EFFECT OF RAW AND ROASTED BENGAL GRAM ON SOME PHYSIOLOGICAL PARAMETERS IN ALBINO RATS

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LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

I. INTRODUCTION

Coronary heart disease and the catastrophic complications arising as its consequence are major problems facing modern society. Unlike in the advanced western countries, precise data on mortality and morbidity caused by heart disease covering the entire country is not available. It is however on the increase in societies moving towards civilisation (WHO, 1974). Statistics from major hospitals reveal that coronary heart disease is becoming a modern epidemic (WHO, 1978).

Incidence of cardiovascular disease varies widely among persons from different geographical regions and of different habits and mode of life from the same region. While this has brightened hopes, unless more information on this relationship is available it cannot be fully met (Malhotra, 1970).

Coronary Heart Disease is known to be the foremost cause of death (about 40%) among medical emergencies in the Madras Government Hospital (Senthilnathan, 1979). There is a growing body of though which considers sudden death as an entity intimately connected with Coronary Heart Disease (Thompson, 1979). In fact Coronary Heart Disease results in more deaths and disability than cancer, diabetes and all infections and accidents combined (Heart News, 1977). What is most disturbing, is that rapid industrialisation, improved standards of living, effective control of infections

diseases and better health care, should result in increases in the proportion of chronic diseases like cardiovascular diseases limiting further technological and economic advances (Pisa, 1977).

Coronary Heart Disease debilitates and incapacitates people at the height of their professional careers. It deprives the country of intelligentia of seasoned and experienced leaders, which liability to a developing nation (WHO, 1974 and Thorogood, 1980).

Specialists from the world over agree that the basic cause of Coronary Heart Disease is atherosclerosis (Heart News, 1977 and 1978). Atherosclerosis results from a series of changes in the intima of arteries caused by total accumulation of fatty material and firbous tissue, disrupting its normal architecture (Kagan, 1977).

In the light of several experimental, epidemiological, and clinical investigations, it is generally accepted that the risk of developing Coronary Heart Disease in families and population groups is positively correlated with the level of serum cholesterol (Carnie, 1976; Padmavati, 1977; Stamler, 1978; Gopalakrishnan, 1979 and Marmot, 1979). This risk, independent of other risk factors is relatively small at levels less than 230 - 250 mg/100 ml but increases progressively with each increment in the plasma cholesterol above this level depending upon age (American Journal of Clinical Nutrition, 1973 and Heart News, 1977).

In the light of several experimental, epidemiological, and clinical investigations, it is generally accepted that the risk of developing Coronary Heart Disease in families and population groups is positively correlated with the level of serum cholesterol (Carnio, 1976; Padmavati, 1977; Stamler, 1978; Gopalakrishnan, 1979 and Marmot, 1979). This risk, independent of other risk factors is relatively small at levels less than 220 - 250 mg/100 ml but increases progressively with each increment in the plasma cholesterol above this level depending upon age (American Journal of Clinical Nutrition, 1973 and Heart News, 1977).

The factors contributing to atherosclerosis and Coronary Heart Disease are already present in adolescents and young people. Preventive measures directed towards proper nutrition and healthy life habits seem particularly important in this respect (WHO, 1974; Pisa, 1977 and Thorogood, 1980).

Serum cholesterol concentration can be successfully reduced in a great majority of people by dietary modifications alone (Heart News, 1976). Several studies have widely emphasized the effects of different components of the daily diet on the levels of cholesterol in the body. Role of nutritional factors such as type and concentration of dietary fat, type of carbohydrate and dietary fibre in altering plasma cholesterol and triglycerides have been widely published (Zeiser, 1973 and Krithevsky et al., 1975).

More recently dietary protein has been recognised for its ability to alter plasma cholesterol levels. Animal protein has been associated with high and vegetable proteins with low cholesterol attributed to amino acid composition (Carroll et al., 1975 and Yadav et al., 1977).

Pulses which are the major sources of protein in Indian dietaries, have been of interest because of their ability to modify cholesterol concentrations. Hypo-cholesterolemic effect of Bengal gram has been related to the presence of the flavanoid Biochain A (NIN, ICMR, 1978).

Mathur et al., (1960) reported the effect of bengal gram as its binding of bile acids inducing increased excretion of natural steroids and decreased endogenous production. Reddy (1963) and Malathi (1967) observed the same.

Soni et al., (1978) has observed the morphological similarity of Bengal gram with estrogen in histochemical studies of rabbit tissues.

Chithralekha (1980) studied the effect of red gram and horse gram on cholesterol level using albino rats and observed that horse gram had a better effect in lowering the cholesterol level. She concluded that this may be due to the fibre content. The limitation of the study was lack of time.

While a few studies have been conducted to evaluate the effect of pulses, the effect of processed legumes on

the physiological parameters has not been fully exploited. The main objective of the present investigation was to evaluate the effect of raw and roasted Bengalgram, one of the most commonly consumed legumes on some physiological parameters in albino rats. Specifically the cholesterol and triglyceride lowering effect of raw and roasted Bengalgram and the histopathological changes of heart, aorta and liver was investigated.

II. REVIEW OF LITERATURE

The literature pertaining to this study on the effect of raw and roasted Bengal gram on some physiological parameters in albino rats is discussed under the following headings:

A. Effect of non-nutritional risk factors in atherosclerosis

B. Role of dietary factors in increasing the cholesterol and triglyceride levels

C. Nutritional and related factors lowering cholesterol and triglyceride levels

D. Perspective of pulses in preventing hyper cholesterolemia.

A. Effect of Non-nutritional Factors Predisposing to Coronary Heart Disease:

Analysis of the epidemiological data accumulated as a result of extensive studies, has led to the formulation of certain "risk factors", some major and some minor, some reversible and some irreversible. Discussed here are some of the major non-nutritional risk factors.

Non-Nutritional risk factors in Atherosclerosis:

Hyper Cholesterolemia:

Cholesterol a precursor of steroid hormone and bile acid is an incriminated major factor in atherosmatous ..

arterial disease (Mc Intyre et al., 1973; Thompson, 1970; Cooper et al., 1980). Elevated level is a risk factor in cardiac heart disease (American Journal of Clinical Nutrition, 1973; Heart News, 1977). Studies in United States and elsewhere have characterised hypercholesterolemia as a risk factor in the development of coronary heart disease(Journal of American Dietetic Association, 1974) and myocardial injarction (Carlson et al., 1972). Elevated blood cholesterol levels are believed to result in more rapid and severe development of atherosclerosis (Nutrition Reviews, 1973).

Hypertriglyceridemia:

Elevation of serum triglyceride levels are independently related to atherosderotic vascular disease in the population as a whole (Keen and Jarrett, 1973). U.S. studies have recognised the increased serum triglyceride as a risk factor in coronary heart disease. Plasma triglycerides were more important risk factor than cholesterol(JADA, 1974). Increased serum triglyceride is associated with coronary heart disease (Deeds, 1973). Abnormal content of apo C-iii-2 is believed to have contributed to the patient's severe hypertriglyceridemia (Lennet, 1979).

Hypertension:

Director General, WHO has stated that hypertension is a silent, secret threat to the health of people around the world (Heart News, 1978 and Lakshmikanthan, 1978). Hypertension accounts for 15 - 20% of cardiac cases in India (Heart News, 1978).

Essential hypertension has been associated with high prevalence of raised aortic lesions and increased heart weight (WHO Chronicle, 1974). High blood pressure became an important risk factor after 50 years of age (Carlson and Bottiger, 1973; JADA, 1974). Long standing hypertension definitely predisposes the human subjects to heart diseases (Ramachandran et al., 1974; Khandekar and Shivde, 1976).

Diabetes Mellitus:

Diabetes is associated with increased classification of coronary artery and in combination with hypertension is associated with increase in raised coronary lesions (WHO Chronicle, 1974).

Obesity:

Obesity is related to accepted atherogenic triat including hypertipidemia (Geriatrics, 1979). Obesity is associated with reduced life expectancy and is largely due to excess cardiovascular mortality. Cardiovascular risk of obesity is largely attributed to atherogenic accompaniments (Simons, 1979). Concentration of serum cholesterol and

triglycerides were greater when androgenic feature were present and were correlated with adiposity (Allard and Goulet, 1968). In comparison of the obese with thin subjects the former were found to have more coronary atherosclerosis (WHO Chronicle, 1974). Obesity is a predominant factor predisposing to hyper cholesterolemia, hyper triglyceridemia diabetes mellitus, hypertension and therefore emerges as a important cause of atherosclerosis (Mann, 1974 and Ganapathy, 1979).

Physical activity:

Reduced physical activity has been recognized as a risk factor in coronary heart diseases. Lack of physical activity may be the major factor determining hypertriglyceridemia (JADA, 1974).

"Hardwork never hurts a healthy human heart" was the dictum preached by the late internationally known cardiologist Dr. Paul Dudley White. Exercise provides much more than a way of spening calories. It helps in active circulation and lower the cholesterol level. It stimulates the pacemaker and so fats are burnt rapidly. It provides an outlet for tensions (Heart News, 1975 and Havefeld et al., 1979). Hickey et al. (1975) are of opinion that exercise does not have a direct effect on coronary mortality and morbidity but that the effect is mediated by lower level of other risk factors.

Dr. Paul emphasised that vigorous usage of large muscle retard atherosclerosis by vigorous pumping of blood and keep the vien clear of clots.

Cigarette smoking:

1980 had been dedicated by WHO and International Society and Federation of Cardiology as "Antismoking" year. Slogan given by WHO is "Smoking or Health, the choice is yours". 25% of death from cardio vascular disease attributed to smoking (Heart News, 1980). Viswanathan (1979) points out that mortality ratio in heavy smokers is twice that of non-smokers.

Smoking enhances high blood pressure and serum cholesterol. Medical scientists are of the opinion that smoking initiates sympathetic discharge of certain chemical compounds which may be involved in pathogenesis of acute coronary heart disease events (Heart News, 1980).

Gopalakrishnan (1979) suggests that permeability of plasma lipid into the arterial wall may be increased. Chemically by carbon monoxide as a result of smoking.

Alcohol consumption:

Both cigarette smoking and alcohol consumption in regular and excessive amounts can cause myocardial damage (M.J.Mulky, 1979). Excessive alcohol consumption may have a direct toxic effect on myocardium resulting in alcohol cardiomyopathy. This has been linked with 3 major risk

factors of heart disease; lipeprotein level, blood pressure and smoking(Ronald E.Laporte et al., 1980). Kagan (1977) showed that alcohol consumption was associated with aortic and coronary clasification. Association of Cigarette Smoking with coronary lesion was due to associated alcohol consumption (WHO Chroniele, 1974).

Coffee drinking habit:

Little et al (1976) found a positive correlation between daily intake of coffee and the serum cholesterol level. It has been found that people drinking more than 5 cups of coffee per day run about twice as great risk of having atherosclerotic myocardial infarction as people drinking no coffee atall (Veasey, 1972). Caffeine rise the cholesterol level (1978).

Sex:

Women in child bearing age seem to be protected from cardiac heart disease, attributed to ovarian hormones (Grande, 1975 and Gerden et al., 1978).

Oral contraceptives:

Oestrogen containing oral contraceptives tend to raise plasma lipids and lipoprotein while combination type contraceptives rise plasma cholesterol and triglycerides (Young, 1976). Bradley et al. (1978) report that reduction of high density lipo protein level in women using oral contraceptives depends on type and dosage.

Compared to non users, users of oral contraceptives showed increased cholesterol, triglycerides, LDL cholesterol, VLDL Cholesterol but HDL cholesterol levels were similar. These were positively associated with the quantity of oestrogen component of the oral contraceptive preparation (Lancet, 1979).

