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Effect of atherogenic diets deficient in vitamin B12, choline, and protein of physiological parameters of the rat

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EFFECT OF ATHEROGENIC DIETS DEFICIENT IN VITAMIN B_{12}, CHOLINE, AND PROTEIN ON PHYSIOLOGICAL PARAMETERS OF THE RAT

by

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INTRODUCTION

The increase in the incidence of heart disease in man during the past two decades stimulated research workers to investigate the etiology and methods of prevention of atherosclerosis. There are probably factors other than diet, such as exercise, disease or stress, which may support or initiate the development of atherosclerosis. In this project the dietary parameters were of prime concern.

In many of the previous research reports, the investigators were concerned with the addition of agents which are not normally present in diets. Some of these agents are propylthiouracil, bile salts, and cholic acid. These agents have been shown to be atherogenic when supplemented to diets. However, a question which arises concerning the applicability of such reports on these agents is "Would the metabolic responses to the particular diets be the same if these atherogenic agents were deleted?" It is doubtful that the same response could be obtained without such agents. The presence of such agents in diets could enhance or inhibit the natural metabolic responses. Evaluation of various dietary factors without "inductive agents" becomes necessary.

Another experimental variable of importance is the level of the various dietary nutrients. Many research workers have utilized extreme levels of protein, fat, and carbohydrate. Extremes of any nutrient can be a biological stress and produce a result which is characteristic for that particular unnatural condition. The dietary variables of each project should be limited to the normal realm for each experimental animal.

Although the development of atherosclerosis in the rat on a highly "inductive diet" is not exactly identical with that in the human, studies conducted with the rat may present valuable information concerning atherosclerosis. Furthermore, it is impossible to conduct the number of experiments, to utilize the complete histology, and to control all the parameters as carefully with humans as with the experimental laboratory animal.

Many research workers have reported that atherosclerosis in the rat is extremely difficult to induce on a "non-inductive" diet. The apparent natural resistance of the rat to the development of atherosclerosis can be beneficial. If atherosclerosis can be controlled by natural parameters in the rat, then it is possible that similar conditions may control the development in the primate.

Atherosclerosis is associated with alterations in lipid metabolism. Characteristics of atherosclerosis are hypercholesteremia, hyperlipemia, and arterial plaque formation. In studying the natural occurring atherosclerosis, 1) rat experimental units, 2) normal nutrient levels without inductive agents such as cholic acid, and 3) long duration trials were incorporated into the research project.

REVIEW OF LITERATURE

Dietary Lipids

Keys *et al.* (1955) demonstrated that dietary cholesterol did not influence the serum cholesterol of man as much as the dietary fat. Serum cholesterols were 25 to 50 per cent greater in men receiving approximately 40 per cent of the calories from fat as compared with 20 per cent.

Mann *et al.* (1955) reported that the rural central American who exists on a vegetable and low fat diet has a lower serum cholesterol value than the urban Guatemalan or the North American. Keys (1964) stated that populations existing on diets high in saturated fat exhibit a high incidence of atherosclerosis. The serum cholesterol was directly related to the level of saturated fat in the diet.

Lowenstein (1964) found that a foreign tribe with a high intake of fat had a low incidence of ischemic heart disease and low serum cholesterol. This apparent conflict of concepts was attributed to the amount of physical activity.

Dayton *et al.* (1961) showed that a 30 per cent coconut oil diet enhances the movement of plasma cholesterol into the aortic wall of rats. The movement of plasma cholesterol was diminished considerably in animals supplemented with 30 per cent safflower oil.

Maruhama (1965) found that alloxan diabetic rats responded differently to cholesterol and fat supplementation than normal animals. The high cholesterol diet caused an increase in plasma cholesterol of diabetic rats but not in the normal rats. Diets rich in unsaturated fat and cholesterol increased total plasma cholesterol in the normal rat, but decreased total cholesterol in the diabetic rat. Diets high in unsaturated fats without cholesterol decreased the plasma triglyceride in the normal but caused elevated plasma triglycerides levels in the diabetic animals.

Scott et al. (1964) demonstrated an accelerated plaque development when rats were fed propylthiouracil and sodium cholate with a diet high in unsaturated fatty acids. These workers also found an elevated serum lipid phosphorus in animals fed butter fat.

