auther observed that, although ammonia was generated in both pure culture of *Klebsiella* sp. and mixed culture but no formation of polyamines. These findings disagree with the findings of Vince *et al.* (1976) and Smith and Bryant (1979) who reported that, the mixture of organisms may modify the behavior of one organism in the presence of another in complex ecosystem of the gut. Also, it is generally accepted that ammonia is often the preferred and main nitrogen source for growth of gastrointestinal tract bacteria (Bryant, 1974), but it seems that it is not

essential for the formation of polyamines as shown in the present results. Hence, precursors amino acids such as arginine, ornithine, lysine, histidine, tyrosine, glutamic acid, aspartic acid, tryptophane, leucine and valine must be present in the media so as to be decarboxylated and thereby form their corresponding polyamines (Gauges, 1980). In addition, he mentioned that decarboxylase was specific for a single amino acid and these enzymes were produced optimally *in vitro* at acidic pH values, which reflect the attempt of the bacteria to buffer their environment. Also, Tabor and Tabor (1985) and Noack *et al*. (1998) mentioned that enterobacteria such as *Escherichia coil* and *Klebsiella* sp. produced cadavarine and the factor that induced cadavarine synthesis has not yet been identified.

Therefore, this point need further investigation by that certain amino acids are introduced to the media under acidic condition so as to *in vitro*. detect the formation of polyamines

say that *Escherichia coli* Also, from this experiment one can prefers neutral condition for growth (pH 7.2) but not alkaline condition Therefore, (pH 8.59), whereas *Klebsiella* sp. survived alkaline condition. this may be to some extent explain the domination of *Klebsiella* sp. upon *Escherichia coli* when gum arabic was ingested by chronic renal failure subjects or healthy ones, especially in uremic patients which they have a colonic pH higher than normal and their faeces contain little or no urea unless antibiotics are given (Brown *et al*., 1971).

5.5. The effect of feeding gum arabic on human blood and faecal compositions:

In general, fibres have been reported to affect the absorption and excretion of minerals (Heaton and Pomare, 1974; Persson *et al*., 1975;

Southgate *et al*., 1976; Demigne and Remesy, 1985; Tuling *et al*., 1987) as well as decreasing serum nitrogen concentration and increasing faecal nitrogen excretion (Bliss *et al.*, 1996). Hence, in the present work healthy and chronic renal failure subjects were chosen to reflect the effect of feeding 25g/day gum arabic for 28-days, on the blood and faecal composition.

Electrolytes are ions that exist in body fluids. In the extracellular fluid the major cations are sodium, phosphorus and calcium (DeVita and Michelis, 1993) whereas, potassium is the major intracellular. The expected concentrations of sodium, potassium, calcium and phosphorus in healthy individuals varies between 136 to 145mmol/L, 3.8 to 5.5mmol/L, 9.0 to 10.0mg/dl and 2.5 to 4.5 mg/dl respectively. In the present study the electrolytes levels of both subjects to some extent laid within the normal range either in pre-gum or post-gum arabic ingestion periods; although the level of K^{+1}, Na^{+1}, and P^{+5} was increased in more than 50% in chronic renal failure subjects, whereas healthy subjects showed only that increase for P^{+5}. It seems that gum arabic in a dose of 25g/day was not effective in reduction or increasing in blood mineral content in both subjects. This may be attributed to low viscosity and dose of gum arabic**;** since high viscous fibres and high dose have a pronounced effect on mineral absorption from the intestine (Jenkins *et al*., 1976; 1978; Ross *et al.,* 1983; Demigne and Remesy, 1985). In addition, although gum arabic has the cations, Ca^{+2} (6000ppm), P^{+5} (3.665ppm), K^{+} (7500ppm) and Na^{+} (6.315ppm) (Buffo *et al*., 2000) but they don't affect the blood mineral composition obviously, even in chronic renal failure patients who have impaired kidneys.

In comparison of the present results with those obtained by other investigators on different subjects and conditions (types and doses of fibres and duration periods) more or less they were similar:-

Cummings *et al.* (1979) observed that 36g/day pectin, calcium cations remain unchanged whereas their faecal excretion was increased. This was attributed to that pectin has uronic acid which form complex with calcium and other cations at the upper part of the gut and released in the large bowel as pectin was metabolized. Ross *et al*. (1983) feeding 25g/day gum arabic to five healthy men for three weeks. No significant change has occurred between pre-gum arabic and post-gum arabic in hematological measurement values. Sodium changes from 140 to 140mmol/L, potassium 3.9 to 4.0mmol/L and phosphorus from 0.97 to 0.96mmol/L.Tulung *et al.* (1987) their studies on the effect of 15% gum arabic on rats revealed that, the concentration of potassium, calcium and magnesium were higher in the caecum after adaptation to gum arabic diet. This reflects the cation–binding capacity of this gum. Parillo *et al*. (1988) observed that phosphorus level was significantly increased from 1.23 to 1.45mmol/L due to high carbohydrate and high–fibres diet, and conversely serum calcium 4.6 to 4.7, potassium 4.8 to 5.2 and sodium 133 to 138mmol/L were not changed significantly. Demigne and Remesy (1985) fed 50% mixed fibres diet, which contained pectin, gum, crude potato starch an the fibres components of wheat bran and soya seed cake. Moderate absorption of sodium cations and a large absorption of potassium, magnesium and calcium along a favorable concentration gradient were observed. This shows that fibres somewhat displace the main site of mineral absorption.

For the non-protein nitrogenous (NPN) compounds, a normal person has base-line blood urea nitrogen of 14-36 mg /dl, whereas the normal range for serum creatinine is of 1.0 to 2.0mg/dl. These ratios vary rather widely in healthy persons, and influenced by diverse factors such as dietary intake or protein and the state of hydration (Woo and Henry, 1993).

In the present work the blood urea nitrogen and creatinine of volunteers under study were both decreased in a very low percentage in both subjects, although chronic renal failure patients were under low conservative management. Also, no obvious correlation between the pH, total acidity, volatile fatty acids content in the faeces and blood urea nitrogen in both subjects, although the nitrogen content was increased in both subjects during the study periods. These findings reflect that either chronic renal failure patients do not follow the dietary programs or the dose of gum arabic and the adaptation periods were not enough to show a significant decrease in their blood urea nitrogen. In addition to this, the digestibility of dietary fibres vary considerably between individuals due to individual differences in the gut microflora or variables in the transit time (Southgate and Durin, 1970; Walters *et al*., 1975; Southgate *et al*., 1976).

Hence, this confirms that subject under such type of study must be hospitalized to insure that subject has received the same treatment so as to get well obvious results, without day-to-day variation in dietary intake quantitatively and qualitatively, i.e. standardized diet.

In contrast with the present results, Demigne and Remesy (1985) observed that mixed fibres diets reduced arterial urea 10% and there is a substantial reabsorption of ammonia despite the lower caecal ammonia

and the acidic pH in the caecum. Parillo *et al*. (1988) observed the effect of high carbohydrates, high-fibres and low protein diet in diabetic patients with moderate chronic renal failure. This diet induced a significant increase in faecal nitrogen losses, but it was not affected serum urea nitrogen 11.8 to 9.7mmol/L, and serum creatine 292 to 318mmol/L. Whereas, Bliss *et al*. (1996) observed that chronic renal failure patients under low-protein diet and supplementation of 50g/day gum arabic, their faecal bacterial mass and nitrogen content significantly increased with concomitant decrease in serum nitrogen 50 to 44 mg/dl while creatinine insignificantly different (4.4 to 4.5 mg/dl).

A study report about the supplementation of 50g/day gum arabic was conducted at the Khartoum Dialysis and Kidney Transplantation Centre in 1998. Three groups were studied. Group (A) 28-chronic renal failure patients on regular hemodialysis, group (B) 19-chronic renal failure patients under low protein diet and group (C) 9-normal volunteers. For group (A) 9-patients showed very good results, three of them were stopped from dialysis. For group (B) after two months, the blood urea nitrogen and serum creatinine were decreased by 43% and 31% respectively. Group (C) after ten days, the blood urea nitrogen and serum creatinine were decreased by 17.6% and 10.3% respectively.

Also, in the present study diamines and polyamine were not detected in serua of both subjects before and after ingestion of gum arabic. This may be attributed to that, the amines produced in the gut are absorbed and enter the portal blood system where they act on by a ammonia oxidase produced by the liver which deaminate a large proportion of the monoamines, thereby, rendering them not–toxic and reducing their concentration in the portal blood and low in the venous blood (Gaugas, 1980). Also, it is difficult to detect polyamines in plasma

or urine prior to acid hydrolysis of the sample, because they were conjugated to a carrier molecule (Gaugas, 1980; Seidle and Snyder, 1989). Ninty five per cent of both putrescine and spermidine were conjugated within five minutes injection in plasma of both normal and cancer patients (Gaugas, 1980). In addition, Swendseid *et al.* (1978) expect that the formation of the metabolic important polyamines spermidine and spermine might be impaired since both *in vivo* and *in vitro* evidence indicate a reduction in the catabolism of ornithine via decarboxylation in chronically uremial rat or metabolized to other products (Bardocz *et al*., 1995).