Hardness of drinking water and trace elements:

Certain elements for example, chromium, copper, iron, manganese, silicon, vanadium and zinc exert beneficial effect on cardio vascular system while cadmium, cobalt and lead have detrimental effects (Masironi, 1973).

Allen and Klevay (1978) report that copper deprivation in rats induce hyper cholesterolemia. Taves (1978) opine that fluorine content reduced mortality due to CHD.

Clayton (1977) observed in England a high negative correlation between cardio vascular mortality and water calcium. Crawford et al (1977) have established well the association between soft water and cardiological disorders.

Other factors:

Sir William Oster (70 years ago) as quoted by Srikantia (1971) list stress and strain of modern living and rich diet as aetiology for atherosclerosis.

Peresdaht (1979) hypothesizes poverty in childhood and adolescence followed by later prosperity result in high cholesterol values. Prolonged stay in high altitudes has marked cholesterollowering effect (Srivatsava et al., 1977).

B. Role of Dietary Factors in Increasing the Cholesterol and Triglyceride Levels:

1. Type and concentration of fat:

Both quantity and quality of dietary fat have an important bearing on cholesterol levels (Grande, 1975). Population eating high saturated fat have higher incidence of hyperlipidemia and coronary heart disease than those who do not (Zeiser, 1973; Grande, 1973; Williams et al., 1976; Lorin et al., 1977 and Kritchevsky et al., 1977).

Bartov et al. (1973), Nakadate, (1975); Padmavati(1977); Honour et al. (1978); Sreekumar et al. (1978); Goldsmith et al (1978); Jacotot and Girardet (1979) reveal butter, lard, palmoil, soya bean oil, hydrogenated groundnut oil, cocoonut oil and mustard oil are highly atherogenic. Padmavati (1977) reported that diets that include a combination of fats from animal origin such as dietary products (butter, cream, cheese etc.) have cholesterol raising effect.

Jacotat et al (1979) reveal that cholesterol esters increase in the order butter, groundnut oil, palm oil, sunflower oil, rape seed oil, soyabean oil and hydrogenated soyabean oil in its rats fed for one year.

Saturated fat diet results in the formation of oleic and palmitic acids that ultimately give rise to esters of fatty acid with cholesterol which are highly atherogenic (Gopalakrishnan, 1979).

Lauric, myristic and palmitic are more atherogenic (Vergroesen, 1972 and Gopalakrishnan, 1979). Diet with butter replaced by margerine results in significant fall of cholesterol in adolescents (Vergroesen, 1972).

Diet predominant in cholesterol rich foods raise serum cholesterol (Turner, 1978).

Type of carbohydrate:

Major source of carbohydrate are cereals, pulses, sugar, roots and tubers (Grande, 1975).

Yudkin et al (1964), (1967) and Arhens (1974) reveal that sucrose is an important factor in the development of CHD. Influence of carbohydrate on serum lipid levels is exerted largely on triglyceride fraction (NIN, 1978).

Kritchevsky et al (1978) proved that cholesterol free purified synthetic diets containing cocconut oil (hydrogenated) with either glucose, fructose, sucrose or starch to be hypercholesteroleuric and hyper triglyceridemic. He also reported that serum cholesterol nearly doubled on diets containing fructose or sucrose and increased by about 25% on a diet containing glucose.

Westmann et al (1978) have conveyed that long term feeding of lactose containing diet to rats is a contributory

factor in the accumulation of cholesterol in serum and liver. Truswell (1978) says pure sugar or wheat and potato raise plasma lipid in man compared with carbohydrate from fruits, vegetables and legumes.

Dietary protein:

Animal protein has been associated with high and vegetable protein with low cholesterol level, this differential effect is thought to be related to amino acid composition (Carroll et al., 1975 and 1976; Yadava et al., 1971). Solomon (1978) reiterate that diet with L-Histidine or orocanic acid induce hhypercholesterolemia.

Leelamma et al (1978) reported that in rats fed different sources of protein, the highest concentration of sterol in faeces was in rats fed soyabean and lowest in those on beef with normal diet.

Hakadate (1975) opine that high casein diet (31%) give serum cholesterol value half that of 11% diet.

Soni et al (1978) state that pulses such as peas and lentil raise cholesterol level in albino rats.

Hermus (1972) showed that a mixture consisting of 6 parts of casein, four parts of gelatin, 6 parts fish protein concentrate and 4 parts soyabean protein gave a lower level of plasma cholesterol than casein, when fed in cholesterol free purified diet.

Soyabean protein at 40% level caused a reduction in serum cholesterol than those fed on diets containing 40% of animal protein (ICMR, 1963). Narinderrnath (1963) state that inclusion of 5% vegetable protein (wheat gluten) in basal diet having casein caused a marked reduction in serum cholesterol.

Vitamins:

Mccully (1979) and Gruberg et al. (1979) implicate that pyridoxine deficiency leads to atherosclerosis. Wilson et al (1978) reported that diets deficient in vitamin E may predispose to atherosclerosis and hypercholesterolemia. Edman et al (1974) have theorised diet induced hyper cholesterolemia in rats on vitamin A free diet, however response is affected by salt mixture used.

Deficiency of coenzyme Q diminish heart's pumping ability due to deficiency of bioenergetics (Science Digest, 1979).

Minerals:

Masironi (1973) says that cadmium, cobalt and lead have a detrimental effect. Allen et al (1978) and Science Digest (1979) attribute that a deficiency of copper intensifies coronary heart disease. Purlaoaty and Altura (1980) found lower level of magnesium increased arterytens leading to vaso constriction and death.

Klevay (1974) and (1976) hypothesized and imbalance in Zn/Cu contributes to the risk of CHD. Lewandowicz et al. (1978) inform that serum zinc levels lower in patients with myocardial infarction.

C. Nutritional and Related Factor Lowering Cholesterol and Triglyceride Levels:

Fats

Poly unsaturated fat decreases cholesterol(Vergroesen, 1977; Durrington et al (1978). Vegetable oils like groundnut oil, gingelly oil, sunflower oil are rich sources of poly unsaturated fatty acids (O'Brien et al., 1977; Sreekumar, 1978; Senthilnathan, 1979 and Jacotot et al., 1979). Rao et al (1977) report sardine oil and safflower oil decrease serum cholesterol within 33-44 days of feeding in male rats. Van Lossomzy et al (1978) report increase in high density lipoprotein cholesterol and decrease in sery triglycerides with a muskeral diet supplemented with linoleic acid.

Premakumari and Sundaravalli (1976) point out serum cholesterol lowers more with safflower oil than with groundnut oil.

Jacotot (1979) shows that rats given groundnut oil and safflower oil had significantly low cholesterol levels than rats given palm oil or hydrogenerated soynbean oil. Serum triglycerides showed a similar trend.

Keys (1970) and Turner (1978) suggest that population consuming diets low in saturated fat have lower incidence of coronary heart disease. Simons et al. (1978) reinstate that plasma cholesterol level were lower in vegetarian than non-vegetarian foods.

Carbohydrates

Mac Donald (1973) states that cream, glucose and starch diet resulted in increase in triglyceride level. Fructose or starch resulted in increase in cholesterol than glucose diet.

Berget et al (1975) and Caster et al (1978) reveal fructose lowers plasma cholesterol.

Normal subjects and patients with minor glucose intolerance given diet with 80% carbohydrate showed a great increase in cholesterol and triglyceride of plasma liporprotein fraction concentration relative to the protein content. Garri, a flour form cassava, a staple food in Nigeria decreases plasma cholesterol concentration (Ononogbu et al., 1978).

Erdman (1979) reveals that 2% gelled agar lowers the liver cholesterol in albino rat fed 1% cholesterol diet.

The insoluble pectinaceous fibres interact with metals and lipids. The hydrogen ion form and neutralised forms of pectinaceous fibers containing specific cations were investigated in conjunction with acidic and neutral lipids (Abstract of Papers, Minnesota, U.S.A., 1978).

Proteins:

Carroll and Carroll et al. (1978) state replacement of animal protein with plant protein decreases the plasma cholesterol.

Von Lossonezy et al (1978) reported that a fish diet to human volunteers reduced plasma cholesterol and triglycerides.

Turner (1978) suggests leanmeat, fish, poultry are beneficial in checking lipid levels in the bood.

Nair et al (1971) and Mann (1977) reveal Yogurt and milk have cholesterol lowering properties.

Kritchevsky et al (1979) reported that rabbits on a casein containing purified diet converted cholesterol to bile acids at a slower rate than rabbits on a chow diet.

Hamilton and Carroll (1978) showed in general that proteins of vegetable origin were less hypercholesterolemic than those of animal origin.

Soni et al (1978) says that Bengal gram has been reported to have hypocholesterolemic action, the other common Indian pulses have been screened for similar effects.

Mathur et al (1969) restates the same and justifies this effect to be due to increased excretion of cholesterol as bile acids and neutral sterols and decreased synthesis in liver.

Hypocholesterolaemic effect of Bengal gram which is well justified may be mediated throughout the biochanin present in the legume (ICMR, 1977).

Algal protein decreases cholesterol level has been found by CFTRI (Central Food Technological Research Institute) workers (Hindu, 1979). Dein et al., (1979) arrived at the same.

Vitamins:

Shaffer (1970) cites certain facts and hypotheses that ascorbic acid deficiency is a contributing factor in the development of myocardial, aortic and cerebral atherosclerosis.

Chauhan and Sarkar (1978) states that neutral and phospholipid fractions were increased in plasma and liver of cholesterol fed scorbutic animals as compared to pair-fed controls.

Ivanow (1975) states that lipoic acid increases the uptake of oxygen and decreases total cholesterol.

Vitamin A retards the accumulation of cholesterol (Erdman, 1974). Activated 7-dehydrocholesterol decreases serum cholesterol concentration (Journal of Food Science, 1978).

Wilson et al., (1978) reported that diets deficient in vitamin E may predispose to atherosclerosis and hypercholesterolemia.

Charman et al. (1973) maintain that large doses of nicotinic acid reduce both free and esterified cholesterol levels. Some has been reported by ICMR (1962). Oster (1977) substantiated the theory that damage to the artery wall by xanthine oxidase could be treated by folic acid.

Fibre:

Kichar et al. (1976) made an observation on the effect of high fibre diet and found by increasing fibre (3%) in the diet the glucose level came down by 23% cholesterol by 25% and triglycerides by 15%.

Moore (1976) showed that a diet containing 20% butter fat and 19% wheat straw was much less hypercholesterolemic than one containing butter fat and either cellulose or cellophane.

Malinow et al. (1979) showed that animals fed on commerical chow and cholesterol with alfalfa had their veins clear as against those given a diet without alfalfa showing characteristic athromatous changes.

Cellulose and bran are capable of binding bile acid cholesterol or fat and might be the cause of increased steroid excretion (Van Berkeyn et al., 1979).

Onion, Garlic:

Saimani et al. (1979) found that individuals who totally abstained from onion and garlic had significantly

higher levels of serum cholesterol, triglycerides, B-lipoproteins and phospholipids.

Onion:

Gupta et al. (1966), Mittal et al. (1974), Basarkar et al (1975), Bordia et al (1977) report that onion as such and essential oil of onion reduce cholesterol levels significantly.

Garlic:

Active principle sulphide of allyl possesses marked cholesterollowering properties (Bordia et al., 1977; Augusti, 1977; Jain et al., 1978 and Sainani et al., 1979).

Spinach stimulate intestinal microflora to form more coprostanol from cholesterol (Iritani et al., 1978).

Ginger:

Gujral et al. (1978) states that ginger reduce serum and hepatic cholesterol by increasing cholesterol excretion in faeces.

Turmeric:

Curcumin from turmeric decrease the cholesterol level (Basarkar et al., 1975).

Saponins:

David Unkenfull (1978) revealed that saponins induce association between fibre and bile salts, particularly with bile salts and with sterols in general.