Connor et al. (1964) have shown with six men fed controlled natural diets, that the level of dietary cholesterol greatly influences the serum cholesterol and phospholipids regardless of the quantity and degree of saturated dietary fatty acids. The removal of dietary cholesterol caused a decrease of 38 mg/100ml in serum cholesterol when the diet contained a large quantity of saturated fat. The concentration of serum lipids remained relatively stable during periods of cholesterol-free diets with either a saturated fat (Iodine No. 63) or an unsaturated fat (Iodine No. 100). Bronte-Stewart (1964) demonstrated that the absorptive surface of the gastrointestinal tract may influence the degree of hypercholesteremia. Patients with ischemic heart disease exhibited prolonged lipemia after an oral test diet. Intravenous lipid was cleared as fast from ischemic patients as from control patients. Evaluation of the lipemia clearing mechanism implicates the digestive system in the development of hypercholesteremia and atherosclerosis.

Olson et al. (1958a) found with rats that levels of dietary fat (butterfat, corn oil or lard) from 6 to 42 per cent did not influence the hypocholesteremia induced by feeding a low choline diet.

In work conducted by Swell and Flick (1953), the blood cholesterol, predominately in the ester fraction, increased when 25 per cent of the rat diet was lard. When fat was fed as oleic or stearic acid the blood cholesterol and cholesterol ester both decreased in diets containing or not containing cholesterol. The authors postulated that oleic or stearic acid inhibited the absorption of cholesterol from the diet.

Moore and Williams (1964a) demonstrated that rabbits fed either a 20 per cent butterfat ration or a low fat-high starch diet developed more atherosclerosis than animals fed 20 per cent maize oil, or 10 per cent maize and 10 per cent butterfat, or 10 per cent maize-starch or a commercial diet. The diets which produced the highest percentage of atheromas had a serum phospholipid to cholesterol ratio of one or less. The linoleic to oleic acid ratios of the diets which produced the least atheromas were two to three times greater than in atherogenic diets. The authors concluded that diets high in linoleic acid maintain a sufficient serum phospholipid level to prevent the development of atheromas.

Hildreth et al. (1951) demonstrated in an experiment conducted on three human males that the serum cholesterols could be decreased by decreasing the dietary fat. Diets which contained low cholesterol but normal quantities of fat produced elevated serum cholesterols.

Groom (1959) autopsied 267 Negroes from Haiti and South Carolina. He found that the Negroes from South Carolina had almost doubled the average severity of atherosclerosis. He related much of this to the difference in tempo of life. However, upon evaluation of the average American Negro diet, the following facts were revealed: 1) the caloric intake was 30 per cent higher, 2) the protein intake from animal origin was 83 per cent greater, 3) the fat intake from animal sources was five times higher and 4) the cholesterol intake was six to seven times greater than that consumed by the Negro from Haiti.

Okey et al. (1959) tested 13 different dietary fats with iodine numbers ranging from 10 (coconut oil) to 113 (corn oil). In general, the fat with the lowest iodine number produced the lowest liver cholesterol values in rats. The cholesterol fed female rats had consistently higher serum cholesterol values than the male rats. The fatty acid moiety of the liver cholesterol was characteristic of the composition of dietary fat. These workers also demonstrated a high concentration of tetraenoic acid in the cholesterol esters of rats fed coconut oil, which indicates a synthesis of arachidonic from non-dietary origin.

Bronte-Stewart et al. (1956) demonstrated an elevated serum cholesterol in humans fed butter, beef drippings, beef muscle and eggs. Marine oils and ground nut oil decreased the serum cholesterol values. Sunflower seed oil and pilchard oil consistently reduced the serum cholesterol in diets containing cholesterol or animal fat.

Kinsell and associates (1952) produced a prolonged decrease of serum cholesterol and lipid phosphorus by replacing the normal diet for patients with a diet free of carbohydrate, high in vegetable fat and containing sodium caseinate. The total drop of serum cholesterol averaged 100 mg/100 ml while the lipid phosphorus decreased 5 mg/100 ml. The daily addition of 60 grams of cholesterol to a diet high in vegetable fat did not elevate the serum cholesterol. Carbohydrate free diets supplemented with fats from dairy origin or egg yolk produced serum cholesterol and phospholipid values nearly identical with the patients on the normal diet.

Anderson et al. (1957) confirmed previous research by showing that the level and type of dietary fat have a central role in the regulation of serum cholesterol. A reduction in dietary fat (meat and butter) reduces sharply the level of serum cholesterol. When corn oil, olive oil and cottonseed oil were supplemented into low fat diets a greater decrease in the serum cholesterol level was observed, while coconut oil had no effect. The decrease in the serum cholesterol was primarily associated with the decrease of cholesterol in the β-lipoprotein fraction.