Controversially Saito *et al*. (1983) measured the serum levels of four polyamines (putrescine, spermidine, spermine and cadavarine) in normal subjects and in patients with chronic renal failure. Their results showed that, when compared to normal subjects, the serum levels of all four polyamines are significantly elevated either in nondialyzed patient with chronic renal failure or in dialyzed patients.

Summary and conclution:

This work was conducted in 2000, which is deals with the comparative study between commercial gum arabic samples (collected from local markets of Khartoum State) and authentic samples in the scope of their physico-chemical properties and bacterial load to determine the purity and safetiness of the commercial samples.The effect of ingestion of 25g/day processed gum arabic for 28-days on the levels of

urea, creatinine, haemoglobin percentage, minerals (Na^{+1}, K^{+1}, P^{+5}, and Ca^{+2}) in the blood of healthy and chronic renal failure subjects. The effects of this dose on the contents of protein and volatile fatty acids in their faeces and polyamines in their blood as metabolic products in their colon. The domination of *Klebsiella* over *E. coli* after ingestion of gum arabic by those subjects.

The proximate analysis for each 25 samples from the commercial and authentic *Acacia senegal* gum revealed that the moisture percentage was more in the commercial samples (12.62) than in the authentic ones (10.40). The ash percentage was less in the commercial samples (3.49) than in the authentic ones (3.85). The pH value was high in the commercial samples (4.82) than the authentic ones (3.85). The specific rotation was (- 24.45) for the commercial samples and (- 32.90) for the authentic ones. The nitrogen content was less in the commercial samples (0.29) than the authentic ones (0.31). The protein content was less in the commercial samples (1.91) than the authentic ones (2.02). All values were significantly different, except the nitrogen and the protein, but inspite of this they were laid within the range specified by the JECFA.

The bacterial load for 25 samples of gum arabic nodules (20 commercial and 5 authentic) was more in the outer surface of the nodules than the cortex, which was completely sterile. With gum processing the bacterial load was decreased. It was ranged from zero to $3.6x10^3$CFU/g for the commercial samples; zero to $0.2x10^3$CFU/g for the authentic ones; zero to $1.2x10^3$CFU/g for the ready crushed forms and more less, zero to $0.2x10^3$CFU/g in the fine powder ones. The major bacterial isolates from gum arabic were Gram-positive *Bacillus* spp.; *B. mycoides* and *B. licheniformis* represent the highest percentage of isolates in all forms of gum arabic samples studied in this work. Whereas, Gram-positive cocci;

Staphylococcus caseolyticus, represent the minor bacterial isolates. Coliforms were not detected.

The effect of ingestion of 25g/day gum arabic for 28-days by healthy and chronic renal failure subjects revealed that when compared with the pre-gum arabic periods. No side effects were observed except few abdominal troubles either in the first period or in the second one. Reduction in haemoglobin level was observed in 70% of healthy subjects and in 33.3% of chronic renal failure ones. K^{+1} level was increased in 30% and decreased in 20% of healthy subjects, whereas, in chronic renal failure ones increased in 55.5% and decreased in 33.3%. Na^{+1} level was increased in 30% and decreased in 40% of healthy subjects, whereas, in chronic renal failure ones increased in 66.6% and decreased in 33.3%. Ca^{+2} level was increased in 70% and decreased in 20% of healthy subjects, whereas, in chronic renal failure ones increased in 44.4% and decreased in 33.3%. P^{+5} level increased in all healthy subjects and in 66.6% of chronic renal failure ones. Urea level was decreased in 20% and increased in 40% of healthy subjects, whereas in chronic renal failure ones decreased in 33.3% and increased in 22.2%. Creatinine level was decreased in 10% and increased in 60% of healthy subjects, whereas in chronic renal failure ones decreased in 44.4% and increased in 33.3%. Total acids level was increased in 66.6% and decreased in 22.2% of healthy subjects, whereas in chronic renal failure ones increased in 44.4% and decreased in 33.3%. Volatile fatty acids level was increased in 44.4% and decreased in 11.1% of healthy subjects, whereas in chronic renal failure ones increased in 22.2% and decreased in 44.4%. Nitrogen content was increased in 33.3% and decreased in 11.1% of healthy subjects, whereas in chronic renal failure ones increased in 55.5% and decreased in 11.1%. All these changes were insignificant and laid within the normal

range. Formation of polyamines was not observed in the sera of these subjects either before or after ingestion of gum arabic. The domination of *Klebsiella* sp. over the other *Enterobacteriaceae* (*E. coli, Pseud.* sp. *and Staph.* sp.) was not due to *Klebsiella* bacteriocine effect but it was suggested to be due to the lowering in pH when gum arabic was fermented in the colon or due to the release of urease enzyme from *Klebsiella* sp. and hence formation of ammonia which elevate the pH of the colon.

The study has concluded that, gum arabic which is sold in local markets of Khartoum State was suggusted to be not mixed with other types of gums since their physico-chemical properties laid within the limits specified by JECFA. It was safe due to absence of coliform isolates in either raw or processed forms but its effects in a dose of 25g/day for 28-days in healthy and chronic renal failure patients were not obvious for blood minerals, urea and creatininine levels.

Therefore, more comparative studies are needed to show the effects of gum arabic when mixed with other fermentable fibres such as guar gum, pectin and oligosaccarides or nonfermentable fibres such as wheat bran, to obtain safe cheap available therapy for chronic renal failure disease as well as prevention of its occurance through decreasing the kidney load via increasing faecal nitrogen excretion, decreasing serum nitrogen concentration, and re-functioning of the kidney through formation of volatile fatty acids and polyamines.

RECOMMENDATIONS:

It is recommended to consume gum arabic in the raw form by chronic renal failure patients than the processed one. It should be mixed with other fibres to improve its physiological action in human body.

The subjects must be fed at least three to four weeks before the experiments on standard free fibre diet, then fibres are added to highlight their effects. Beside this, subjects must be hospitalized to keep all variables constant through the study period.

It is recommended that preventive medicine must specify measurement for better life. Food composition table should contian dietary fibres, since many claims have been made for the beneficial effects of dietary fibres in prevention the aetiology of certain common

diseases such as cancer, renal diseases, diabetes,..…etc. In addition, more than 25g/day of fibre must be taken by each subject to regulate intestinal absorption of nutrients instead of drugs.

Greater variety of bread, potatoes, vegetables and fruits must be offered at prices that encourage their consumption to prevent the occurance of such nutritional diseases.

References

Abboud, O. I. (1982). Chronic renal failure in the Sudan. An M. D. Thesis ;University of Khartoum-Sudan.

Alder, H., Margoshes, M., Snyder, L. R. and **Spitzer, C. (1977).** Rapid chromatographic method to determine polyamines in urine and whole blood. J. Chromatog. 143: 125 – 136.

Ali, Affra, Y. (2000). Gum arabic as supportive therapy for chronic renal failure (CRF) disease. B.Sc. honors degree in Family Science; Ahfad University for Women; Omdurman-Sudan.

Ali, N. E. S. (1996). Nitrogen and specific rotation as qualifying indices for gum arabic derived from *Acacia senegal* (L) Willd. An M.Sc. thesis; University of Khartoum-Sudan

Allison, C. and **Macfarlane, G. T. (1989).** Influence of pH, nutrient availability and growth rate on amine production *by Bacteriodes fragilis* and *Clostridium perfringens*. Appl. Environ. Microbiol. 55: 2894 – 2898.

Ames, B. N. and **Dubin D. T. (1960).** The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J. Biol. Chem. 235: 769 – 775.

Anderson, **D. M. W**. and **Dea, I. C. M. (1968).** Studies on uronic acid materials: Some unusual forms of the gum arabic from *Acacia senegal* (Willd.). Carbohyd. Res. 6: 104 – 110.

Anderson, D. M. W., Dea, I. C. M., Karamalla, K. A. and **Smith, J. F. (1968).** Studies on uronic acid materials: An analytical study of different forms of the gum from *Acacia senegal* (Willd). .103 – Carbhyd. Res. 6: 97

Anderson, **D. M. W**., **Howlett, J. F**. and **McNab, C. G. A. (1985).** The amino acid composition of the proteinaceous component of gum arabic, *Acacia senegal* (L) (Willd). Food Additives and Cotaminants 2: 159 – 164.

Anderson, D. M. W. and **Karamalla, K. A**. **(1966).** Intranodule variation and the acidic component in *Acacia nilotica* gum. Carbohyd. Res. 2: 403 – 410.