Probucol is a lipophilic bis-phenol that lowers serum cholesterol in several species. Compound SQ 10,501 lowered plasma cholesterol in rats fed normal or hyperlipidemic diets by blocking the biosynthesis of cholesterol.

Oral neomycin decrease the serum cholesterol by increasing fecal bile acid and sterol excretion (Lerner et al., 1978).

D. Perspective of Pulses in Preventing Hyper Cholesterolemia

Bengal gram (Cicer arietinium) has been shown to have a hypocholesteremic effect in several species of animals including (Mathur et al., 1963). It has been suggested that this may be related to the presence of biochanin A (5, 7-dihydroxy 4'-methoxy isoflavone and formonovetin (7-hydroxy 4'-methoxy isoflavone) both of which are known to have estrogenic properties (Bradbury and White, 1951, NIN, ICMR, 1978).

Hypocholesteremic effect of Bengal gram is due to increased excretion of cholesterol as bile acids and neutral sterols and decreased synthesis in liver (Mathur et al., 1969).

Polish and Hungarian workers have reported that flavanoids reduce the concentration of lipids (including cholesterol) in blood and tissues (Szabo et al., 1970) Kadykow et al., 1978).

Hypocholesteremic effect of bengal gram which is well justified may be rediated through the biochanin present in the legume (ICMR, 1977).

Reddy (1963) while evaluating the effect of egg yolk consumption by rabbits on serum cholesterol observed lowering effect of bengal gram on serum cholesterol(Malathi et al., Reddy (1967) observed the same.

Madhavan (1971) observed the morphological similarity of bengal gram with oestrogen in histochemical studies of rabbit tissues.

Soni et al (1978) reported that bengal gram to have hypocholesteremic action and other common Indian pulses have been screened for similar effects.

III. EXPERIMENTAL PROCEDURE

The main objective of this investigation was to analyse and evaluate the cholesterol and triglyceride altering properties and the histological changes of heart, aorta and liver tissues in albino rats fed raw and roasted Bengal gram.

Accordingly the procedure adopted in this investigation covered the following aspects:

A. Selection and processing of legumes

B. Formulation of diets

C. Selection and grouping of the animals

D. Feeding the groups and evaluating the effects of processed Bengal gram

A. Selection and Processing of Legumes

An average Indian diet which is predominant in cereals, pulses form the major source of protein (Panikar, 1974 and Devadas, 1979). Most pulses contain about 20 - 25 per cent of protein. Subbulakshmi et al (1976) reveal that green gram, chikpea, lentils, cowpea, beans, peas etc. are widely used in diets. In northern as well as southern regions of India, Bengal gram and roasted Bengal gram are very commonly consumed. Proteins from vegetable sources have earned much publicity because of their ability to alter cholesterol levels (Carroll et al., 1975) and Yadav et al., 1977).

Studies with Bengal gram (Mathur et al., 1964; Madhavan, 1971; Mathur, 1971 and Reddy, 1979) green gram, black gram, peas and lentils. Soni et al., (1973) have been done to reveal that all these lower cholesterol levels.

This study was aimed at experimenting with the raw and roasted Bengal gram (cicer arietenum). Practically all legumes are consumed only after they have been subjected to some form of processing or other such as heating, boiling, roasting, soaking, sprouting, dehydration and autoclaving (Babu, 1976 and Chandrasekhar, 1978). All these methods are known to improve palatability, alter digestibility, decrease a nutritional factors, reduce gas producing agents and also covert some vital constituents of the pulses into simpler compounds which are ultimately beneficial nutritionally (Liener, 1962; Ray, 1969; 1970; Jaya et al., 1975; Babu, 1976 and Chandrasekhar, 1978).

In this study the selected pulse namely Bengal gram was soaked in water for half an hour to remove its husk and dried, then ground into flour. Roasted Bengal gram was made by soaking the gram for half an hour in water and then roasting it to a temperature of 110°C and cooling and grinding it in the flour. The quantity of flour required for the whole study was prepared in one lot.

B. Formulation of Diets:

The processed pulses were first analysed for nitrogen

content by macrokejldahl method (NIN, 1971), in triplicates and the amount of protein (N_2 x 6.25) was taken as the basis for formulating diets.

Totally three diets were formulated for the whole experimental period. These were, an atherogenic diet, a raw Bengal gram diet and roasted Bengal gram diet.

Atherogenic diet provided skim milk protein at 18 per cent level. The experimental diets however provided skim milk protein at 6 per cent levels, the rest of the 10 per cent being derived from Bengal gram. Thus the pulse diets or the experimental diets also provided protein at 16 per cent levels.

In the atherogenic diet the source of fat was coconut oil at a level of 30 per cent (Sreekumar and Kamp, 1978). In the other diets namely raw and roasted bengal gram diets, ground nut oil at nine per cent level was used as a source of fat as an average Indian diet contains about 10 g of vegetable oil (Achaya, 1978). The reason for using ground nut oil as a source of fat in the second phase of the study was to stabilize cholesterol levels as far as possible (Landes et al., 1974).

The vitamin and mineral mixture were supplied at two per cent and four per cent levels respectively to supply adequate quantities of these nutrients. The rest of the weight was made up with corn starch.

All the diets were prepared at the start of the experiment and stored in the deep freeze.

The detailed composition of these diets is presented in Table I and the formulation of mixture is given in Appendix I.

TABLE I

PERCENTAGE COMPOSITION OF THE ATHEROGENIC AND EXPERIMENTAL DIETS

	Atherogenic	Experimental	
		Raw Bengal gram	Roasted Bengal gram
Skim milk powder	55	24.39	24.39
Vitamin	2	2	2
Mineral	4	4	4
Starch commercial	19	10.61	16.17
Fat	20 (Coconut oil)	9	9
Raw Bengal gram flour	—	50	—
Roasted Bengal gram flour	—	—	44.44
Total	100	100	100

C. Selection and Grouping of Animals:

Twelve male albino rats were selected from the laboratory stock colony and grouped into three groups.

A number of scientists investigating the effects of different foods on the lipid profiles seem to have selected animals of different ranges of weight for their studies for example Mathur et al., (1978) used rats weighing between 150 - 200, Yadav et al., (1977), between 100 - 120 g, Sreekumar and Kurup (1978) used rats weighing 80 g, Martin et al., (1979) albino rats between 100 - 150 g, and Anusuya Devi et al. (1979) albino rats weighing 200 - 220 g.

In this study rats weighing 140 - 170 g were selected. The study was conducted in two phases. In the first phase 12 male albino rats were weighed and housed in individual cages. All the animals were placed on an atherogenic regimen for a period of 60 days. Food and water were provided adlibitum. At the end of this period one of the groups was sacrified and the liver, heart and aorta were removed and preserved for the histopathological study.

In the second phase of the study, the other two groups were fed experimental diets. Group II was provided the raw Bengal gram diet and Group III roasted Bengal gram diet. The diet was fed adlibitum for 45 days and animals were sacrificed at the end of the phase, organs, heart, aorta, liver were removed and preserved for histopathological study.

D. Conducting the Feeding Trial and Evaluating the Effect of Processed Bengal Gram:

Criteria for evaluation:

1. Food intake

2. Weight gain

3. Cholesterol levels

4. Triglyceride levels

5. Histopathological study of selected tissues.

1. Food intake:

The animals were given food and water adlibitum every day according to the dietary schedule presented in Table II.

TABLE II

DETAILS OF DIETS PROVIDED TO DIFFERENT GROUPS

Group No.	No. of rats	Ist Phase (60 days)	II Phase(45 days)
I	4	Atherogenic diet	—
II	4	Atherogenic diet	Raw Bengal gram diet
III	4	Atherogenic diet	Roasted Bengal gram diet

Food for a particular group of rats were weighed out the separately mixed with sufficient water and pressure cooked to a semisolid consistency. Weighing in individual cups provided more accuracy than weighing in bulk and pouring it into individual cups.

The food intake of the animals was assessed by recording the amount of food consumed every day. The left over food was cleaned off hair and excreta, placed in separate numbered watch glasses and heated in the oven overnight w till completely dry. Spilt food for individual rats was also

collected and pooled in with the left over diet. Food intake was calculated by subtracting the left over food from the quantity of food given to each animal.

2. Weight gain:

A record of the weight gain in all the rats was assessed by weighing the animals weekly. This was done to observe the relative growth promoting abilities of each of the diets.

3. Cholesterol levels:

The cholesterol altering properties of pulses is well known. Studies have proved that Bengal gram (Mathur et al., 1964; Mathur, 1971, Madhavan, 1971 and Reddy et al., 1979) lowers cholesterol levels significantly in all mammals including man.

Cholesterol levels were estimated before starting the experiment and then twice every month till the end of experimental period.

4. Triglyceride levels:

Since triglyceridemia is also a risk factor in atherosclerosis, attempt was made in measuring serum triglyceride levels. At random one rat was selected before the experiment and blood was estimated for the normal level. The procedure is given in Appendix II. Then at the end of atherogenic period, and of the experimental period on two

diets again blood was collected from the rats and serum triglyceride was estimated.

Blood was collected in the live animals by severing one and a half centimeters of the tail from the tip with a sharp blade. The cut end was then lowered into a clean test tube. A piece of cotton wool dipped in xylene was rubbed over the whole length of the tail, without touching the wound, to facilitate in an easy flow of blood. Xylene was used to dilate the major tail vein. About 4 ml of blood was collected by this method from each animal. After collection of blood the wound was plugged with a piece of cotton dipped in tincture iodine. Estimations for the cholesterol and triglycerides were done within three days of collecting blood, in triplicates.

Final estimation was done for the blood drawn by direct cardiac puncture after anaesthetizing the animal.

5. Histopathological Study of Selected Tissues:

After sacrificing theanimals, the heart along with the main blood vessels and aorta was removed cleaned off blood by blotting it on cotton and preserved in 10 per cent formalin. Whole liver was also removed and preserved similarly.

Studies were prepared by fixing, dehydrating, impregnating in wax, embedding, cutting and staining the tissues with haematoxylineosin. These were viewed under microscope. The procedure is given in Appendix III.

IV. RESULTS AND DISCUSSION

The results obtained on this study pertaining to the effect of raw and roasted Bengalgram on some physiological parameters in albino rats is discussed under the following headings:

A. Food intake
B. Weight gain
C. Cholesterol levels
D. Triglyceride levels
E. Histopathological changes of the selected tissues.

A. Food Intake:

The twelve male albino rats selected were divided into three groups according to their weights and designated as groups I, II and III.

In the first phase all the twelve rats were fed an atherogenic dietary regimen and were observed regularly for changes in blood lipid levels and external symptoms of essential fatty acid deficiency.

Around the 42nd day the rats started developing essential fatty acid deficiency symptoms like loss of hair on the head and trunk, scaling of the skin in the tail region were observed. The rats became lethargic, food intake was diminished and anorexia observed in all the

animals. By the 54th day, three rats developed severe fatty acid deficiency and urine was spotted with blood in these animals. Even the intake of water started decreasing. Hence it was decided to end the first phase of the study namely feeding of the atherogenic diet after 60 days. Similar observation of development of essential fatty acid deficiency with atherogenic diet in 60 days has been observed by Burr and Burr (1926). Figure 1 shows the loss of hair and scaling of skin in the deficient rat.

The mean food intake of the rats on atherogenic and experimental diets is given in Table III. Weekly food intake of the individual rats are appended in Appendix IV.