Nath *et al*. (1959) demonstrated in rats that coconut oil had little influence upon the serum cholesterol level. Cholesterol supplementation to the coconut oil diet produced significant increases in the serum cholesterol values and enhanced the essential fatty acid deficiency. Cholic acid supplementation to a cholesterol-coconut oil diet further enhanced the serum cholesterol values. Swell *et al*. (1955) fed soybean oil (hydrogenated to different iodine numbers) to rats on purified cholesterol-free diets. It was concluded that the blood cholesterol level varies directly with the iodine number. Free fatty acids were more effective in raising the blood cholesterol than neutral fats. Scott *et al*. (1961) found that the serum lipid phosphorus values rose rapidly in butterfat supplemented rats.

Deuel *et al*. (1954) demonstrated an increase in liver cholesterol and a decrease in plasma cholesterol in rats that were fed a 15 per cent hydrogenated coconut oil diet. After a period, the plasma cholesterol values returned to near normal.

Aftergood et al. (1957) placed weanling rats on purified diets containing 24 per cent casein, 0.24 per cent choline, bile salts, and either 15 per cent lard or 15 per cent cottonseed oil. Differences in the plasma cholesterol and liver lipids were not apparent unless cholesterol was present in the diet. It was also demonstrated that the females had higher plasma cholesterol and lower liver cholesterol than the males. Lard supplementation increases carcass lipids (cholesterol) more than cottonseed oil.

Avigan and Steinberg (1958) compared the serum cholesterol response to the addition of either 20 per cent hydrogenated coconut oil or 20 per cent corn oil to a commercial basal rat diet. The hydrogenated coconut oil produced higher serum cholesterol values than the corn oil. However, both oil groups had higher serum cholesterol values than the basal diet. Corn oil increased the esterified liver cholesterol whereas the hydrogenated coconut oil demonstrated no response.

Portman et al. (1956) demonstrated an increase in serum cholesterol values of the cebus monkey when a greater percentage of total calories were derived from corn oil. A greater increase in serum cholesterol values was observed when a hydrogenated oil replaced the corn oil. Kinsell and Micheals (1955) reported that human serum cholesterol and phospholipid values consistently decreased when vegetable oils replaced the animal fat. Libert and Rogg-Effront (1962) demonstrated after four months on an "inductive diet" containing cholesterol, sodium cholate and propylthiouracil that no significant differences in total serum esterified fatty acids or serum cholesterol occurred with rats fed peanut meal, lard, margarine or corn oil.

Moore and Williams (1964b) showed with rabbits that hydrogenated coconut oil elevated the lipid plasma fractions four to five times greater than the values obtained by supplementation with other fats. These workers also demonstrated that the phospholipid to free cholesterol ratio was correlated with the development of atherosclerosis. If the ratio were greater than one, atherosclerosis was not as pronounced, but when the ratio was less than one severe atherosclerosis developed. Schrade et al. (1961) found that the fatty acid content of the hyperlipemic human serum contained a greater porportion of palmitic, palmitoleic and oleic than linoleic or arachidonic. The changes in the fatty acid content were more severe as the lipemia increased.

Fatty Acids

Alfin-Slater et al. (1954) found that the liver and adrenal cholesterol increased and the plasma cholesterol decreased in rats fed a fatty acid deficient diet. The increase in liver cholesterol was attributed to the ester fraction. The esterification with a more saturated acid such as oleic may account for the accumulation of cholesterol in the liver, because it is unavailable for other tissue metabolism. Peifer and Holman (1955) found that a characteristic essential fatty acid deficiency syndrome could be produced in two weeks by supplementing low fat diets (essential fatty acid deficient) with cholesterol. However, if the low fat diet was not supplemented with cholesterol three months were required to produce the same characteristic syndrome.

Swell et al. (1955) demonstrated the importance of fatty acids on the absorption of cholesterol in the rat. The addition of saturated fatty acids to the diet caused greater increases in serum cholesterol than unsaturated fatty acids. The dietary supplementation of cholesterol and cholesterol butyrate produced higher serum cholesterol values than when cholesterol oleate or cholesterol linoleate was fed. The speed at which the fatty acid is cleared from the cholesterol molecule in decreasing order is butyrate, oleate, and linoleate. The addition of bile salts caused increased levels of serum cholesterol.

Fisher and Kaunitz (1964) demonstrated in rats that medium chained saturated fatty acids (C_6 - C_{10}) exhibited a hypocholesteremic potential. The medium chained saturated fatty acids decreased the liver cholesterol below the level established by the long-chained saturated fatty acids.