Anderson, D. M. W. and **Stoddart, J. F. (1966).** Study on uronic acid materials: The use of molecular-sieve chromatography in studies on *Acacia senegal* gum (gum arabic).Carbhyd. Res. 2: 104 –114.

Anderson, H. H., Murayama, F. and **Abreu, B. E. (1947).** Pharmacology and Experimental Therapeutics. Univ. California Press. Los Angeles, page 1. Cited in **Glicksman, M.** and **Sand, R. E.** (1973). In: Whistler, R. L. (Editor); Industrial gums Polysaccharides and their derivatives. 2nd. Edit. Academic Press. New York, San Francisco, London.

Anderson, I. H., Levine, A. S. and **Levitt, M. D. (1981).** Incomplete absorption of the carbohydrates in all-purpose wheat flour. New Eng. J. Medi. 34: 891 – 892.

Anderson, J. W. and **Chen, W. J. L. (1979).** Plant fibre**:** Carbohydrate and lipid metabolism . Am. J. Clin. Nutr.32: 346 – 363.

Anderson, M. W. D. and **MaCDonald, F. J. (1987).** Degradative studies of gum arabic. Food Additives and Cotaminants 4: 247 – 255.

Antonis, A. and **Bersohn, I. (1962).** The influence of diet on faecal lipids in South African White and Bantu Prisoners. Am. J. Clin. Nutr. 11: 142 – 148.

A.O. A. C. (1984). Official methods of analysis of the Associationof Official Analytical Chemistry 14th Edit. Washington D. C.

of **A.O. A. C. (1990).** Official methods of analysis of the Association Official Analytical Chemistry 15th Edit. Washington D. C.

Aries, V., Growther, J. S., Drasar, B. S., Hill, M. J. and **Williams, R. E. O. (1969).** Bacteria and the aetiology of cancer of the large bowel. Gut 10: 334 – 335.

Assimon, S. A. and **Stein, T. P. (1994).** Digestible fibre (gum arabic), nitrogen excretion and urea recycling in rats. Nutr.10: 544 – 550.

Awad El-karim, M. M. E. (1994). Analytical studies on some crude and processed gum arabic samples with regard to quality aspects. An M.Sc. thesis; University of Khartoum-Sudan

Bardocz, S., Duguid, T., Brown, D. S., Grant, G. Pusztai, A., White, A. and **Ralph, A. (1995).** The importance of dietary polyamines in cell regeneration and growth. Br. J. Nutr. 73: 819 – 828.

Bardocz, S., Grant, G., Brown, D. S., Ralph, A. and **Pusztai, A. (1993).** Polyamines in food-implications for growth and health. J. Nutr. Bioch. 12: 114 – 121.

Barrow,G. L. and **Feltham, R. K. A. (1993).** In: Cowan and Steel's manual for identification of medical bacteria. 3rd. Edit. Cambridge University Press.

Bartels, H. and **Bohmer, M. (1971).** Eine mikromethode zur kreatininbestimmung. Cli. Chim. Acta 32: 81 – 85.

Bartos, F., Bartos, D., Grettie, D. and **Campbell, R. (1977).** Polyamine levels in normal human serum: Comparison of analytical methods. Biochem. Biophys. Res. Commun. 75: 915 – 919.

Bashier, Nawal, M. (1996). Dietary management and complains of patients with end-stage renal failure on maintenance hemodialysis therapy in Khartoum Dialysis and Kidney

Transplantation Centre. An M.Sc. thesis; University of Khartoum – Sudan.

Batshaw, M. L., Brusilow, S., Waber, L., Blom, W., Brubakk, A. M., Burton, B. K. Cann, H. M., Kerr, D., Mamunes, P., Matalon, R., Myerberg, D. and Schafer, I. A. (1982). Treatment of inborn errors of urea synthesis activation of alternative pathways of waste nitrogen synthesis and excretion. N. Engl. J. Med. 306: 1387 – 1392.

Benamouzig, R., Mahe, S., Luengo, C., Rautyreau, L. and **Tome, D. (1997).** Fasting and postgrandial polyamine concentrations in the human digestive lumen. Am. J. Clin. Nutr. 65: 766 – 770.

Betain, H. G., Linehan, B. A., Bryant, M. P. and **Holdeman, L. V. (1977)**. Isolation of a cellulytic *Bacteroides* sp. from human faeces. Appl. Environ. Microbiol. 33: 1009 – 1010.

Bjorck, I., Nyman, M. and **Asp, N. G. (1984)**. Extrusion cooking and dietary fibre: Effects on dietary fibre content and degradation in the rat intestinal tract. Cereal Chemistry. 61: 174 – 179.

Bliss, D. Z., Stein, T. P., Schleifer, C. R. and **Settle, R. G. (1996).** Supplementation with gum arabic fibre increases faecal nitrogen excretion and lowers serum urea nitrogen concentration in chronic renal failure patients consuming a low protein diet. Am. J. Clin. Nutr. 63: 392 – 398.

Blunt, H. S. (1926). Gum arabic, with special reference to its production in the Sudan. Oxford University Press, New York. Cited in **Glicksman, M.** and **Sand, R. E .** (1973). In: Whistler, R. L. (Editor); Industrial gums: Polysaccharides and their derivatives. 2nd. Edit. Academic Press. New York, San Francisco, London.

Bokhary H. A. , Hassib, A. M. and **Suleiman A. A. A. (1983).** Gamma irradiation effects on carbohydrate composition, growth of microorgnisms and ESR spectra of gum arabic (*Acacia senegal* L.) . J. of Food Protec. 46: 585 – 588.

Bowman, W. H., Tabor, C. W. and **Tabor, H. (1973).** Spermidine biosynthesis, purification and properties of propylamine transferase from *Escherichia coli*. J. Biol. Chem. 248: 2480 – 2486.

Brachet, P. and **Tome, D. (1992).** Putrescine uptake by rabbit intestinal brush membrane vesicles. Biochem. Int. 27: 465 – 472.

British Nutrition Foundation (1990). Complex carbohydrate in foods: The reports of the British Nutrition Foundation's Task Force. (London: Chapman and Hall).

Brown, C. L., Hill, M. J. and **Richards, P. (1971).** Bacterial ureases in uraemic men. The Lancet 2: 406 – 408.

Bryant, M. P. (1974). Nutritional features and ecology of predominant anaerobic bacteria of the intestinal tract. Am. J. of Clin. Nutr.27: 1313 –1319.

Buffo, R. A., Reineccius, G. A. and **Oehlert, G. W. (2000).** Factors affecting the emulsifying and rheological properties of gum acacia in beverage emulsions. Food Hydrocolloids.15: 53 – 66.

Burkitt, D. P., Walker, A. R. P. and **Painter, N. S. (1972)**. Effect of dietary fibre on stools and transit-times and its role in the causation of disease. The Lancet 2: 1408 – 1412.

Burkitt, D., P. Walker, A. R. P. and **Painter, N. S. (1974).** Dietary fibre and disease. J. Am. Med. Assoc.229: 1068 –1074.

Chaney, A. L. Marbach, E. P. (1962). Modified reagents for determination of urea and ammonia. Clin. Chem. 8: 130 – 132.

Chen, W. L. and **Anderson, J. W. (1981).** Soluble and insoluble plant fibre in selected cereals and vegetables. Am. J. Clin. Nutr. 34: 1077 – 1082.

Cowan, S. T. and **Steel, K. J. (1977).** Manual for the identification of medical bacteria. 5th Edit., Cambridge University Press.

CowGill, G. R. and **Anderson, W. E. (1932).** Laxative effects of wheat bran and washed bran in healthy man. A comparative study. J. Am. Med. Assoc. 98: 1866 – 1875.

Cruichshank, R. , Duguid, J. P. Marmion, B. P. and **Swain, R. H. (1975).** Medical Microbiology. 12th Edit. vol. (2). London, New York.

Cummings, J. H. (1980). Dietary fibre and large bowel cancer. Proc. Nutr. Soc. 40: 7 – 14.

Cummings, J. H. (1981). Dietary fibre. Br. Med. Bull. 37: 65 – 70.

Cummings, J. H., Southgate, D. A. T., Branch, W. J., Wiggins, H. S., Houston, H., Jenkins, D. J. A., Jivraj, T. and **Hill, M. J. (1979)**. The digestion of pectin in the human gut and its effect on calcium absorption and large bowel function. Br. J. Nutr. 41: 477 – 485.

Dekker, R. F. H. and **Richards, G. N. (1973).** J. of the Scie. of Food and Agric. 24: 375 – 379. Cited in **Nyman, M. N.** and **Asp, N. G.** (**1982**). Fermentation of dietary fibre components in the rat intestinal tract. Br. J. Nutr. 47: 357 – 366.

Demigne, C. and **Remesy, C. (1985).** Stimulation of absorption of volatile fatty acids and minerals in the caecum of rats adapted to a very high fibre. J. Nutr. 115: 53 – 60.