Figure 1

NORMAL AND ESSENTIAL FATTY ACID DEFICIENT RAT

TABLE III

MEAN FOOD INTAKE OF THE RATS ON ATHEROGENIC AND EXPERIMENTAL DIETS

Group	Phase		Food intake in grams					Average
			Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	
I	First	W*	73.55	70.48	77.90	68.14	—	72.44
		D*	10.50	11.21	11.09	9.53	—	10.63
II	First	W	74.70	75.73	82.69	67.19	68.14	73.69
		D	10.67	10.82	11.81	9.60	9.73	10.53
	Second	W	80.05	87.95	104.80	82.50	88.20	88.7
		D	11.44	12.56	14.97	11.79	12.6	12.67
III	First	W	70.56	74.33	64.34	69.46	—	69.62
		D	10.08	10.60	9.18	9.92	—	9.95
	Second	W	77.18	77.33	83.48	93.37	—	82.84
		D	11.03	11.05	11.093	13.34	—	11.84

W* – Average food intake per week

D* – Average food intake per day

It was noted that the food consumption on an average during the first phase was around ten grams. The average intake in Group I was 10.63 g., in group II 10.83 and in group III 9.95 g. It was also recorded that the intake in most of the rats showed a sudden fall, then a gradual increase and again a decrease at the end of the experimental phase I. This could probably have been the cause of development of essential fatty acid deficiency due to a high concentration of coconut oil in the diet. This is in line with the results obtained by Burr and Burr (1929) who stated that, if hydrogenated coconut oil is added to the diet consumptous scaliness of the skin develops markedly over the tail scomor and growth ceases earlier.

Since the first phase namely the atherogenic diet phase ended after eight weeks when considerable increase in cholesterol and triglyceride levels were evidenced by all the rats and when essential fatty acid symptoms were observed, three rats were sacrificed from group I in order to study the histopathological changes due to atherogenic diet. The remaining rat of group I(Rat No.4) (Table III) was grouped in Group II and fed raw Bengal gram diet during the second phase of the experiment. The second phase of the experiment lasted for 45 days.

In the second phase of study group II was fed with raw Bengal gram diet and group III with roasted Bengal gram

diet. During this period the food intake increased in all the animals to a maximum and the average intake was higher than the atherogenic phase. A higher mean food intake was recorded by rats on raw Bengal gram diet (12.67 g.) than the rats on roasted Bengal gram diet (11.84 g). On an average group II showed an increased food intake of 15.1 g. per week and group III 13.2 g. per week from the atherogenic phase. Figure 2 represents the food intake of the rats during the two phases.

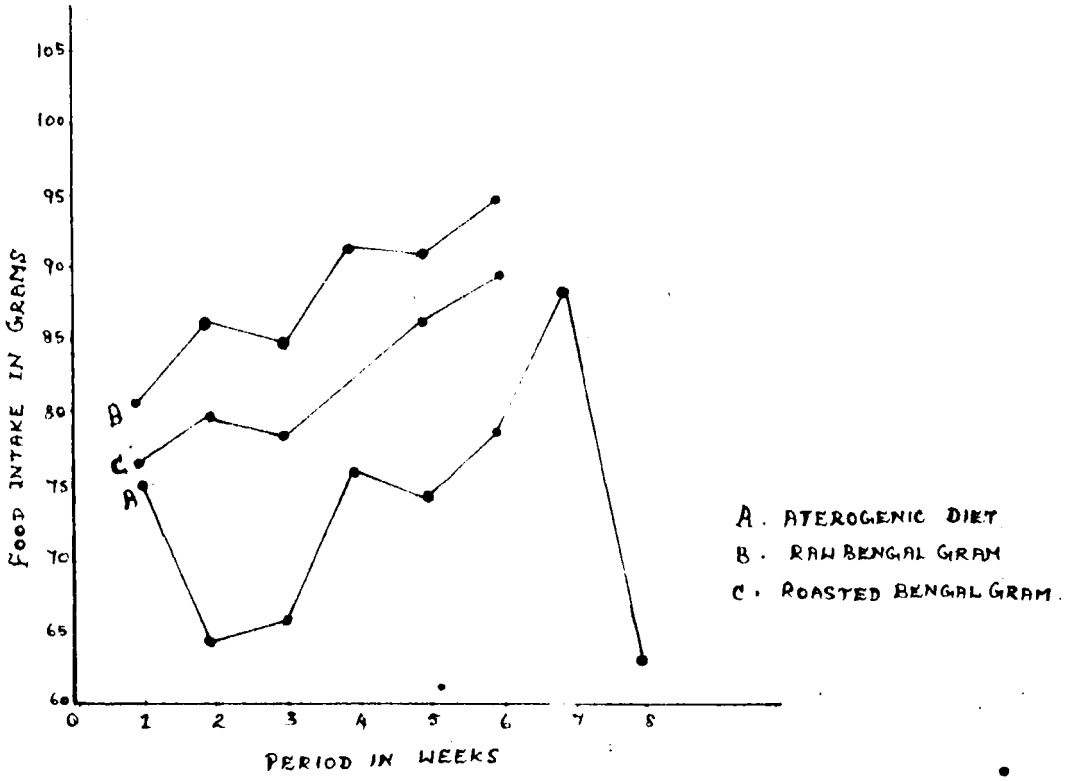
B. Weight Gain:

Table IV depicts the total weight gains of the rats in different groups and in the two phases of the study. Weekly gain in weight of individual rats is given in Appendix V.

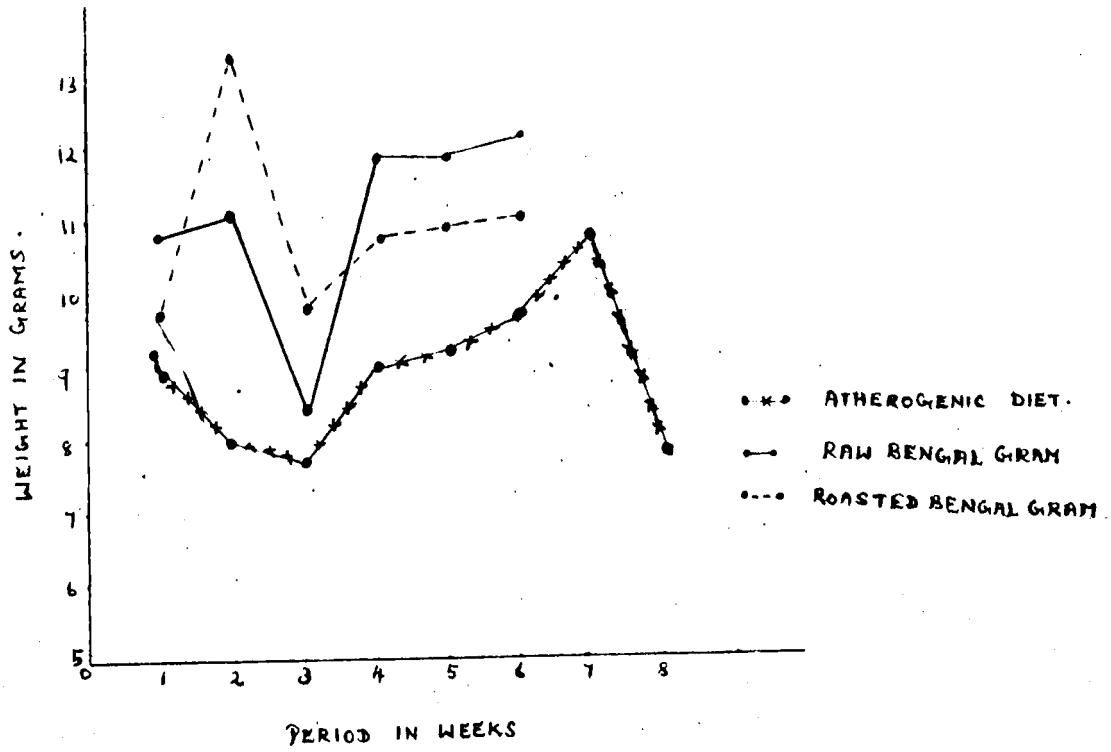
TABLE IV

TOTAL WEIGHT GAIN(GRAM) OF THE RATS IN THE TWO PHASES OF THE EXPERIMENTANT

Rat	Group I (First phase alone)	Group II First	Group II Second	Group III First	Group III Second Phase
1	69.56	70.41	60.29	54.63	54.78
2	70.4	58.34	57.79	35.54	35.63
3	67.5	77.13	77.16	57.77	61.98
4	63.86	63.7	57.1	72.18	72.3
5	—	63.86	62.11	—	—
Average gain/ week	8.479	8.336	10.48	6.879	9.36



PATTERN OF FOOD INTAKE AND WEIGHT GAIN



In the first phase of the study during which the animals were maintained on an atherogenic diet the total weight gain over the eight week period ranged from 54 to 78 g. The animals in groups I, II and III registered an average weight gain of 8.479 g., 8.336 g. and 6.879 g per week respectively during this phase. It was found that group I registered the maximum gain in body weight.

It was found that as the food intake increased upto seventh week the body weight also increased showing a positive correlation ($r = +0.7217$) and then started decreasing probably due to essential fatty acid deficiency.

In second phase of the study the animals registered considerable increases in body weights, especially the group on raw Bengal gram diet. In both the groups the weight gain was marked compared to the phase I. The average weight gain for the group II and group III in the second phase of the experiment was 10.46 g. and 9.38 g. per week respectively. Figure 2 represents the pattern of weight gain in the two phases.

It was interesting to note that soon after the shift over ofrom the first phase, the food intake of the animals in both the experimental diets increased as also the weight gain indicating a highly positive correlation ($r = +0.840$ for raw Bengal gram diet and $r = +0.710$ for roasted Bengal gram diet) and hence the value of pulses as potential sources of growth promoting factors.

C. Cholesterol Levels:

The cholesterol levels in all the three groups of rats were measured at the start of the experiment and then once a month during phase I and then onwards every fortnight in phase II. The mean initial blood cholesterol level was found to be 126 mg/100 ml. The mean cholesterol levels of the rats are presented in Table V. The details of the cholesterol values for individual rat is appended in Appendix VI.

TABLE V

MEAN CHOLESTEROL LEVELS IN mg/100 ml OF BLOOD IN THE TWO PHASES*

Group	Atherogenic		Experimental		
	30 days	60 days	15 days	30 days	45 days
I	367.5	488.25	—	—	—
II	210.65	377.50	398.75	166.00	154.00
III	272.75	407.00	312.50	163.75	132.50

*Mean initial level was 126 mg/100 ml.