Mead and Fillerup (1957) fed C-labelled stearate, oleate, and linoleate to rats. Stearate and oleate were found in the plasma triglycerides. The linoleate was found in the plasma phospholipids. In contrast to much of the literature, Klein (1958) demonstrated that the cholesterol esters of the liver 1) increased with low fat diets, 2) decreased with normal dietary fat and 3) increased with linoleic acid supplementation.

Kinsell et al. (1958) obtained lower plasma cholesterol levels when purified ethyl and glycerol esters of linoleic were supplemented to the human diet. Purified oleic acid did not produce a decrease in plasma cholesterol levels. These workers concluded that linoleic is the main ingredient of vegetable oil which produces the hypocholesteremic effect.

In evaluating the influence of dietary fats and degree of saturation on the development of hypercholesteremia in humans, McOsker et al. (1962) reported that fats which contain 25 per cent or less saturated acids and contained a polyunsaturated to saturated ratio of greater than 0.5 were hypocholesteremic. Butterfat which had the highest percentage of saturated fats and the lowest polyunsaturated to saturated ratio produced the highest serum cholesterols of the fats tested.

Erickson et al. (1963) reported that varying the polyunsaturated to saturated fatty acid ratio from 1.6 to 0.1 in a cholesterol free diet the human plasma cholesterol levels were not altered. However, when these same diets were supplemented with cholesterol, an elevation in the plasma cholesterol resulted.

Keys et al. (1959) supplemented pure arachidonic and oleic acids in human diets. They found that arachidonic caused an elevation of serum cholesterol which remained elevated several days after withdrawal of the oral supplement. The increase in the total serum cholesterol occurred in the β-lipoprotein fraction of the serum.

Karmen et al. (1963) fed C^{14} labeled palmitic, stearic, oleic and linoleic acids to rats by gastric intubation. The process of fatty acid absorption and lymph chylomicron triglycerides formation demonstrated no specifity for any of the four acids. However, the chylomicron cholesterol ester formation displayed a marked affinity for oleic acid.

Peifer and Holman (1959) supplemented essential fatty acids to weanling rat diets containing hydrogenated coconut oil. The addition of 1 per cent corn oil or 0.5 per cent linoleate caused greater growth responses when the hydrogenated levels of coconut oil were increased. As the levels of hydrogenated oil were increased with either essential fatty acid deficiency or essential fatty acid supplementation, polyunsaturated (trienoic) acids accumulated in the heart. The rats fed essential fatty acids had a lower heart trienoic content, but the level was increased 20-fold when supplemented with hydrogenated coconut oil.

Quackenbush and Pawlowski (1960) demonstrated that supplemented linoleic acid decreased the total plasma cholesterol of rats fed a cholesterol supplemented diet or a low-fat diet. These workers also demonstrated that the female rats had higher plasma cholesterol values than the male rats.

Böttcher and van Gent (1961) demonstrated on human aortas that as the degree of atherosclerosis increased, the percentage of unsaturated acids in the phospholipids decreased. The greatest change occurred in the arachidonic acid. Of the saturated fatty acids, palmitic seemed to have preference; however, it displayed no regular tendency.

Sinclair (1956) reported that the cholesterol becomes esterified with saturated fatty acids when either dietary essential unsaturated fatty acids are unavailable or excess saturated fatty acids are available. He also stated that the phospholipids become more saturated and are not able to leave the plasma for metabolism by the tissue. The increased levels of phospholipids then initiate thrombosis.

Choline

Griffith and Wade (1939) first described the choline deficiency syndrome in young rats. The syndrome is characterized by a hemorrhagic enlargement and degeneration of the kidney, regression of thymus, and an enlargement of the spleen. The deficiency is prevented by amounts of choline too small to influence the deposition of liver fat. Griffith (1940) characterized the choline deficiency in rats as follows: 1) female rats were least affected, 2) older rats were less susceptible, 3) kidneys were hypertrophied, and 4) choline supplementation hastened recovery.

Engel (1942) demonstrated that weanling rats fed 5 mg. of choline per day did not develop the hemorrhagic kidney lesions characteristic of choline deficiency. The lesions and death occur within 7-14 days after weanling rats have been placed on choline deficient diets. Ridout et al. (1954b) supplemented hypolipotropic diets with choline, betaine, inositol, and vitamin B_{12}. They found that choline, betaine, and vitamin B_{12} would prevent the characteristic serum cholesterol drop on hypolipotropic diets. These workers concluded that factors which control liver lipids also control serum cholesterol.