DeVita, M. V. and **Michelis, M. F. (1993).** Perturbation in sodium balance: Hyponatremia and hypernatremia. In: Preuss H. G.: edit. Clinicsin Laboratory Medicine: Renal function. Philadelphia, W. B. Saunders Company, page 134. In Henry, J, B, (1996). Clinical diagnosis and management by laboratory method.19th Edit. Page 147.

Difco, (1972). Manual of dehydrated culture media and reagents for microbiological and clinical laboratory procedures. 9th Edit. printed in U.S.A.

Dowling, R. H., Riecken, E. O., Lows,J. W. and **Booth, C. C. (1967).** The intestinal response to high bulk feeding in the rat. Clin. Scien. Lond. 32: 1 – 9.

DuDley, H. W. and **Rosenheim, O. (1925).** Notes on spermine. Biochem. J. 19: 1034 – 1036.

Durrington, P. N., Manning, A. P., Bolton, C. H. and **Hartog, M. (1976).** Effect of pectin on serum lipids and lipoproteins, whole-gut transit-time and stool weight. Lancet 2: 394 – 396.

Durrington, P., Wicks, A. C. B. and **Heaton, K. H. (1975).** Effect of bran on blood lipids. Lancet 2: 133.

Eastwood, M. A. (1969). Dietary fibre and serum–lipids. Lancet 2: 1222 – 1225.

Eastwood, M. A. (1975). Vegetable dietary fibre potent pith. J. R. Soc. Health 95: 188 – 190.

Eastwood, M. A., Brydon, W. G. and **Anderson, D. M. W. (1986).** The effect of the polysaccharides composition and structure of dietary fibres on caecal fermentation and faecal excretion. Am. J. Clin. Nutr. 44: 51 – 55.

Eastwood, M. A., Fisher, N., Greenwood, C. T. and **Hutchinson, J. B. (1974).** Perspective on the bran hypothesis. The Lancet 1: 1029 – 1033.

Eastwood, M. A., Kirkpatrick, J. R., Mitchell, W. D., Bone, A. and **Hamilton, T. (1973).** Effects of dietary supplements of wheat bran and cellulose on faeces and bowel function. Brit. Med. J. 4: 392 – 394.

Eastwood, M. A. and **Passmore, R. (1975).** Human nutrition and dietics, 8th Edit., page 409.

Eastwood, M. A. and **Passmore, R. (1983).** Dietary fibre. The lancet 2: 202 – 206.

Eastwood, M. A., Robertson, J. A., Brydon, W. G. and **McDonald, D. (1983).** Measurement of water-holding properties of fibre and their faecal bulking ability in man. British J. of Nutr. 50: 539 – 547.

Editorial, (1983). High-carbohydrate high-fibre-diets for diabetes mellitus. Lancet 1: 741 – 742.

Elsenhans, B., Blume, R. and **Caspary, W. F. (1981).** Long-term feeding of unavailable carbohydrate gelling agents. Influence of dietary concentration and microbiological degradation on adaptive responses in the rat. Am. J. of Clin. Nutr. 34: 1837 – 1848.

Englyst, H. N. and **Cummings, J. H. (1985)**. Digestion of the polysaccharides of some cereal foods in the human small intestine. Am. J. of Clin. Nutr. 44: 51 – 55.

Englyst, H. N. and **Cummings, J. H. (1986)**. Digestion of carbohydrates of banana (*Musa paradisica sapientum*) in the human small intestine. Am. J. of Clin. Nutr. 44: 42 – 50.

Englyst, H. N. and **Cummings, J. H. (1987)**. Digestion of the polysaccharides of potato in the small intestine of man. Am. J. of Clin. Nutr. 45:423 – 431.

Fabiny, D. L. and **Ertingshausen, G. (1971).** Automated reaction-rate method for determination of serum creatinine with centrifuge. Clin. Chem. 17: 696 – 700.

FAO, Rome (1986). Food and nutrition. Paper No. 34, page 93 – 95.

FAO, Rome (1990). Food and nutrition. Paper No. 49, page 23 – 25.

Findlay, J. M., Smith, A. N., Mitchell, W. D. and **Eastwood, M. A. (1974)**. Effects of unprocessed bran on colon function in normal subjects and in diverticular disease. Lancet 1: 146 – 149.

Finegold, S. (1977). Faecal microbial flora in seventh day adventist populations and control subjects. Am. J. Clin. Nutr. 30: 1781 – 1792.

Fujita, Y., **Okuta, T., Rikimaru, T., Ichikawa, M., Miyatani, S., Kajiwara, N. M., Date, C., Minamide, T., Koishi, H., Alpers, M. P.** and **Heywood, P. I. (1986).** Studies of nitrogen balance in male highlanders in Papua New Guinea. J. Nutr. 116: 536 – 544.

Gabb, S. (1997). Gum arabic production in Sudan: A brief introduction. Sudan Foundation. Ecnomics File Number 13, London WCIV 9LD, United Kingdom.

Gamst, O. and **Try, K. (1980).** Determination of serum-phosphate without deproteinization by ultraviolet spectrophotometry of the phosphomolybdic acid complex.. Scand. J. Clin. Lab. Invest. 40: 483 – 486.

Gaugas, J. M. (1980). Polyamines in biological research. A Wiley-Interscience Puplication. Chichester NewYork, Bribane, Torinto.

Gayed, S. T. and **Hassan, H. M. (1964).** A preliminary study on the suitability of gum arabic as medium for fungal growth. Mycologia 18: 1 – 10.

Ginty, D. D., Osborne, D. L. and **Seidel, E. R.** (**1989**). Putrescine stimulate DNA synthesis in intestinal epithelial cells. Am. J. Physiol. 257: G145 – G150.

Giordano, C., DePascale, C. Cittadini, D. and **Grescenzi, A. (1968).** Incorporation of urea N^{15} in amino acids of patients with chronic renal failure on low nitrogen diet. Am. J. Clin. Nutr. 21: 394 – 400.

Glicksman, M. (1969). Gum technology in the food industry. New York and London, Academic Press. Page 97 – 111.

Glicksman, M. and **Sand, R. E . (1973).** In: Whistler, R. L. (Editor); Industrial gums: Polysaccharides and their derivatives. 2nd. Edit. Academic Press. New York, San Francisco, London.

Grande, F., Anderson, J. T. and **Keys, A. (1965).** Effect of carbohydrates of leguminous seeds, wheat and potatoes on serum cholestorol concentration in man. J. Nutr. 86: 313 – 317.

Hajj-Elmahi, A. A. (2001). Evaluation and composition of the nutritional status of patients suffering from chronic renal failure in the Sudan. A thesis of qualification degree, Faculty of Education-Univesity of Khartoum - Sudan.

Hanson, P. J. and **Parsons, D. S. (1977).** Metabolism and transport of glutamine and glucose in vascularly perfused rat small intestine. Biochem. J. 166: 509 – 519.

Harborne, J. B. (1973). Phytochemical methods. Published by Chapman and Hall Ltd., London. Page 181.

Harrison, G. A. (1931). The spermine in human tissue. Biochem. J. 25: 1885 – 1892.

Harvey, R. F., Pomare, E. W. and **Heaton, K. W. (1973).** Effects of increased dietary fibre on intestinal transit. Lancet 1: 1278 – 1280.

Haslett, C., Chilvers, E. R. Hunter, J. A. A. and **Boon, N.A. (1999).** Principles and practice of medicine.18th Edit. London Newyork. Page 429 – 439.

Heaton, K. W. (1973). Food fibre as an obstacle to energy intake. Lancet 2: 1418 –1421.

Heaton, K. W., Manning, A. P. and **Hartog, M. (1976)**. Lack of effect on blood lipid and calcium concentrations of young men on changing from white to whole meal bread. Brit. J. Nutr. 35: 55 – 60.

Heaton, K. W. and **Pomare, E. W. (1974).** Effect of bran on blood lipids and calcium. Lancet 1: 49 – 50.

Heller, S. N., Hackler, L. R., Rivers, J. M., Vas Soest, P. J., Roe, D. A., Lewis, B. A. and **Robertson, J. (1980).** Dietary fibre: the effect of particle size of wheat bran on colonic function in young adult men. Am. J. of Clin. Nutr. 33: 1734 – 1744.

Herbst, E. J. and **Snell, E. E. (1949)**. Putrescine and related compounds as growth factors for *Hemophilus parainfluenzae*7901. J. Biol. Chem. 181: 47 – 54.

Hill, M. J. , Growther, J. S., Drasar, B. S., Hawksworth, G., Aries, V. and **Williams, R. E. O. (1971).** Bacteria and aetiology of cancer of large bowel. Lancet 1: 95 – 100.

Holdman L., V. and **Moore, W. E. C. (1972).** Anaerobe laboratory manual. Anaerobe Laboratory Puplishers, Virginia, page20 - 36.