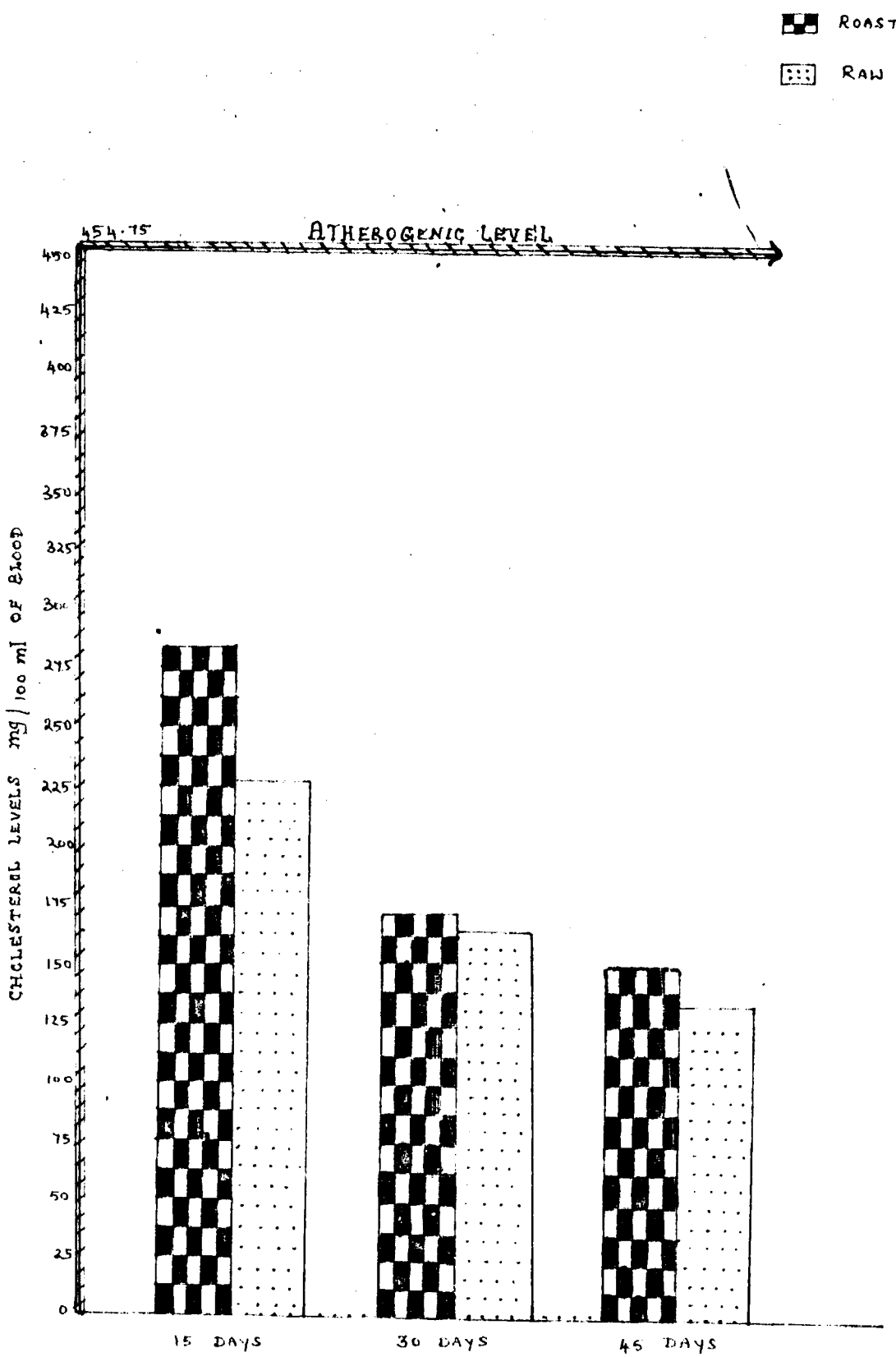
When serum cholesterol levels in the individual were considered (Appendix VI) during the atherogenic diet phase in all the groups it ranged from 170 mg to 310 mg/100 ml. of blood in the first month and 300 mg/100 ml to 628 mg/100 ml. of blood during the second month end. On an average during the first 30 days, the serum cholesterol levels in groups I,

II and III were 367.5 mg/100 ml, 310.65 mg/100 ml, 373.75 mg/100 ml of blood respectively. At the end of the phase I (60 days) it further increased to 498.25 mg/100 ml, 377.5 mg/100 ml. and 497 mg/100 ml. respectively for the three groups.

In the second phase of the study rats in groups II and III were placed on raw and roasted Bengal gram diet respectively. The diets were continued for 45 days. After fifteen days the cholesterol levels had fallen from the previous levels.

The average cholesterol levels in the animals of group II had fallen from 377 mg/100 ml to 298.75 mg/100 ml and for group III from 497.5 mg/100 ml to 212 mg/100 ml of blood. After thirty days the average cholesterol levels had decreased further to 166 mg/100 ml and 163.75 mg/100 ml for groups II and III respectively. The cholesterol levels further came down to 154 mg/100 ml and 132.5 mg/100 ml of blood at the end of forty five days. These details are depicted in Figure 13.

The statistical comparison of cholesterol levels between the atherogenic phase and experimental phase in groups II and III were done using paired 't' testing. Comparisons in the cholesterol values between the raw and roasted Bengal gram and between 15th, 30th and 45 days in phase II were also made using 't' testing. The data is presented in Table VI.



HYPO CHOLESTEREMIC EFFECT OF RAW AND ROASTED BENGAL GRAM.

TABLE VI

STATISTICAL COMPARISON OF CHOLESTEROL LEVELS BETWEEN PHASES AND NUMBER OF DAYS

Source of comparison	't' values
Between the experimental groups	
Roasted Bengal gram diet (15 days) Vs Raw Bengal gram diet (15 days)	4.6498**
Roasted Bengal gram diet (30 days) Vs Raw Bengal gram diet (30 days)	0.2457
Roasted Bengal gram diet (45 days) Vs Raw Bengal gram diet (45 days)	1.7874
Between the days	
Raw Bengal gram diet	
15 days Vs 30 days	0.6707
15 days Vs 60 days	10.2606**
30 days Vs 45 days	1.925
Roasted Bengal gram diet	
15 days Vs 30 days	0.2342
15 days Vs 45 days	6.8698**
30 days Vs 45 days	3.0478
Paired 't' test	
Atherogenic diet Vs Raw Bengal gram diet	0.965
Atherogenic diet Vs Roasted Bengal gram diet	2.391

**Significant at one per cent level.

The observation in the present investigation further brings out the fact that roasted Bengal gram has much more beneficial effect than the raw sample and hence the value of simple processing technique used for homescale processing of commonly consumed legumes in lowering the cholesterol levels.

It is evident that there is a highly significant ($P < 0.01$) difference for both roasted and raw Bengal gram in the cholesterol levels when compared to the atherogenic phase of the same animals.

When the raw and roasted samples were compared for the cholesterol lowering effect it is evident that there is highly significant difference between the groups in 15 days period ($P < 0.01$). However by the 30th day and 45th day, the statistical analysis between the experimental groups was not much significant though the animals on roasted Bengal gram diet showed reduced levels of cholesterol than the animals on raw Bengal gram diet.

Though there was significant difference in the cholesterol levels between the atherogenic and the experimental groups, statistically it was not significant.

When days were compared for both raw and roasted samples, as the periodicity increased there was significant difference in blood cholesterol levels. This again implies that daily consumption of pulse protein has cholesterol lowering effect. These results are in tune with the reported

results in the literature (Hamilton and Carroll, 1978 and Soni et al., 1978).

Mathur et al. (1977) restates the same and justifies this effect to be due to increased excretion of cholesterol as bile acids and neutral sterols and decreased synthesis in liver.

When the food intake and cholesterol level of the animals was compared, it was found that in the atherogenic phase, as the food intake increased the cholesterol level also increased showing a positive correlation ($r = +0.211$). In the experimental phase, as the food intake increased, the cholesterol level came down as shown by negative correlation ($r = -0.1585$ for raw Bengal gram diet and $r = -0.0396$ for roasted Bengal gram diet) and hence the effective use of pulses in our daily diet. Table VII presents the relationship between food intakes, weight gain and cholesterol level in both the phases.

TABLE VII

STATISTICAL COMPARISON OF GROUPS FOR FOOD INTAKE WEIGHT GAIN AND CHOLESTEROL LEVELS

Groups compared	Correlation coefficient 'r'
Food Intake Vs Cholesterol levels	
Atherogenic diet	+0.3111
Raw Bengal gram diet	-0.1585
Roasted Bengal gram diet	-0.0396
Weight Gain Vs Cholesterol levels	
Atherogenic diet	+0.0264
Raw Bengal gram diet	-0.2670
Roasted Bengal gram diet	-0.1594

Similarly, the relationship between weight gain and cholesterol level was also calculated. The experimental groups showed negative correlationindicating the possible role of pulses in reducing the blood cholesterol level which is one of the risk factor in atherosclerosis.

D. Triglyceride Levels:

Elevated fasting plasma concentration of triglycerides is an independent and important risk factor than cholesterol in myocardial infarction (Carlson and Bottiger, 1972 and JADA, 1974). Serum triglyceride are hyperbolically related

to HDL - cholesterol (Lancet, 1979). Hence in the present study it was also though of interest to evaluate the triglyceride levels of randomly selected rats of all the groups. For this purpose one rat was sacrificed during the end of each phase from each group and comparison was made with rat on stock diet. The data obtained is given in Table VIII.

TABLE VIII

TRIGLYCERIDE LEVELS OF THE SELECTED RATS

S.No.	Diet	Triglyceride level mg/100 ml of blood
1.	Stock diet	37
2.	Atherogenic diet	68
3.	Raw Bengal gram diet	42
4.	Roasted Bengal gram diet	38

Triglyceride levels showed a similar trend like cholesterol. Among the experimental diets roasted Bengal gram diet showed triglyceride level on par with normal level whereas the triglyceride levels of raw Bengal gram diet was slightly higher. However, both these values were much lower than the atherogenic diet phase. This further implies that roasted Bengal gram has greater beneficial effect in bringing down the risk factor namely triglyceride levels associated with atherosclerosis.

5. Histopathological Changes of the Selected Tissues:

The liver, heart and aorta of the animals sacrificed at the end of the first and second phases respectively were subjected to histopathological examination. section of the liver, the apex section of the heart to study the coronary arteries and the section of the aorta itself were taken. Studies in mammals including man reveal that deposition of lipids cholesterol is mainly evident in the coronary arteries and aorta.

The findings of the study is discussed phasewise.

Phase I:

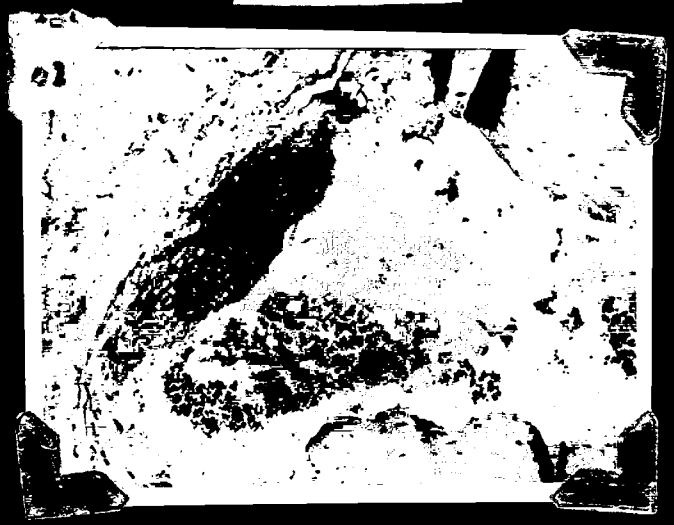
In the first phase of the experimental period, all the animals were maintained on an atherogenic diet. Microscopic examination of the heart section through the apex of the heart (Figure 4 & 5) showed the coronary artery with accumulation of fat cells in the initial lining beneath the endothelial lining. These fat cells are seen as signet ring cells containing a clear cytoplasm and nuclei pushed peripherally. The base showed no coronary vessels in the section. This coronary atherosclerosis is invariably associated with aortic atherosclerosis which is an important digenerative disease of arteries which may lead to strokes and thrombosis.

The aorta of this group showed local thickening of walls and these thickened areas showed vacuolated spaces between the elastic lamellae. These interstitial vacuoles are suggestive of lipid accumulation. These vacuoles are

Figure 4:

ACCUMULATION OF FAT CELLS IN THE INTIMAL LINING OF THE CORONARY ARTERY

Figure 5:



LOCAL THICKENING AND PLAQUE FORMATION OF AORTA SHOWING ATHEROSCLEROSIS

more towards the lumen and extending into the media also. The plaques are established lesions of atherosclerosis. They increase the thickness of intima and encroach on the lumen of the artery. Figure 5 depicts the details of the finding.

This is indicative of the fact that a high coconut oil diet of the order of 30 per cent given for a period of 60 days is capable of inducing plaque formation in the arteries of the rat. This is in line with the results of Narita et al. (1979) who have indicated that a dietary regimen containing cholesterol 3 per cent, coconut oil 1.5 per cent and thiouracil 35 mg/100 gm body weight provided for a period of two months induced sclerotic plaque formation in the aorta and also raised cholesterol levels to the order of 350 - 300 mg/100 ml from an initial level of 86.97 ± 5.02 mg/100 ml.