Forbes et al. (1965) demonstrated that with a choline deficiency in rats the β-lipoprotein (low density) cholesterol and triglyceride and the serum lipid phosphorus are decreased. Choline deficiency did not affect the serum cholesterol content of the α-lipoproteins, but depressed the serum triglyceride content. Scott et al. (1964) demonstrated that choline deficiency did not alter the serum fatty acids of the rat.

Rice et al. (1956) demonstrated in rats that choline does not influence the absorption of cholesterol. Weiss et al. (1952) placed rats on a purified diet containing 20 per cent fat, 18 per cent casein, and 0.25 per cent bile salts.

Choline supplementation to the diet produced marked hypercholesteremia, while at the same time decreasing the hepatic sterol content. Zilversmith and Diluzio (1958) demonstrated that a single dose of choline to animals fed a choline deficient diet resulted in an increased liver fat oxidation and liver phosphatide synthesis. The addition of choline to livers derived from choline deficient animals increased the incorporation of P^{32} into the phosphatide molecule.

Harper (1958) demonstrated in rats that the dietary deficiency of choline can be alleviated by providing methyl group donors such as betaine or methionine. Folic acid and vitamin B_{12} supplementation decreases the severity of choline deficiency. The evidence, as indicated by Harper (1958) suggests that choline enhances the transportation of fat from the liver and the rate of oxidation of fat in the liver. Young et al. (1957) demonstrated that betaine and monomethylaminoethanol were just as effective as equimolar amounts of choline in the presence or absence of vitamin B_{12} for both growth and fat mobilization.

Wilgram and Hartroft (1955) demonstrated coronary arterial lipidosis, aortic sclerosis, and myocardial necrosis in choline deficient rats. The addition of cholesterol to choline deficient animals aggravated the cardiovascular lesions observed without increasing the serum cholesterol values. When cholesterol was supplemented to adequate choline diets, no changes were observed in the cardiac lesions.

Stetten and Salcedo (1945) fed various fatty acids to choline deficient rats. As the length of the fatty acids decreased (from 18C to 4C) the percentage of fatty livers increased. No severe fatty livers were observed when the ethyl esters of fatty acids (12 carbons or less) were fed to the rats.

Fischer and Garrity (1954) found that protein synthesis and blood protein concentration cannot be sustained in young rats fed choline deficient diets. The choline deficiency is enhanced because the amino acids are utilized first for the synthesis of protein rather than for choline.

It was demonstrated in 1954 by Wilgram et al. that rats fed a choline deficient diet developed fat deposits in the myocardial muscle. In a choline deficient diet, lard and beef fat produced a greater number of cardiac lesions than corn oil or coconut oil. By feeding rats a choline deficient diet, Hartroft et al. (1952) demonstrated stainable lipid in the endothelial cells of the intima of the aorta and necrosis and calcification of the small plaques in the large vessels.

Nino-Herrera et al. (1954) found that either additional protein or threonine and choline were required to prevent fatty livers in rats fed low-protein and choline free diets, thus indicating the presence of two distinct deficiences. Horning and Eckstein (1946) demonstrated an increase in the transport of radioactivity of serum phospholipids and a slight increase in liver phospholipids by supplementing choline to a lipogenic diet for rats.

Ridout *et al*. (1954a) fed various levels of cholesterol (0.2, 0.4, 0.8 and 1.6 per cent) to rats on hypolipotropic diets. When the cholesterol content was 0.2 per cent, sufficient choline and betaine were able to prevent the deposition of glycerides and cholesterol esters in the liver. Dietary choline failed to prevent the accumulation of liver cholesterol esters with diets rich in cholesterol. Inositol supplementation was without effect on the deposition of fat in the rat livers.

Best and Ridout (1936) showed a drop in the rat liver glyceride and cholesterol content when choline was supplemented to a high cholesterol diet. When cholesterol is removed from the diet, choline supplementation increases the rate at which cholesterol esters are removed from fatty livers. Best and Ridout (1933) demonstrated in the liver fat a decrease from 10 per cent to 4 per cent with choline supplementation to a 20 per cent fat and cholesterol diet.

Wilgram *et al*. (1955, 1957) demonstrated with rats on a choline deficient diet that the α-lipoprotein, β-lipoproteins and serum cholesterol values are decreased. These workers observed that cardiovascular lesions occurred without hypercholesteremia, hyperlipemia, or hyper-β-lipoproteinemia.