Holt, S., Heading, R. C., Carter, D. C., Prescott, L. F. and **Hothill, P. (1979).** Effect of gel fibre on gastric emptying and absorption of glucose and paracetamol. Lancet 2: 636 – 639.

Holtta, E. (1977). Oxidation of spermidine and spermine in rat liver: purification and properties of polyamine oxidase. Biochemistry. 16: 91 – 100.

Howes, F. N. (1949). Vegetable gums and resins. Published by the Chronica Botonica Company of Walltham, Mass U.S.A.

Huth, K. and **Fettel, M. (1975).** Bran and blood lipids. Lancet 2: 456.

Idris, O. H. (1989). Physico-chemical and microbiological characterization of *Acacia senegal* (L.) Willd. gum (Gum Arabic). An M.Sc. thesis, University of Khartoum - Sudan.

Ishag, K. E. A. (1977). Chemotaxonomic studies on Sudan *Acacias*. An M. Sc. thesis, University of Khartoum - Sudan.

Jackson, A, A. (1983). Amino acids**:** Essential and nonessential . Lancet 1: 1034 – 1037.

Jacobs, L. R. and **Lupton, J. R. (1984).** Effect of dietary fibres on rat large bowel mucosal growth and cell proliferation. Am. J. Physiol. 246: G6378 – G6385.

JECFA (1997). Superseding specifications prepared at the 44th JECFA (1995), published in Food and nutrition paper No. (52) Addendum 3(1995).

JECFA/FAO/WHO (1999). Compendium of Food Additive Specification. Joint Expert Committee on Food Additives FAO, Food and nutrition paper No. (52) Rome.

Jenkins, D. J. A., Hill, M. S. and **Cummings, J. H. (1975)$_a$**. Effects of wheat fibre on blood lipids, faecal steroid excretion and serum iron. Am. J. Clin. Nutr. 28: 1408 – 1411.

Jenkins, D. J. A., Leeds, A. R., Newton, C. and **Cummings, J. H. (1975)$_b$**. Effect of pectin, guar gum and wheat fibre on serum-cholesterol. Lancet 1:1116 – 1117.

Jenkins, D. J. A., Leeds, A. R. and **Gassull, M. A. (1976).** Unabsorbable carbohydrates and diabetes: decreased postprandial hyperglycemia. Lancet 2: 172 – 174.

Jenkins, D. J. A., Wolever, T. M. S., Hockaday, T. D. R., Leeds, A. R., Howarth, R., Bacon, S., Apling, E. C. and **Dilawari, J. B. (1977).** Treatment of diabetes with guar gum. Lancet 2: 779 – 780.

Jenkins, D. J. A., Wolever, T. M. S., **Leeds, A. R., Gassull, M. A., Haisman, P., Diliwari, J., Goff, D. V., Metz, G. L.** and **Alberti, K. G. (1978).** Dietary fibres: fibre analogues and glucose tolerance: importance of viscosity. Brit. Med. J. 1: 1392 – 1394.

Johnson, I. T. and **Gee, J. M. (1986).** Gastrointestinal adaptation in response to soluble non-available polysaccharides in the rat. Br. J. of Nutr. 55: 497 – 505.

Jones, J. D. and **Burnet, P. C. (1974).** Creatinine metabolism in humans with decreased renal function: creatinine deficit. Clin. Chem. 20: 1204 – 1212.

Karamalla, K. A., Siddig, N. E. and **Osman, M. E. (1998)**. Analytical data for *Acacia senegal* var. senegal gum samples collected between 1993 and 1995 from Sudan. Food Hydrocolloids 12: 373 –378.

Karamalla, K. A. (1965). Ph.D. thesis. Edinburgh University.

Kay, R. M. (1982). Dietary fibre. J. Lipid Res. 23: 221 – 242.

Kay, R. M. and **Truswell, A. S. (1977).** Effect of citrus pectin on blood lipids and faecal steroid excretion in man. Am. J. Clin. Nutr. 30: 171 – 175.

Kelsay, J. L. (1978). A review of research on effects of fibre intake on man. Am. J. Clin. Nutr. 31: 142 – 159.

Keys, A., Anderson, J. T. and **Grande, F. (1960).** Diet-type (fats constant) and blood lipids in man. J. Nutr. 70: 257 – 261.

Keys, J. E., Van-Soest, P. J. and **Young, E. P. (1970).** J. of Animal Scie. 31: 1172 – 1177. Cited in **Nyman, M.N.** and **Asp, N. G. (1982).** Fermentation of dietary fibre components in the rat intestinal tract. Br. J. Nutr. 47: 357 – 366.

Kneib, B., Raymondjean, M., Bogdanovsky, D., Bachner, L. and **Shapira, G. (1979).** An improved and easy technique for polyamine determination in biological samples. J. of Chromatogr. 162: 547 – 559.

Kortkiewski, M. (1984). Effect of guar gum on body weight, hunger-rating metabolism in obese subjects. Br. J. of Nutri. 52: 97 – 105.

Kritchevsky, D. and **Story, J. A. (1975).** *In vitro* binding of bile acid and bile salts. Am. J. Clin. Nutr. 28: 305 – 306.

Kumagai, J. and **Johnson, L. R**. **(1988).** Characteristics of putrescine uptake in isolated rat enterocytes. Am. J. Physiol. 254: G81 – G86.

Leeds, A. R. Ralphs, D. N. L., Ebied, F., Metz, G. and **Dilawari, J. B. (1981).** Pectin in the dumping syndrome: reduction of symptoms and plasma volume changes. Lancet 1: 1075 – 1078.

Livesey, G. (1992). The energy values of dietary fibre and sugar alcohols for man. Nutr. Res. Rev. 5: 61 – 84.

Lupton, J.R., Coder, D. M. and **Jacobs, L. R. (1985).** Influence of luminal pH on rat large bowel epithelial cell cycle. Am. J. Physiol. 249: G382 – G388.

Lupton, J. R. and **Kurtz, P. P. (1993).** Relationship of colonic luminal short-chain fatty acids and pH to *in vivo* cell proliferation in rats. J. Nutr. 123: 1522 – 1530.

Macfarlane, G. T. and **Macfarlane, S. (1997).** Human colonic microbiota: ecology, physiology and metabolic potential of intestinal bacteria. Scand. J. Gastroenterol. 32 : 3 – 9.

Mahan, L. K. and **Escott-Stump,S. (1996).** Food nutrition and diet therapy. 9th. Edit. Page 782.

Malcolm,D. W. (1936). "Report on gum and gum arabic," Government Printery, Dar-es-Salam, Republic Somalia. Cited in **Glicksman, M.** and **Sand, R. E. (1973).** In Whistler, R. L. (Editor); Industrial gums: Polysaccharides and their derivatives. Sec. Edit. Academic Press New York San Francisco London.

Malke, H., Starke, R., Jacob, H. E. and **Kohler, W. (1974).**Bacteriocine-like activity of group-A *Streptococci* due to the production of peroxide. J. Med. Microbiol.7: 367 – 374.

Maytum, C. K. and **Magath, T. B. (1932).** J. Am. Med. Ass.99: 2251 – 22. Cited in **Glicksman, M.** and **Sand, R. E .** (1973). In Whistler, R. L. (Editor); Industrial gums: Polysaccharides and their derivatives. 2nd. Edit. Academic Press. New York, San Francisco, London.

McBurney,M. I. and **Thompson, L. U. (1989).** *In vitro* fermentabilities of purified fibre supplements J. Food Sci. 54: 347 – 350.

McCance, R. A. and **Widdowson, C.M. (1948).** The digestibility and absorption of the calories, proteins, purines, fats and calcium in whole meal wheaten bread. Brit. J. Nutr.2: 26 – 41.

McCormack, S.A. and **Johnson, L. R. (1991).** Role of polyamines in gastrointestinal mucosal growth. Am. J. Physiol. 260: G795 – G806.

Merchant, I. A. and **Packer, R. A. (1977).** Sterilization by hot air oven. In: Sterilization and Disinfection. Veterinary bacteriology and virology. 7th Edit. Iowa State, University Press, Ames and Iowa. U. S. A. page 85.

Miles, A. A. and **Misra, S. S. (1938).** The stimulation of the bactericidal power of the blood. J. Hyg. Gamb 38: 732 – 748.

Miller, T. L. and **Wolin,T. J. (1979).** Fermentation by saccharolytic intestinal bacteria. Am. J. Clin. Nut. 32: 164 – 172.

Ministry of National Planning (M.N.P.) (1980). Gum arabic development, restocking of the gum belt in the Sudan final report.

Mobley, H. L. T., Cortesia, M. J., Rothenthal, L.E. and **Jones, B. D. (1988).** Characterization of urease from *Campylobacter pylori.* J. of Clin. Microbio. : 831 – 836.