In the first phase all the rats showed high increments in cholesterol levels (300 mg/100 ml) which might be one of the reasons why plaque formation had developed in the aorta.

The liver tissue in this group differed from the normal in that it showed a sparse distribution of fat cells indicating the fatty degeneration of the liver. Glycogen vacuolation was not prominent showing the fatty liver and this could go on to fibrosis and cirrhosis.

Figure 6

FATTY DEGENERATION OF THE LIVER

Figure 7

FAT CELLS BENEATH THE ENDOTHELIAL LINE IN THE CORONARY ARTERY

Figure 6 indicates the fatty degeneration of the liver.

Phase III

Group III

Animals in group II were maintained on an atherogenic diet in the first phase of the study and subsequently placed on a raw Bengal gram diet during the second phase. Microscopic examination of the coronary artery of the heart showed a considerable accumulationof fat cells with eccentric nuclei just beneath the endothelial line (Figure 7). Similar vacuolated cells were distributed sparsely in the deeper layers also.

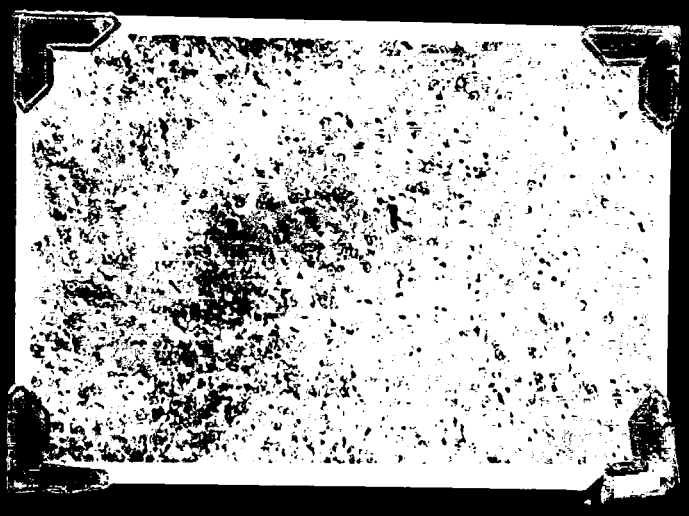
The heart showed myocarditis or inflammation of myocardium with focal destruction of myocardial fibres in one isolated area. This is presented in Figure 8. The destroyed myocardial fibres, replaced by an accumulation of cells with vesicular nuclei probably reticulo endothelial cells was seen. The heart showed congestion and mild gemmulation.

The liver of this group revealed fatty vacuolation and degeneration in a significant manner (Figure 9). There was sparse but significant distribution of fat cells in the liver indicative of mild fatty degeneration.

Figure 6:

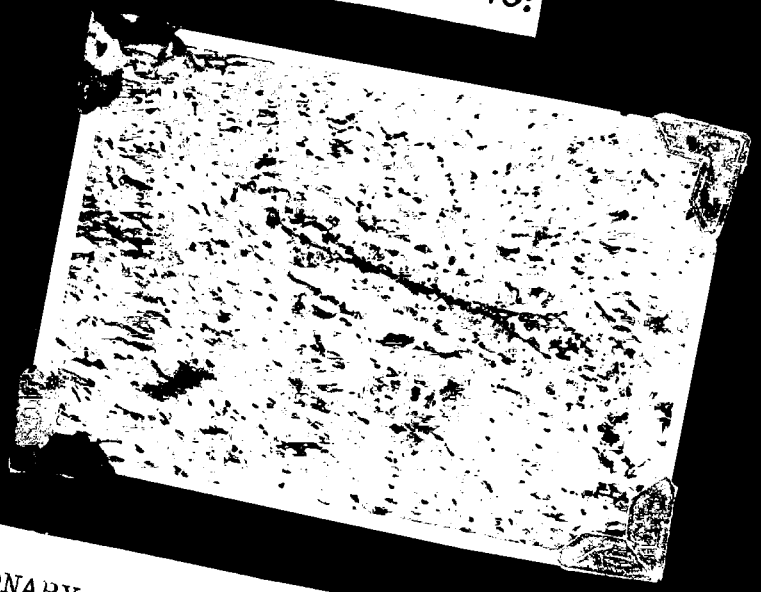
HEART SHOWING MYOCARDITIS WITH FOCAL DESTRUCTION OF MYOCARDIAL FIBRES

Figure 9:



LIVER SHOWING FATTY VACUOLATION

Figure 10:



CORONARY VESSELS WITH NORMAL HISTOLOGY

Group III:

Rats in this group were provided with atherogenic diet for 60 days and then subsequently switched over to roasted Bengal gram diet for 45 days.

All the sections through the heart appeared to be normal. The coronary vessels showed normal histology with no fat cells or interstitial vacuolation in the artery walls (Figure 10 11). There was congestion and granulation.

Aorta and lung showed no apparent change but there was more granulation attributed to high protein content of the diet. Figure 11 12 represents these details.

In the liver, comparatively less fat cells were seen and there was no fatty degeneration (Figure 12 13). Glycogen Vacuolation characteristic of normal and healthy liver tissue) was very prominent.

The above cited observations and discussions in the present investigation vividly brings out the fact that pulses are indeed effective sources of growth promoting factors and in fact roasted Bengal gram has even more beneficial effect than the raw sample in bringing down the cholesterol level which is one of the most important risk factors in coronary heart disease.

The histopathological picture also revealed that from a very atherogenic picture, the roasted Bengal gram restored

FIGURE 17

[illegible] AORTA AND LUNG TISSUE

Figure 19:

[illegible] HEALTHY LIVER TISSUE

the heart, liver and aorta to a normal histology. It significantly modified the fatty infiltration from what it was in the atherogenic phase. It thus brings out the possible role of processing like common heat processing method namely, roasting in fully bringing out the potential of legume proteins. Hence the role of these legumes specially in the vegetarian diets and in maintaining a lesser risk level in human consuming such diets.

V. SUMMARY AND CONCLUSION

This study was carried out to investigate the relative effect of raw and roasted Bengal gram on some physiological parameters like changes in food intake, weight gain, cholesterol levels, triglyceride level and histopathological alterations in the heart aorta and liver of albino rats.

Twelve male albino rats weighing 140-170 g were grouped on their weight basis in to three different groups and designated as Group I, II and III. In the first phase all the rats were administered atherogenic diet for 60 days. The food intake and weight gain were recorded for all the rats. Immediately after the completion of first phase the rats of group I were sacrificed. After sacrificing the animals, the heart, aorta and liver were removed and preserved for histopathological analysis.

In the second phase of the study group II and III were transferred to raw Bengal gram diet and roasted Bengal gram diets respectively and the experiment was carried out for 45 days.

The serum cholesterol level was analysed every month during the atherogenic phase and every fifteen days during the second phase and also at the beginning and termination of each phase. One rat in each group was randomly selected and serum triglyceride level was estimated at the end of each phase.

In the second phase of the study group II and III were transferred to raw Bengal gram diet and roasted Bengal gram diets respectively and the experiment was carried out for 45 days.

The serum cholesterol level was analysed every month during the atherogenic phase and every fifteen days during the second phase and also at the beginning and termination of each phase. One rat in each group was randomly selected and serum triglyceride level was estimated at the end of each phase.

The animals were sacrificed at the end of the study and liver, heart and aorta of the animals were subjected to histopathological study.

The results of the study revealed the following facts:

1. Coconut oil in the concentration of 30 per cent as a source of fat in the diet reduces the food intake of rats.

2. Prolonged feeding of atherogenic diet for 60 days brought out the development of symptoms of essential fatty acid deficiency.

3. The average daily food intake registered in group I was 10.63 g, group II 10.53 g, and 9.95 g in group III, during the first phase of the study. In the second phase, group II and group III registered 12.67 g. and 11.84 g. respectively.

4. The rats in group I, II and III registered an average weight gain of 8.48g, 8.34 g and 6.88 g per week respectively during the first phase and in the second phase 10.48 g. and 9.36 g per week respectively for group II and III showing positive correlation between food intake and weight gain in all the groups in both the phases.

5. At the end of phase I, the cholesterol level raised to 488.25 mg/100 ml, 377.5 mg/100 ml and 497.0 mg/100 ml respectively for group I, II and III showing the possible role of coconut oil in increasing the blood cholesterol level.

6. At the end of the study the cholesterol level came down to 154 mg/100 ml and 132.5 mg/100 ml for group II and III respectively showing the beneficial effect of Bengal gram especially that of roasted Bengal gram in the diet.

7. Though the cholesterol lowering effect was much appreciable in roasted Bengal gram diet as against the raw Bengal gram diet, the fall in cholesterol level between raw and roasted sample was significant ($P < 0.01$) only for the 15 days' level and not for 30 or 45 days.

8. When the days were compared for both raw and roasted samples, as the periodicity increased there was statistically significant difference in the cholesterol levels within the sample.

9. Roasted Bengal gram diet showed triglyceride level (36 mg/100 ml) in line with normal value (37 mg/100 ml) whereas that of raw Bengal gram diet was slightly higher.

10. Feeding coconut oil alone for a period of 60 days induced plaque formation in the aorta, accumulation of fat cells in the intimal lining of the coronary artery, interstitial vacuoles in the aorta and sparse distribution of fat cells in the liver. Glycogen vacuolation was not prominent in the lever.

11. The histopathological results of raw Bengal gram diet revealed considerable accumulation of fat cells in the coronary artery. The heart showed myocarditis. Fatty vacuolation and degeneration of the liver was significant.

12. In the roasted Bengal gram diet, microscopical section of the heart, aorta and lung showed no apparent change. Glycogen vacuolation in the liver was very prominent.

The above discussions clearly putforth the facts describing the possible role of pulses in our daily diet and the beneficial effect of roasted Bengal gram in bringing down the cholesterol level, serum triglyceride level and restoration of normal histology of the organelles like live, heart and aorta.

Hence the role of Bengal gram in the dietaries as an antiatherogenic factor.

Some of the future avenues open for investigation in this and related areas are:

1. This study needs to be further ascertained with human subjects to fully understand the underlying consequences, as dietary patterns are never rigid in human beings.

2. One could possibly study on the effect of different concentrations of pulses and their responses in rats.

3. Another challenging area needing thorough investigation is the effect of different cereal - pulse - fat combinations.

4. Many other cooking methods can be adopted and the relative effect of those methods assessed.

5. Female rats could also be used for the investigation to study the control of pulses on their lipid profiles.

6. Different lipo protein fractions could be assessed.

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APPENDICES

APPENDIX – I

COMPOSITION OF VITAMIN AND MINERAL MIXTURES

Vitamin mixture per 1 g.	
Vitamin A	100 I.U.
Vitamin D	100 I.U.
Vitamin E	10 I.U.
Vitamin K	.50 mg
Thiamine	.50 mg
Riboflavin	1.0 mg
Pyridoxine	.40 mg
Pantothenic acid	4.0 mg
Niacin	4.0 mg
Choline	200 mg
Inositol	35 mg
Para amino benzoic acid	10 mg
Vitamin B_{12}	2.0 mg
Biotin	0.02 mg
Folic acid	0.2 mg

Mineral mixture per 100 g	
Sodium chloride	13.93 g
Potassium dihydrogen orthophosphate	38.9 g
Calcium carbonate	38.14 g
Magnesium sulphate	5.73 g
Ferrous sulphate	2.7 g
Manganese sulphate	.401 g
Potassium iodide	.070 g
Copper sulphate	.0477 g
Zinc sulphate	.064 g
Cobalt chloride	.023 g

APPENDIX - II

DETERMINATION OF TRIGLYCERIDE LEVEL IN SERUM

Triglyceride elevation in serum indicate hazardous development of Atherosclerosis particularly of Coronary Arteriosclerosis. Physiologically it is found elevated in angina pectoris exogenous hypertriglyceridemia, enzymatic defect in infants and associated with prebetalipoproteinaemia in diabetic and alcoholic lipaemia.