Blumenstein (1964) studied phospholipid metabolism in rats fed purified diets supplemented with and without guanidocetic acid or choline. The total liver lipid phosphorus content remained normal on diets deficient in lipotropic factors. There was, however, a change in the ethanolamine and the choline containing phospholipids in the livers of the animals fed the deficient diet. Guanidoacetic acid hastens the choline deficiency by being methylated and converted to creatine. This methylation decreases the methyl pool.

Protein

Channon and Wilkinson (1935) demonstrated in rats that the liver glycerides vary inversely with the protein content of the diet. As the percentage of dietary protein increases, the percentage of liver fat decreases irrespective of the action of choline. The protein content of the two diets was 5 and 30 per cent. Authors suggest that the amino acid balance may explain the differences. Best et al. (1936) demonstrated that casein exerts a choline like action in white rats. The lipotropic factor present in casein exerts a greater response in high fat diets than in low fat diets.

It is demonstrated by Harper et al. (1954) that neither adequate dietary methionine nor choline could reduce the liver fat accumulation on low protein diets. The fat content of the liver could be decreased by increasing the protein content of the diet. It was suggested that the protein acts in two ways: 1) supplying excess methionine which spares choline and 2) reduces the imbalances of amino acids which may contribute to the development of the fatty liver.

Fillios and Mann (1954) fed soyaprotein to rats with corn oil and sucrose. Although not reported, their work appears to demonstrate that as the dietary level of protein increased serum cholesterol values increased. Methionine supplementation to soyaprotein decreased the serum cholesterol values. The serum cholesterol values obtained from diets containing soyaprotein and supplemented methionine were nearly identical to those obtained with casein.

In preliminary work, Okey and Lyman (1956) found that higher levels of protein resulted in greater decrease of liver cholesterol in male rats than in female rats. In verifying this work, these workers found that castration of males decreased liver cholesterol, and estradiol administration to the castrated male further decreased liver cholesterol. They observed that food restriction and hormone treatment increased the serum cholesterol values in the high protein groups.

Hypercholesteremia has been demonstrated by Jones and Huffman (1956) when dietary protein in form of casein is fed to rats at levels either below or above 12-18 per cent. A group of rats fed 40 per cent casein developed hypercholesteremia without thiouracil or cholic acid supplementation.

The hypercholesteremia is associated with a decrease in α-lipoprotein and an increase in lower density lipoproteins.

Moyer et al. (1956) fed rats either casein or soya-protein with an inductive diet containing lard, cholesterol and cholic acid. Irrespective of the type of protein, the serum cholesterol values decreased with increased levels of dietary protein.

Fillios et al. (1956) reported that a casein level of 10 per cent in an "inductive diet" containing cholesterol, sodium cholate and thiouracil produced the highest serum cholesterol in rats. When the casein was increased to 60 per cent the serum cholesterol level was depressed. Serum phospholipid levels were not changed by the level of protein.

Olson et al. (1958a) demonstrated in young male rats that diets low in methionine and choline were hypocholesteremic. There was a decrease in both the α- and β-lipoproteins. These workers also demonstrated that the hypolipemia was prevented by supplementing casein to a hypolipotropic diet.

In a long duration experiment, Jones et al. (1957) fed casein at various levels to rats. They found that the level of casein did not influence coronary atheromatosis. Methionine supplementation did not influence atherosclerotic lesions in casein fed animals, but did produce an exaggerated hypercholesteremia. However, methionine supplementation decreased the hypercholesteremia response observed in the chow-fed controls.

Keys and Anderson (1957) evaluated dietary protein levels and serum cholesterol of humans. They found no changes in serum cholesterol by varying the dietary protein from 8.6 per cent of calories to 17.7 per cent of calories with a constant fat intake. In these experiments a dietary cholesterol level of 1000 mg/day did not influence the serum cholesterol. Olson et al. (1958b) studied the effects of altering the dietary protein level in the human. A reduction in dietary protein, without altering fat content or total calories, resulted in a hypocholesteremia and a hypo-β-lipoproteinemia. Their data suggest that alteration of dietary protein, particularly animal protein, does have an influence on the regulation of the serum lipids.

In contrast to previous work, Nath et al. (1958) demonstrated that the serum cholesterol of rats was the lowest when the animals were fed a 40 per cent casein diet and highest when the casein level was either 6 or 69 per cent. The diets of these rats contained 20 per cent hydrogenated coconut oil, 1 per cent cholesterol, and 0.5 per cent cholic acid.