Mohammed, Hanan, H. (2000). Microbiological examination of gum arabic *Acacia senegal* L. (Willd.) and its degradability by bacteria and fungi. An M.Sc. thesis. University of Khartoum - Sudan.

Moore, W. E. C. and **Holdeman, L. V. (1974)$_a$**. Human faecal flora. The normal flora of 20 Japanese – Hawaiians. Appl. Microbiol. 27: 961 – 979.

Moore, W. E. C. and **Holdeman, L. V. (1974)$_b$.** Specific problems associated with the isolation and identification of intestinal bacteria in faecal flora studied. Am. J. Clin. Nutr. 27: 1450 – 1455.

Morris, D. R. and **Koffron, K. L. (1969).** Putrescine biosynthesis in *Escherichia coli*. J. Biol. Chem. 244: 6094 – 6099.

Morris, D. R. and **Pardee, A. B. (1966).** Multiple pathways of putrescine biosynthesis in *Escherichia coli*. J. Biol. Chem. 241: 3129 – 3135.

Mortensen, P. B., Holtug, K. and **Rasmussen, H. S. (1988).** Short-chain fatty acid production from mono- and disaccharides in a faecal incubation system: Implications for colonic fermentation of dietary fibre in humans. J. Nut. 118: 321 – 325.

Noack, J., Kleessen, B., Proll, J., Dongowski, G. and **Blaut, M. (1998).** Dietary guar gum and pectin stimulate intestinal microbial polyamine synthesis in rats. J. Nutr. 128: 1385 – 1391.

Nyman, M.N. and **Asp, N. G. (1982).** Fermentation of dietary fibre components in the rat intestinal tract. Br. J. Nutr. 47: 357 – 366.

O'Grady, F. W. (1966). Differences in ammonia production by faecal bacteria of patients with hepatic encephalpathy. Proc. Roy. Soc. Med. 59: 1246.

Olson, E. R. (1993). Influence of pH on bacterial gene expression. Mol. Microbiol. 8: 5 – 14.

Osborne, D. L. and **Seidel, E. R. (1989)**. Microflora–derived polyamines modulate obstruction – induced colonic mucosal hypertrophy. Am. J. Physiol. 256: G1049 – G1058.

Osborne, D. L. and **Seidel, E. R. (1990).** Gastrointestinal luminal polyamines: Cellular accumulation and enterohepatic circulation. Am. J. Clin. Nutr. 21: G576 – G584.

Osman, E. M. (1998). Microbiological and physiochemical studies on gum arabic quality and safety. M.Sc. thesis, University of Khartoum - Sudan.

Osman, M. E. Williams, D. A., Menzies, A. R. and **Phillip, O. G. (1993).** Characterization of commercial samples of gum arabic. J. Agric. Food Chem. 41: 71– 77.

Osol, A. and **Farrar, G. E. (1955**). The dispensatory of the United States. J. F. Lippincott, Philadelphia, 25th Ed., page 1. Cited in **Glicksman, M.** and **Sand, R. E .** (1973). In Whistler, R. L.

(Editor); Industrial gums: Polysaccharides and their derivatives. 2nd. Edit. Academic Press. New York. San Francisco London.

Oxoid, (1973). Manual of culture media, ingredients and other laboratory services. 3rd. Edit. , published by Oxoid Limited.

Painter, N. S. (1971). Treatment of diverticular disease. Br. Med. J. 2: 156 – 160.

Painter, N. S., Almeidia, A. Z. and **Colebourne, K. W. (1972).** Unprocessed bran in treatment of diverticular disease of the colon. Br. Med. J. 2: 137 – 140.

Painter, N. S. and **Burkitt, D. P. (1971)**. Diverticular disease of the colon: A deficiency disease of Western civilization. Br. Med. J. 2: 450 – 454.

Palmer, G. H. and **Dixon, D. G. (1966).** Effect of pectin dose on serum cholesterol levels. Am. J. Clin. Nutr. 18: 437 – 442.

Parillo, M., Riccard, I. G., Pacioni, D. Lovine, C. Contaldo, F. ,Isernia, C. , DeMarco, F. , Perrotti, N. and **Rivellese, A. (1988).** Metabolic consequences of feeding a high-carbohydrate, high-fibre diet to diabetic patients with chronic kidney failure. Am. J. Clin. Nutr. 48: 255 – 259.

Paul, A. A., Southgate, D. A. T., McCance and **Widdowson's (1978)**. The composition of human foods. 4thedn. London: HM Stationary Office. Cited in **Eastwood, M. A.** and **Passmore, R.** (**1983**). Dietary fibre. The lancet 2: 202 – 206.

Payler, D. K., Pomare, E. W. Heaton, K. W. and **Harvey, R. F. (1975).** The effect of wheat bran on intestinal transit. Gut. 16: 209 – 213.

Pegg, A. E. and **Ashman, H. G. W. (1969).** On the role of S-adenosyl-L-methonine in the biosynthesis of spermidine by rat prostate. J. Biol. Chem. 244: 682 – 693.

Pegg, A. E., Conover, C. and **Wrona, A. (1978).** Effects of aliphatic diamines on rat liver ornithine decarboxylase activity. Biochem. J. 170: 651 – 660.

Pegg, A. E. , and **Hibasami, H. (1979).** The role of S-adenosylmethionine in mammalian polyamine synthesis. In:

Transmethylation, edited by Usdin, E. , Borchardt, R. T. and Creveling, C. R. New York: Elsevier/ North Holland, page 105 – 116.

Pegg, A. E. and **McCann, P. P. (1982).** Polyamines metabolism and function. Am. J. Physiology. 243: C212 – C221.

Pendreigh, D. M., Heasman, M. A., Howitt, L. F., Kennedy, A. C., MacDougall, A. I., MacLeod, M., Robson, J. S. and **Stewart, W. K. (1972).** Survey of chronic renal failure in Scotland. Lancet 1: 304 – 307.

Persson, I., Raby, K. Fonns–Bech, P. and **Jensen, E. (1975).** Bran and blood- lipids. Lancet 2: 1208.

Phillips, G. O. (1998). *Acacia* gum (Gum arabic): A nutritional fibre, metabolism and calorific value. Food Additives and Contaminant 15: 251 – 264.

Phillips, G. O. and **Williams, P. A. (1993).** The specification of the gum arabic of commerce. Food Hydrocolloids: Structures, properties and functions, edited by K Nishinari and E. Doi New York, Plenum Press, page 45 – 63.

PiePmeyer, J. L. (1974). Use of unprocessed bran in treatment of bowel syndrome. Am. J. Clin. Nutr. 27: 106 – 107. irritable

Prather, E. S. (1964). Cellulose on serum lipids in young women. J. Am. Dietet. Assoc. 45: 230. Cited in **Kelsay, J. L. (1978).** A review of research on effects of fibre intake on man. Am. J. Clin. Nutr. 31: 142 – 159.

Quemener, V., Havouis, R. Khan, N. A., Martin, C. Bouet, F. and **Moulinoux, J. P. (1995).** Determination of erythrocyte polyamines as a predictive method in tumour diagnosis. An animal study with chemically induced tumours. Anticancer Res. 15:2517 – 2522.

Rab, S. M. and **Baseer, A. (1976).** Acute ostemalacia amongst healthy and pregnant women in Pakistan. Lancet 2: 1211 – 1213.

Raina, A. and **Janne, J. (1975).** Physiology of the natural polyamines putrescine, spermine and spermidine. Med. Biol. 53:121 – 147.

Records of Federal Ministry of Health (1995 – 1999). In-patient classification, cases and deaths by States. The Democratic Republic of the Sudan, Ministry of Health, Statistics and Research Department .Annual Statistical Report.

Richards, P. (1972). Nutritional potential of nitrogen recycling in man**.** Am. J. Clin. Nutr. 25: 615 – 625.

Richards, P., Brown, C.L. Houghton, B.J. and **Thompson, E. (1971).** Synthesis of phenylalanine and valine by healthy and Aramaic men. Lancet 2: 128 – 134.

Richards, P., Gibson, M., Ward, E. E., Wrong, O. and **Houghton, B. J. (1967).** Utilization of ammonia nitrogen for protein synthesis in man, and the effect of protein restriction and uraemia. Lancet 1: 845 – 849.

Risetti , U. and **Mancini. G. (1954).** Acta Neurol.(Naples) 9: 391 – 395.Cited in **Tabor, H., Tabor, C. W.** and **Rosenthal, S. M. (1961).** The biochemistry of polyamines**:** spermidine and spermine. Ann. Rev. Biochem. 30: 579 – 604.

Roediger, W. E. (1980). Role of an aerobic bacteria in the metabolic welfare of the colonic mucosa in man. Gut 21: 793 – 798.

Rolandelli, R. H., Koruda, M. J., Settle, R. G. and **Rombeau, J. L. (1986)**. The effect of internal feedings supplemented with pectin on the healing of colonic anastomoses in the rat. Surgery 99: 703 – 707.