Principle:

Triglycerde is measured after hydrolysis by estimating its glycerol content. The commonest procedure involves oxdation of glycerol to formaldehyde which is measured colorimetrically with chromatorepic acid. The lipid extracted from serum must therefore be freed from other sources of glycerol, phospholipids, in particular and from glucose which on oxidation can also yield the same. After absorption of serum by neolite mixture triglyceride level is measured.

Collection of Sample:

About 5 ml of venous blood is obtained after 12 hour fast as triglyceride levels are increased and widely, variable after meals. Prolonged venous stasis can increase lipeprotein concentration. As serum lipid depends partly on diet the subject should have received his habitual diet for 2 weeks prior to sampling. Centrifuge the Blood at 2,000 - 3,000 r.p.m. for 3 minutes. Separate the serum and keep it ready for the tests.

Reagents: (To be used for one test)

*Zerolite (activated)	..	1 g.
Chloroform	..	9.6 ml
Working Standard (0.05 mg/ml)	..	0.8 ml
**Alcoholic potassium hydroxide	..	1.0 ml.
Alcohol	..	1.0 ml.
0.2 N sulphuric acid	..	2.0 ml.
Sodium Metaperiodate	..	0.4 ml.
Sodium Arsenite	..	0.4 ml.
*** Chromotropic acid solution	..	20.0 ml.

Note:

All the glass wares should be washed on previous day, dried at 110°C kept dry for use.

*Keep the Zerolite in theoven at 110°C for ½ hour and cool in a desiccator before use.

**The 0.5 ml of stock alcoholic KOH and alcohol and make the volume to 2.5 ml and use

***Dissolve the Chromotropic acid from one packet in 25.0 ml distilled water, separately and 75 ml concentrated sulphuric acid to chromotropic acid solution. Store in brown bottle and do not use after 3 weeks.

Procedure:

1. Take a stoppered test tube. Place 1.0 g of the Zerolite in it. Add 0.8 ml Chloroform and shake.

2. Place 0.2 ml serum in the tube on top of the Zerolite. Add 4.8 ml of chloroform. Keep for 10 minutes at room temperature with intermittant shakings (minimum 4 shakings).

3. Filter through whatmann No.1 filter paper.

4. Take one ml filtrate in two stoppered test tubes.

5. Take another set of two stoppered test tubes and place 0.5 ml of standard in each.

6. Evaporate the solven from all the four tubes at 60°C in an electrically heated water bath.

7. To one tube one in each of the unknown and standard and 0.5 ml. Working alcoholic KOH (saponified) and to the other two tubes add 0.5 ml. alcohol(unsaponified).

8. Keep all the tubes at 60°C for about 30 minutes.

9. To all the tubes add 0.5 ml. of 0.2N H_2SO_4 keep in gently boiling water bath for 30 to 40 minutes to remove the alcohol. It is advisable to keep the water level of bath only slightly above the surface of the reaction mixture to avoid evaporation of water from the tubes.

10. Cool to room temperature

11. Add 0.1 ml of Sodium metaperiodate. Keep for 10 minutes.

12. Add 0.1 ml Na arsenite to each tube. The yellow colour of iodine appears and disappears in a few minutes.

13. Add 5.0 ml chromatropic acid to each tube and mix.

14. Cap the tubes tightly or plug with cotton. Keep in vigorously boiling water bath for exactly 30 minutes in the absence of excessive light.

15. After cooling, determine the optical densities at 570 mu. or using green filter.(the colour is stable for several hours).

Calculations:

$$\frac{\text{OD saponified unknown} - \text{OD unsaponified unknown}}{\text{OD saponified standard} - \text{OD unsaponified standard}} \times 100$$

= mg % Triglyceride

(To convert to m.eq/litre multiply the result by 0.00342).

Packing & tests:

Zerolite (activated)	-	5 x 1 g.
Chloroform	-	80 ml
Working Standard	-	5 ml
Alcoholic KOH (Stock)	-	3 ml
Alcohol	-	15 ml
0.2 N H_2SO_4	-	12 ml
Na Metaperiodate	-	3 ml
Na Arsenite	-	3 ml
Chromotropic acid	-	25 mg x 5

Storage:

Reagents except standard could be preserved at room temperature. Do not use the opened vial of standard for more than one day. Do not keep zerolite in refrigerator. Let it gets inactivated. Prepare chromotropic acid every 2-3 weeks.

Normal Values: 37 - 134 mg%(1.3 to 46 m Eq. 1/L)

APPENDIX III

HISTOPATHOLOGICAL TECHNIQUES

The general preparationof tissue consits of :

1. Fixation
2. Dehydration
3. Cleaning
4. Infiltration and Impregnation
5. Embedding or casting or blocking

1. Fixation:

It is essential that tissues be fixed as soon as possible after death or renoval from the body to prevent putrifaction and autolysis. Fixation also helps to preserve, harden, solidify colloid material and helps in optical differentiation of tissue components.

Usually 10 per cent formation or slightly higher concentrations are used to preserve specimens.

Preparation of 10 per cent formalin:

Formalin (40 per cent formaldehyde)	-	10 ml
Distilled water	-	90 ml

Tissues should be completely immersed in the fixation solution.

2. Dehydration:

After fixation delicate tissues need to be dehydrated slowly, starting in 50 per cent ethyl alcohol. The tissues are then placed in 70 per cent, 96 per cent and 100 per cent alcohol for 2 - 4 hours in each solution. The volume of the

reagent should be 50 - 100 times the bulk of the specimen. Before transferring to the next concentration of alcohol the tissue is laid on a piece of filter paper and lightly blotted to remove excess fluid.

3. Clearing of tissues

The most common clearing agents in use are chloroform, benzene, kylene toluene, carbontetra chloride and ceder wood oil.

Small pieces of tissue are cleared within 2-4 hours of immersing in xyline. The tissue becomes clearer as the alcohol is replaced owing to the difference in refractive index.

Tissues for clearing should be lightly blotted during transfer from one reagent to the next. The volume of clearing agent be 50-100 times that of the tissue. Tissues cleared in xylene should be given onechange after 30-60 minutes and transferred to wax when they are seen to be clear (translucent).

4. Impregnation with wax

Impregnation with wax (paraffin) takes place in an oven heated to 54 - 60°C. The temperature of the paraffin wax must be maintained at 58 - 60°C.

After blotting with filter paper, the tissue is transferred from the clearing agent to molten paraffin wax. The volume of the tissue and must be changed at least once

during impegnation. This change is effected by simply lifting the tissue from one pot of wax to the next with warmed forceps.

Casting or blocking:

Tissue is blocked by transferring it from the final wax bath to a mould filled with molten wax, inverting the tissue to free the surface to be cut from air bubbles and oriented so that this surface rests on the base of the mould. The block is then quickly cooled.

Cutting of the paraffin wax embedded sections:

Trimming the block:

When cutting sections on microtomes, blocks must first be trimmed and fixed to wooden fillets. Wax is then removed with a sharp knife until 1/8 inch remains on all sides of the tissue. Great care is necessary to avoid exposing the tissue at any point, and the edges of the block should be made parallel so that an even ribbon of sections result. The surface of the block which is to be cut should be trimmed on the microtome during the preliminary cutting.

Only small flakes of wax should be trimmed at a time; trimming large pieces can lead to splitting of the block and exposure of the tissue; this will entail reblocking in fresh wax.

Fixing the block on the microtome:

When using a microtome with a flat metal stage on which

the microtome with a flat metal stage on which the block can be fixed directly, a wooden handle spatula, or a metal scalpel which has been inserted into a wooden handle is heated in a bunsen burner. When hot, but not red hot this is applied to the underside of the block and to a layer of wax on the stage until the two wax surfaces melt, when they are quickly and firmly pressed together.

The surfaces of the wooden fillet is first serrated with a saw or a scalpel and coated with hot wax. The block of the tissue is then attached to the wood in a manner described earlier. The wooden fillet is then mounted in a clamp type holder on a microtome.

Cutting Technique

1. Fix the block in the block holder on the microtome in such a position that it will be clear of the knife when this is in position.

2. Turn back the feed mechanism on the microtome almost as far as it will go.

3. Insert the appropriate knife in the knife holder and screw it lightly in position, check that the tilt of the knife is set at a correct angle (if this is adjustable). It is intended to use the knife obliquely, the movable knife holder should be adjusted in the desired position.

4. Move the block holder forward or upward or adjust the feed mechanism until the wax block is almost touching the

the knife. Ensure that the whole surface of the block will move parallel to the edge of the knife, and that the leading edge of the block is almost parallel to the edge of the knife in order to ensure a straight ribbon of sections.

5. Tighten all the adjusting screws on the microtome. Faults in section cutting are more frequently due to the looseness of the block or the knife in the early stages of section cutting than to any other reason.

6. To trim the block set the section thickness gauge to about 15 microns and with the rough knife or one end of a m large knife which is kept for trimming in position, operate the microtome until complete sections of the tissues are being cut.

7. Replace the rough knife by a sharp one, or move the knife to a new position; check that it is screwed tightly in position. Rock the feed mechanism back a little to allow for slight differences which always exist in different knives, and even in different parts of the same knife. Apply ice to the surface of the block and wipe the surface free of water; this is optional, but makes flat even sections easier to cut.

8. Set the thickness guage; for routine work 6 microns will give moderable thin sections with ease of cutting.

9. The microtome is now operated until complete sections are being cut.

10. Routine sections may be laid in small card board boxes or directly floated on to slides. Small card board boxes on which the number or the name of the tissue is marked in pencil are useful.

11. Cutting is normally only continued until sufficient perfect sections have been produced. The free end of each ribbon is supported either with five forceps or with the fingers until it is about 12 inches in length; the ribbon is then freed from the knife by bringing a dissecting needle up under the last section - it must not be allowed to touch the cutting edge of the knife. The sections are then laid on black paper and should be fixed in position by gentle pressure with a finger at each end of the ribbon to avoid them being scattered by stray draughts.

Fixing the sections on slides:

During cutting, paraffin wax embeded sections become slightly ceased. Before being attached to slides these creases must be removed and the section flattered, and this is achieved by floating them on warm water (50 - 60°C) by one of the following three methods.

Water bath method:

Thermostatically controlled baths with inside coloured black are maintained at a temperature 5 - 8 degrees below the melting point of the paraffin wax; or

an enamel or glass bowl filled to the brim with a correct temperature may be used. Air bubbles forming on the bottom of the dish must be dislodged with fingers or by vibration.

Sections are divided with a scalpel into lengths convenient to go on a slide, usually single sections or 3-4 small sections. Room must be left at each end of the slide, for a label on one side, and space on the other side to prevent any difficulty when examining them under the microscope. It is preferable to leave 3/4 inch for the label and 1/3 inch at the other end.

The section, or sections are lifted off the bench and on to the surface of the water by inserting the point of a dissecting needle into the wax at one end. Care being taken not to damage the tissue during this preparation.

If creases in the sections do not disappear immediately after contact with water, dissecting needles should be used to tease them out by applying one to each side of a crease with a gentle pressure.