Fillios et al. (1958) demonstrated in rats that either inadequate or excessive amounts of protein resulted in an increased amount of cardiovascular sudanophilia. The diets contained cholic acid as an atherogenic ingredient. Fillios et al. (1959) demonstrated that adenine was more atherogenic than other purine bases. Guanine, uric acid and xanthine produced hypercholesteremia without the cardiovascular changes. Of the pyrimidine bases, uracil caused the greatest hypercholesteremia and cardiovascular sudanophilia. Thymine caused mild changes while cyotsine demonstrated no atherogenic potential.

Methionine supplementation to low protein diets for rats was shown by Bagchi et al. (1963) to elevate the serum cholesterol. These workers also demonstrated that adequate protein, methionine or sulfoxamine administration reduced the sulfhydryl content of the liver and the serum cholesterol level.

Beveridge et al. (1963) placed 65 university students on a strict dietary regime of different protein levels ranging from 5 to 20 per cent of total calories. The diet contained 20 per cent butterfat and cholesterol (500 mg/1950 calories). They found that only the low level of protein (5 per cent of calories) produced hypercholesteremia.

Renaud and Allard (1964) observed fatty streaks and atherosclerotic plaques in rats fed a purified atherogenic diet containing sodium cholate irrespective of the level of dietary protein. The cholesteremic response was greater with lower levels of protein.

In evaluating the desirable protein level for rats, Allison et al. (1964) found that maximum liver weights, protein to DNA ratio and DNA to RNA ratios were obtained when 15 per cent casein was fed. Levels of casein above 30 per cent were stress factors to the animal.

Mendez (1964) demonstrated that rats fed a 5 per cent casein diet plus cholesterol maintained higher serum cholesterol values than those fed 20 per cent casein. The rats fed a 5 per cent casein diet had increased serum lipids after starvation whereas those fed 20 per cent demonstrated a decrease in serum lipids.

Vitamin B_{12}

During the process of fatty acid metabolism, all the even-numbered fatty acids are degraded by two carbon cleavages, but the odd-numbered fatty acids have a three carbon segment remaining. The three carbon section can enter the metabolism by forming a propionyl-Co A compound. The propionyl-Co A is converted to methymalonyl-Co A and finally to the succinyl-Co A compound of the tricarboxylic acid cycle. The conversion to succinyl-Co A has been shown to require vitamin B_{12}.

Marston et al. (1961) have demonstrated that sheep fed a vitamin B_{12} deficient diet could not utilize the propionic group in the tricarboxylic acid cycle. Erfle et al. (1964) reported that the concentration of liver methylmalonyl mutase was decreased in vitamin B_{12} deficient rats. Hsu and Chow (1957) demonstrated that serum cholesterol values of rats decreased when vitamin B_{12} was supplemented to vitamin B_{12} deficient diets. These workers also observed that the adrenal cholesterol content increased in the vitamin B_{12} deficient regime. Fox et al. (1956) demonstrated by chick growth studies that the vitamin B_{12} requirement increased from 2.5 micrograms to 75 micrograms/kilogram of diet with increased dietary fat. Fox et al. (1957) demonstrated that methionine and choline have a vitamin B_{12} sparing effect in chicks. Free methionine was more effective than choline or an equivalent amount of methionine supplied by casein. This work was confirmed by Weissbach and Dickerman (1965) which places vitamin B_{12} as an essential nutrient in the synthesis of methionine from the one carbon pool. Five-methylfolate and homocysteine combine in the presence of a vitamin B_{12} enzyme to form methionine. Buchanan et al. (1964) reported that a methyl transfer block occurs with a deficiency of vitamin B_{12}. These workers attribute the activity of vitamin B_{12} to its ability to regulate and maintain the folate compounds of the tissues.

MATERIALS AND METHODS

Experimental Design

Animals

Three hundred sixty Sprague Dawley white weanling rats[1] were randomly distributed to cages. Two animals were placed in each 8" x 10" cage. Large mesh cages were selected to allow the feces to drop through freely. The females were placed on one side of the rack and the males on the opposite side.

After all the animals were distributed, the rats were individually ear notched for identification. A paper punch with a 1/8" triangle shaped die was used to make a V-shaped notch in the ear. The animals were marked consecutively from 1 to 360.

Division of groups

Since each side of a rack has six columns, each split-plot (basal) diet was assigned to a column. The six basal split-plot diets are:

1. 10% Protein
2. 25% Protein
3. 10% Protein Vitamin B_{12} Deficient
4. 25% Protein Vitamin B_{12} Deficient
5. 10% Protein Choline Deficient
6. 25% Protein Choline Deficient.

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[1] Purchased from Holtzman Co., Madison, Wisconsin.