Rosenthal, S. M., Fisher, E. R. and **Stohlman, E. F. (1952)**. Proc. Soc. Exptl. Biol. Med. 80: 432 – 434. Cited in **Tabor, H., Tabor, C. W.** and **Rosenthal, S. M. (1961).** The biochemistry of polyamines**:** spermidine and spermine. Ann. Rev. Biochem. 30: 579 – 604.

Ross, A. H. M., Eastwood, M. A., Brydon, W. G., Anderson, J. R. and **Anderson, D. M. W. (1983).** A study of the effects of dietary gum arabic in humans. Am. J. Clin. Nutr. 37: 368 – 375.

Ross, A. H. M., Eastwood, M. A., Brydon, W. G., Busuttil, A. and **McKay, L. F. (1984).** A study of the effects of dietry gum arabic in the rat. Bri. J. Nutr. 51: 47 – 56.

Rouf, M. A. and **Lomprey, R. F. (1968).** Degradation of uric acid by certain aerobic bacteria. J. Bacteriol. 96: 617 – 622.

Saito, A. Takagi, T. Chung T. and **Ohta, K. (1983).** Serum levels of polyamines in-patients with chronic renal failure. Kidney Int. Suppl. 16: S234 – S237.

Salvendran, R. R. (1983). The chemistry of plant cell walls. In Birch, G. G. and Parker, K. J. (eds.); dietary fibre; Publishers, Appl. Sicence London page 95 – 148.

Salyers, A. A. (1979). Energy sources of major intestinal fermentative anaerobes. Am. J. Clin. Nutr. 32: 158 – 163.

Salyers, A. A., Vercellotti, J. R., West, S. E. H. and **Wilkins, T. D. (1977)$_a$**. Fermentation of mucin and plant polysaccharides by strains *Bacteroides* from the human colon. App. Environ. Microbiol. 33: 319 – 322.

Salyers, A. A., Vercellotti, J. R., West, S. E. H. and **Wilkins, T. D. (1977)$_b$.** Fermentation of mucins and plant polysaccharides by an aerobic bacteria from the human colon. App. Environ. Microbiol. 34: 529 – 533.

Samejima, K. Kawase, M. Sakamoto, S., Okada, M. and **Endo,Y. (1976).** A sensitive flurometric method for the determination of aliphatic diamines in biological materials by high-speed liquid chromatography. Anal. Biochem. 76: 392 – 406.

Sandstead, H. H., Prasad, A. S., and **Schulert, A. R., (1967).** Human zinc deficiency, endocrine metabolism and treatment. Am. J. Clinc. Nutr. 20: 422 – 442.

Sarhan, S., Knodgen, B. and **Seiler, N. (1989).** The gastrointestinal tract as polyamine source for tumour growth. Anticancer Res. 9: 215 – 224.

Sayer, A. A., Kuritza, A. P. and **McCarthy, R. E. (1985).** Influence of dietary fibre on the intestinal environment proc. Soc. Exp. Biol. Med. 180: 415 – 421.

Scottish Data (1993). Cited in **Haslett, C., Chilvers, E. R. Hunter, J. A. A.** and **Boon, N.B. (1999).** Principles and practice of medicine.18th Edit. London Newyork, Page 429 – 439.

Searcy, R. L., Reardon, J. E. and **Foreman, J. A. (1967).** A new phtometric method for serum urea nitrogen determination. .20 Amer. J. Med.Technol. 33: 15 –

Seidel, E. R. and **Snyder, R. G. (1989).** Pentagastrin induction of spermine/spermidine N-acetyl transferase and mucosal polyamines. Am. J. Physiol. 256: G16 – G21.

Seiler, N. (1971). Identification and quantitation of amines by thin-layer chromatography. J. Chromat. 63: 97 – 112.

Seiler, N. and **Dezeure, F. (1990).** Polyamines transport in mammalian cells. Int. J. Bioch. 22: 211 – 218.

Seiler, N., Sarhan, S., **Grauffel, C., Jones, R., Knodgen, B.** and **Moulinoux, J. P. (1990).** Endogenous and exogenous polyamines in support of tumour growth. Cancer Res. 50: 5077 – 5083.

Seyfried, C. E. and **Morris, D. R. (1979).** Relationship between inhibition of polyamines biosynthesis and DNA replication in activated lymphocytes. Cancer Res. 39: 4861 – 4867.

Shurpalekar, K. S., Doraiswamy, I. R., Sundaravalli, O. E. and **Rao, M. N. (1971)**. Effect of inclusion of cellulose in an atherogenic diet on the blood lipids of children. Nature 232: 554 – 555.

Siddig, N. A. (1996). Nitrogen and specific rotation as qualifying indices for gum arabic derived from *Acacia senegal* (L) Willd. An M. Sc. Thesis, University of Khartoum.

Smith, A. N., Drummon, E. and **Eastwood, M. A. (1981).** The effect of coarse and fine Canadian red spring wheat and French soft wheat on colonic motility in patients with diverticular disease. Am. J. Clin. Nutr. 34: 2460 – 2463.

Smith, C. J. and **Bryant, M. P. (1979).** Introduction to metabolic activities of intestinal bacteria. Am. J. Clin. Nutr. 32: 149 – 157.

Smith, F. and **Montgomery, R. (1959).** The chemistry of plant gums and mucilages. Reinhold, Publishing Corp., New York. Cited in **Glicksman, M.** and **Sand, R. E. (1973).** In Whistler, R. L.

(Editor); Industrial gums: Polysaccharides and their derivatives. 2nd. Edit. Academic Press. New York San Francisco London.

Smith, T. A. (1970)a. Putrescine, spermidine and spermine in higher plants. Phytochemistry 9: 1479 – 1486.

Smith, T. A. (1970)b. The quantitative estimation of putrescine by gas chromatography. Anal. Biochem. 33: 10 – 15.

Sneath, P. H. A., Mair, N. S.,Sharpe, M. E. and **Holt, J. G. (1986).** Bergy's manual systematic bacteriology, vol.2 8th Edit. London, U. K.

Somogyi, M. (1937). A reagent for the copper-idometric determination of a very small amounts of sugars. J. Biol. Chem. 117: 771 – 778.

Southgate, D. A. T., Branch, M. J. and **Hill, B. S. (1976).** Metabolic responses to dietary supplements of bran. Metabolism 25: 1129 – 1135.

Southgate, D. A. T. and **Durnin, V. G. A. (1970).** Calorie conversion factors. An experimental reassessment of the factors used in the calculation of the energy value of human diets. Brit. J. Nutr. 24: 517 – 535.

Sprague, P. H. (1942). Can. Med. Ass. J. 47: 253.Cited in **Glicksman, M.** and **Sand, R. E . (1973).** In: Whistler, R. L. (Editor); Industrial gums: Polysaccharides and their derivatives. 2nd. Edit. Academic Press. New York, San Francisco, London.

Stahl, E. (1969). Thin layer chromatography. A laboratory handbook. 2nd. Edit. Toppan Company Ltd., Tokyo, Jaban. Page 889.

Stanley, M. M., Paul, D., Gacke, D. and **Murphy, J. (1973).** Effects of cholestyramine, metamucil, and cellulose on faecal bile salt excretion in man. Gastroentroerology 65: 889 – 894.

Stern, J. et al. **(1957).** Clin. Chim. Acta 2: 576.

Stephen, A. M. and **Cummings, J. H. (1980).** Mechanism of action of dietary fibre in the human colon. Nature 284: 283 – 284.

Sterne, M, and **Batty, I. (1975).** Pathogenic colisteridia. London and Botson Butterworth Co. Ltd.

Street, C. A. and **Anderson, D. M. W. (1983).** Refinement of structures previously proposed for gum arabic and other *Acacia* gum exudates. Talanta 30: 887 – 893.

Suliman, S. M. (1991). Glomerular changes associated with some tropical diseases in Sudan. An M. D. thesis, University of Khartoum - Sudan.

Suliman, S. M. Beliela, M. H. and **Hamza, H. (1995).** Dialysis and transplantation in Sudan. Saudi J. Kidney Dis. Transplant. 6: 312 – 314.

Swendseid, M. E. Wang, M., Schutz, I. and **Kopple, J. D. (1978).** Metabolism of urea cycle intermediates in chronic renal failure. Am. J. Clin. Nutr. 31: 1581 – 1586.

Tabacco, A. Meiattini, F. Moda. E. Tarli, P. (1979). Simplified enzymic colorimetric serum urea nitrogen determination. Clin. Chem. 25: 336 – 337.

Tabor, C. W. and **Tabor, H. (1976).** 1,4-diaminobutane (putrescine), spermidine and spermine. Ann. Rev. Biochem. 45: 285 – 306.