A clean or albuminized (the albumin is prepared by mixing albumin of the egg with glycerine, and this is applied as a very thin coat into the slides and allowed to dry) slide is half submerged, in the water and with a dissecting needle the sector is brought into contact with it. With the needle the sections is then oriented on the slide by withdrawing the slide and bringing the flattered section with it. It is important that only the wax edge be touched with the needle

and that this operation be carried out while there is still water on the slide. The slide is then set in an upright position to drain. The mounted sections may then be left in the incubator at a temperature of 37°C over night to dry and are ready for staining. Hot stage method: Hot stages are available commercially with a specially shaped metal top which is heated and maintained at 45 - 50°C. A clean or albuminized slide is laid on the hot stage, flooded with distilled water and the section or sections are laid on the surface of the water. Creases should be teased out with needles or forceps and theslides left for a few moments to get warm. The crease will flatten out due to the heat. When completely flat the slide is removed from the hot plate the excess water is drained off and the section oriented into the correct position. It is then returned to the stage, section downards to prevent dust from settling on it and left for 30 minutes. The section is then ready for staining.

Warmed slide method:

A clean or albuminised slide is flooed with distilled water sections are laid on the surface and with the major creases removed the slide, held over a bunsen burner for a second or two to slowly warm the water. If necessary a second heating is given but the wax must not be melted during this operation. The creases will flatten out during this operation, process following which the excess water is

drained off and the slide put into the oven at a temperature of 37°C or 56°C to dry. The section is ready for staining.

Stages in staining and mounting paraffin sections:

1. Removal of wax with xylol
2. Hydration with graded alcohols
3. Staining
4. Dehydration through graded alcohols
5. Clearing with xylol
6. Mounting under a coverslip.

Technique:

1. Removal of wax: Sections are placed in xylol for 1-2 minutes to dissolve the wax.
2. Hydration: The section is taken out of xylol (it should appear quite clear) and it is transferred to absolute alcohol for 1 minute, when it will become opaque.

The section is removed from absolute alcohol drained and placed in 90 per centalcohol, placed in picric alcohol (saturated solution of picric acid in alcohol) for 5-10 minutes, followed by the washing in the slide tray for 10 minutes. The sections are then transferred to 90 per cent alcohol for 3 minutes.

3. Staining:

a) slides are transferred from 90 per cent alcohol, after hydration to haematoxylin and left for 10-40 minutes.

b) Slides after draining off excess haematoxylin, are transferred to the slide washing tray and washed until the sections are blue (when first removed from the haematoxylin there are pink). This takes about 10 minutes in tap water and is known as "bluing sections".

c) Sections are dipped into acid alcohol when they are agitated for a few seconds, and returned to the slide washing tray until blue again. They should be observed under low power of the staining microscope to ensure that they are sufficiently differentiated.

d) If the sections are underfifferentiated it is again returned to the acid alcohol for a short period; rinsed in distilled water and returned to the haematoxylin for 10-15 minutes.

e) Sections which are differentiated and blued are transferred to 1 per cent eosin for 2-4 minutes to counterstain them.

f) Sections are transferred from eosin to the slide washing tray for 3-4 minutes, this will differentiate the eosin.

4. <u>Dehydration:</u>

g) After draining, sections are transferred from the slide washing tray to 90 per cent alcohol where they are agitated for 10 - 15 seconds.

h) From 90 per cent alcohol they are transferred to absolute alcohol where they are agitated for 10 - 15 seconds.

i) They are taken from absolute alcohol I to Alcohol II for 30 seconds.

5. Clearing:

j) Sections should be transferred from absolute alcohol II to xylol I and left until completely clear for 15 seconds.

k) Sections are then transferred to xylol II, when clear, from which they may be mounted.

6. Mounting:

Mountants:

Histological sections which need to be examined for any length of time or to be stored, must be mounted under a cover slip. There are generally two main classes:

i) Aqueous media and

ii) Resinous media

Canada Balsam (refractive index 1.52) a resinous media is a commonly used mountant. It is obtained in a mortar and pestle until free of lumps and dissolved in xylol to 56 - 70 per cent by weight.

l) Rectangular cover slips are wiped with soft, fluffless glass cotton.

m) A cover slip is laid on a blotting paper; the section is removed from the xylol, the surplus

xylol is removed by wiping the back of the slide and around the section, leaving a margin of about 1/8 of an inch; this stage should be quickly completed to avoid the section from drying.

n) One or two drops of Canada Balsam (depending on the size of the coverslip are placed on the section; being laid along the middle of the section to reduce the likelihood of trapping air bubbles.

o) The slide is quickly inverted over the coverslip; one end is placed on the blotting paper and the other end slowly lowered until the balsam touches the coverslip. The balsam quickly spreads under the coverslip and the slide. With the coverslip attached it is again quickly inverted and the coverslip guided into place with a dissecting needle.

An alternative method is to put the mountant on the section as described, place one end of the coverslip on the slide (clear of section) and with the aid of a dissecting needle slowly lower the coverslip in position.

Air bubbles:

Air bubbles may be expressed by gentle pressure on the coverslip with a dissecting needle. When there is more than one air bubble in the mountant it is easier to put the slide

back into xylol (which will remove the coverslip and remove the section.

Routine Staines

Haematoxylin and eosin is the most popular routine stain and the one devised by Ehrlich is the most popular because of its durability, easy fiferentiation and comparitive permanancy.

As a counterstain 0.5 - 1.0 per cent aqueous solution of water soluble eosin is generally preferred to give a more informative picture.

Reagents required for Ehrlich's Alum Haematoxylin stain:

Staining time	30 - 40 minutes
Absolute alcohol	300 ml
Hamatoxylin	6 g
Distilled water	300 ml
Glycerol	300 ml
Glacial acetic acid	300 ml
Potassium alum	in excess

Preparation:

Haematoxylin should be dissolved in alcohol and the other components added in the order given. Finally potassium alum is added while the solution is shaken until threre is a deposit of alum crystals at the bottomof the stock container. Glycerol is said to given more even precise.

staining and to stabilize the stain against over oxidation and evaporation.

Ripening:

After preparationof the stain it must be kept in a loosely plugged bottle in a warm and well lit place (window sill) until the oxidation of the haematoxylin to haematein has taken place (this process is known as ripening). This will take 1-2 months.

APPENDIX IV

FOOD INTAKE (IN GRAMS) OF THE INDIVIDUAL RATS ON THE ATHEROGENIC DIET

Period	Food intake (grams)											
	R_1*	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}
1st week	80.3	68.2	92.6	72.6	77.4	70.3	55.5	70.5	78.8	76.1	99.5	66.5
2nd week	63.5	64.6	73.7	58.9	60.3	66.6	54.4	57.9	63.9	70.8	78.7	59.2
3rd week	64.0	59.0	69.0	69.1	66.5	61.6	64.8	68.3	69.3	70.3	63.2	62.3
4th week	76.4	81.6	83.7	71.2	81.4	80.9	68.7	64.7	74.7	76.7	78.7	65.6
5th week	71.5	86.4	78.5	69.0	69.9	75.3	68.2	69.5	78.3	79.4	83.8	64.7
6th week	79.1	83.9	79.1	73.6	74.3	78.2	73.0	83.5	82.8	79.7	74.1	75.8
7th week	93.7	101.2	88.3	91.0	91.3	85.0	82.2	83.2	83.9	88.8	98.7	81.4
8th week	59.8	82.9	57.7	40.7	57.6	69.0	50.3	61.8	66.9	65.0	77.7	62.0
Total	588.2	627.8	630.8	545.1	564.5	593.8	513.9	555.7	597.6	606.8	661.5	537.5
Mean	73.53	78.48	77.6	68.14	70.56	74.23	64.24	69.46	74.7	75.73	82.69	67.19

*R – Rat number

FOOD INTAKE(IN GRAMS) OF THE INDIVIDUAL RATS ON THE EXPERIMENTAL DIETS

Period	Roasted Bengal gram diet				Raw Bengal gram diet				
	R_1^*	R_2	R_3	R_4	R_1'	R_2'	R_3'	R_4'	R_5'
1st week	64.8	68.6	88.3	84.7	70.4	72.8	105	84.7	81.3
2nd week	67.3	71.7	88.3	88.8	72.9	76.8	105	89	87.5
3rd week	76.8	76.3	68.3	91.5	73.6	89.4	104.2	70.7	85.5
4th week	80.8	80.3	84.7	99.3	86.5	92.6	104.2	81.3	94.5
5th week	84.3	83.6	97.3	96.2	87.3	96	106.2	83	87.3
6th week	89.1	84.5	85.2	98.6	88.6	100.1	104.2	86.3	93.1
Total	433.1	464.0	500.9	560.2	550.7	527.7	638.8	495	529.2
Mean	77.18	77.33	84.48	93.37	91.75	87.95	104.8	82.5	88.2

R^* - Rat Number

APPENDIX V

WEIGHT GAIN (IN GRAMS) OF THE INDIVIDUAL RATS ON THE ATHEROGENIC DIET

Period	Weight gain (grams)											
	R_1	R_2	R_3	R_4	R_5	R_6	R_7	R_8	R_9	R_{10}	R_{11}	R_{12}
1st week	8.78	5.47	10.9	7.4	8.63	7.3	11.3	7.7	8.13	4.71	6.3	9.08
2nd week	6.98	8.3	9.0	6.6	7.0	6.8	8.9	5.2	6.3	4.0	6.14	7.45
3rd week	7.00	6.0	6.3	6.9	7.49	6.6	9.0	7.3	6.5	4.45	6.95	8.34
4th week	8.38	8.5	10.8	7.3	8.2	7.3	8.9	7.5	8.5	5.4	7.75	8.3
5th week	8.95	11.0	9.8	8.3	9.8	7.7	10.03	8.8	6.2	4.0	4.45	9.1
6th week	9.94	9.9	7.5	10.0	10.4	7.74	8.9	9.3	6.6	4.2	9.3	10.94
7th week	11.73	12.1	8.1	9.96	10.5	8.6	11.8	11.7	7.3	4.5	10.48	10.9
8th week	7.83	9.13	5.1	7.4	8.4	6.3	9.3	8.2	5.1	3.68	6.4	8.07
Total	69.56	66.40	67.5	63.86	70.41	58.34	77.13	64.7	54.63	35.54	57.77	73.18
Mean	8.69	7.55	8.43	7.98	8.80	7.29	9.64	8.08	6.73	4.44	7.22	9.02

WEIGHT GAIN (IN GRAMS) OF THE INDIVIDUAL RATS ON THE EXPERIMENTAL DIETS

Period	Raw Bengal gram diet					Roasted Bengal gram diet			
	R_1	R_2	R_3	R_4	R_5	R_1'	R_2'	R_3'	R_4'
1st week	6.35	6.05	12.98	9.8	9.5	7.7	5.3	10.9	10.9
2nd week	9.2	6.07	12.9	10.3	10.27	7.96	5.5	10.9	11.5
3rd week	6.1	9.5	13.78	8.1	10.04	9.1	5.85	8.46	11.8
4th week	11.1	10.2	13.8	9.4	11.1	9.55	6.2	10.49	12.8
5th week	10.9	10.6	13	9.6	10.3	9.97	6.3	10.68	12.6
6th week	11.14	11.07	13.8	9.9	10.9	10.5	6.48	10.55	12.7
Total	60.39	57.79	77.26	57.1	62.11	54.73	35.63	61.98	73.3
Mean	10.06	9.63	12.88	9.5	10.35	9.13	5.94	10.33	12.05

APPENDIX VI

SERUM CHOLESTEROL LEVELS OF THE INDIVIDUAL RATS IN THE TWO PHASES OF THE EXPERIMENT(mg/100 ml of blood)

Group		Atherogenic		Experimental		
		30 days	60 days	15 days	30 days	45 days
I	(1)	250	450	—	—	—
	(2)	310	566	—	—	—
	(3)	280	511	—	—	—
	(4)	230	450	—	—	—
II	(1)	300	420	230	150	120
	(2)	331	420	280	155	135
	(3)	345	638	195	180	150
	(4)	285	520	235	170	135
III	(1)	280	510	375	130	130
	(2)	220	390	290	130	150
	(3)	170	300	305	150	140
	(4)	172.6	310	325	165	170
	(5)	—	—	—	155	190