Tabor, C. W. and **Tabor, H. (1984).** Polyamines. Ann. Rev. Biochem. 53: 749 – 790.

Tabor, C. W. and **Tabor, H. (1985).** Polyamines in microorganisms. Microbiol. Rev. 49: 81 – 99.

Tabor, H. and **Tabor, C. W. (1961).** Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, spermine and related amines. Adv. Enzymol. 36: 203 – 268.

Tabor, H. and **Tabor, C. W. (1969).** Formation of 1,4-diaminobutane and of spermidine by ornithine auxotroph of *Escherichia coli* grown on limiting ornithine or arginine. J. of Biolog. Chem. 244: 2286 - 2292.

Tabor, H. and **Tabor, C. W. (1972).** Biosynthesis and metabolism of 1,4-diaminobutane, spermidine, spermine and related amines. Adv. Enzymol.36: 203 –268.

Tabor, H., Tabor, C. W. and **Rosenthal, S. M. (1961).** The biochemistry of polyamines**:** spermidine and spermine. Ann. Rev. Biochem. 30: 579 – 604.

Tabor, H., Rosenthal, S. M. and **Tabor C. W. (1958).** The biosynthesis of spermidine and spermine from putrescine and methionine. J. Biol. Chem. 233: 907 – 914.

Tagg, J. R. and **Bannister, L. V. (1978).** Fingerprinter B-haemoltic streptococi by their prodution of and sensitivity to bacteriocine-like inhibitors. J. Med. Microbiol. 12: 397 – 411.

Takahashi, M., Benno, Y. and **Mitsuoka, T. (1980).** Utilization of ammonia nitrogen by intestinal bacteria isolated from pigs. Appl. Envirom. Microbiol. 39: 30 – 35.

Titgemeyer, E. C., Bourquin, L, D., Fahey, G. C. and **Garleb, K. A. (1991).** Fermentation of various fibre sources by human faecal bacteria *in vitro*. Am. J. Clin. Nutr.53: 1418 –1424.

Topping, D. L., Illman, R. J. and **Trimble, R. P. (1985).** Volatile fatty acid concentrations in rats fed diet containing gum arabic and cellulose separately and as a mixture. Nutr. Rep. Int. 32: 809 – 814.

Trowell, H. C. (1972). Reveue. Eur. Etud. Clin. Biol. 17: 345. Cited in **Eastwood, M. A., Fisher, N., Greenwood, C. T.** and **Hutchinson, J. B. (1974).** Perspective on the bran hypothesis. The Lancet 1: 1029 – 1033.

Trowell, H. C., Southgate D. A. T., Wolever, T. M. S., Leeds, A. R., Gassull, M. A. and **Jenkins, D. A. (1976).** Dietary fibres redefined. Lanect 1: 967.

Truswell, A. S. and **Kay, R. M.** (**1975**). Absence of effect of bran on blood-lipids. Lancet 1: 922 – 923.

Tulung, B. Remesy, C. and **Demigne, C. (1987).** Specific effects of guar gum or gum arabic on adaptation of caecal digestion to higher fibre diets in the rats. J. Nutr. 117: 1556 – 1561.

Ulman, E. A. and **Fisher, H. (1983).** Arginine utilization of young rats fed diets with simple versus complex carbohydrates. J. Nutr. 113: 131 – 137.

United State Renal Data SystemAnnual Reports (1997). Cited in **Haslett, C., Chilvers, E. R. Hunter, J. A. A.** and **Boon, N.B. (1999).** Principles and practice of medicine.18th Edit. London New York. Page 429 – 439.

Veening, H. Pitt, W. W. and **Jones, G. (1974).** Ion-exchange chromatographic separation and fluorometric detection of urinary polyamines. J. of Chromat. 90: 129 – 139.

Veron, M. (1975). Nutition et taxonomie des *Enterobacteriaceae* et bacteries voisines. I.Methodes detudes desauxanogrammes. Ann. Microbiol. (Paris)126A: 257 – 274. Cited in Grimont and Grimont **(1978).** J. Clin. Microbiol. 8: 73 – 83.

Vince, A., Dawson, A. M., Park, N. and **O'Grady, F. (1973)**. Ammonia production by intestinal bacteria. Gut 14: 171 – 177.

Walker, A. R. P. (1975). Effect of high crude fibre intake on transit time and the absorption of nutrients in South Africa Negro School Children. Am. J. Clin. Nutr. 28:1161 – 1169.

Walser, M. and **Bodenlos, L. J. (1959).** Urea metabolism in man. J. Clin. Invest. 38: 1617 – 1626.

Walter, D. J., Eastwood, M. A., Brydon, W. G. and **Elton, R. A. (1986).** An experimental designe to study colonic fibre fermentation in the rat: The duration of feeding. Br. J. of Nutr. 55: 465 – 479.

Walters, R. L. Baird, I. M. Davies, P. S., Hill, M. J., Drasar, B. S., Southgate, D. A. T., Green, J. and **Morgan, B. (1975).** Effects of two types of dietary fibre on faecal steroid and lipid excretion. Brit. Med. J. 2: 536 – 538.

Wapnick, S., Wicks, A. C. B., Kanengoni, E. and **Jones, J. J. (1972).** Can diet be responsible for the initial lesion in diabetes? The Lancet 12: 300 – 302.

Weaver, R. H. and **Herbst, E. J. (1958).** Metabolism of diamines and polyamines in microorganisms. J. Biol. Chem. 231: 637 – 646.

Weber, F. L., J, and **Fresard, K. M. (1981)**. Comparative effects of lactulose and magnesium sulfate on urea metabolism and nitrogen excretion in cirrhotic subjects. Gastroenter. 80: 994 – 998.

Wickner, R. B., Tabor, C. W and **Tabor, H. (1970).** Purification of adenosylmethionine decarbxylase from *Escerichia coli.* W: evidence for covalently bound pyruvate. J. Biol. Chem. 245: 2132 – 2139.

Wiegand, L. and **Pegg, A. E. (1978).** Effects of inhibitors of S-adenosylmethionine decarboxylase and ornothine decarbolxyase on DNA synthesis in rat liver after partial hepatectomy. Biochim. Biophys. Acta 517: 169 – 180.

Wiliams, R. D. and **Olmsted, W. H. (1936)$_a$.** The effect of cellulose, hemicellulose and lignin on the weight of the stool: A contribution to the study of laxation in man. J. Nutr. 11: 433 – 449.

Williams, R. D. and **Olmsted, W. H. (1936)$_b$.** The manner in which food controls the bulk of the faeces. Ann. Internal. Med. 10: 717 – 727.

Windmueller, H. G. and **Spaeth, A. E. (1978).** Identification of ketone bodies and glutamine as the major respiratory fuels *in vivo* for post-absorptive rat small intestine. J. Biol. Chem. 253: 69 – 79.

Wolpert, E., Philips, S. F. and **Summerskill, W. H. (1970).** Ammonia production in the human colon: Effects of cleansing, neomycin and acetohydroxamic acid. New Engl. J. Med. 283: 159 – 164.

Woo, J. and **Henry, J. B. (1993).** Metabolic intermediates and inorganic ions. In Henry, J, B, **(1996).** Clinical diagnosis and management by laboratory method.19th Edit. page 162.

Wood, B. J. B. and **Allan, M. C. (1982).** Production of alcohol and conversion to vinegar.In source book of experiments for the teaching of microbiology. Publisher a subsidiary of Harcourt Brace Jovanovich, pages 272 – 281.

Wrong, O. (1978). Nitrogen metabolism in the gut. Am. J. Clin. Nutr. 31: 1587 – 1593.

Wyatt, G. M., Bayliss, C. E. and **Holcroft, J. D. (1986).** A change in human faecal flora in response to inclusion of gum arabic in the diet. Br. J. Nutr. 55: 261 – 266.

Wyman, J. B., Heaton, K. W. **Manning, A. P.** and **Wicks, A. C. B. (1976).** The effect on intestinal transit time and the faeces of raw and cooked bran in different doses. Am. J. Clin. Nutr. 29: 1474 – 1479.

Wyman, J. B., Heaton, K. W. Manning, A. P. and **Wicks, A. C. B. (1978).** Variability of colonic function in healthy subjects. Gut. 19: 146 – 150.

Yang, M. G., Manoharan, K. and **Young, A. K. (1969).** Influence and degradation of dietary cellulose in caecum of rats. J. Nutr. 97: 260 – 264.

Yoshizawa, M., Ishibashi, T., Kametaka, M. and **Kandatsu, M. (1973).** Utilization of diammonium citrate by adult rats. Agric. Biol. Chem. 37: 1057 – 1065.

Younes, H. K., Behr, S., Remesy, C. and **Demigne, C. (1995).** Fermentable fibres or oligosaccharides reduced urinary nitrogen excretion by increasing urea disposal in the rat caecum. J. Nutr. 125: 1010 –1016.