The animals were maintained on these diets from June 29, 1963 to February 14, 1964. On February 14, 1964, two racks were selected at random from the three racks for either cholesterol or cholesterol plus hydrogenated coconut oil supplementation. Hydrogenated coconut oil contains a high percentage of the saturated fatty acids and a low concentration of the essential fatty acids.[1] The animals were maintained on these described dietary regimes until the experiment terminated in July of 1964. (See Figure 1 for experimental design.)

Method of handling

The animals were fed diets as shown in Tables 1, 2 and 3. The diets shown in Tables 1, 2 and 3 have been isocalorically balanced by altering the cellulose content of the diet. The caloric density of the diet is 4.0 to 4.2 calories per gram of diet.

After weighing the ingredients, the diets were thoroughly mixed in a Patterson-Kelly Twin Shell Blender[2] for 10 to 15 minutes. The mixed diets were placed in plastic bags and kept in properly marked fibre drums. The animals were fed daily.

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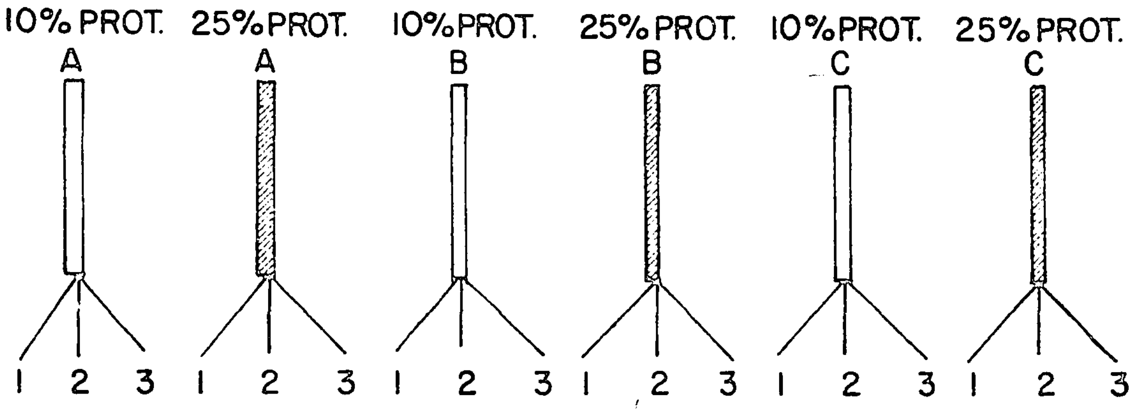
[1]Analysis provided by C. H. Cook of Procter and Gamble, Cincinnati, Ohio, the fatty acid composition is as follows: caproic - trace, caprylic - 4.2%, capric - 4.8%, lauric - 41.5%, myristic - 19.0%, palmitic - 12.0%, stearic - 13.8%, oleic - 4.2% and linoleic - 0.5%. The iodine number is 4.9.

[2]Patterson-Kelley Co., Inc., East Stroudsburg, Pennsylvania.

Figure 1 Experimental design of whole-plot and split-plot treatments

EXPERIMENTAL DESIGN



A = ADEQUATE DIET
B = VITAMIN B_{12} DEF. DIET.
C = CHOLINE DEF. DIET

1 = BASAL DIET
2 = BASAL DIET + 1% CHOLESTEROL
3 = BASAL DIET + 1% CHOLESTEROL WITH 12 % COCONUT OIL.

Table 1 Composition of Basal Diet

	10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
	%	%	%	%	%	%
Casein (devitaminized)[a]	11.8	29.4	11.8	29.4	11.8	29.4
Dextrose[b]	70.0	52.4	70.0	52.4	70.1	52.5
Coconut Oil[c]	8.0	8.0	8.0	8.0	8.0	8.0
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Mineral Mix[d]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin Mix 1[e]	0.1	0.1	---	---	0.1	0.1
Vitamin Mix 2[f]	---	---	0.1	0.1	---	---
Choline Chloride	0.1	0.1	0.1	0.1	---	---
	100.0	100.0	100.0	100.0	100.0	100.0

a. Sheffield Chemical, Norwich, New York.

b. Corn Products, Argo, Illinois.

c. Procter and Gamble, Cincinnati, Ohio.

d. Jones and Foster (1942) NaCL, 13.9%; KH_2PO_4, 38.9%; $MgSo_4$, 5.73%; $CaCO_3$, 38.1%; $FeSO_4$-$7H_2O$, 2.7%; KI, 0.08%; $MnSO_4$-$2H_2O$, 0.44%; $ZnCl_2$, 0.26%; $CuSo_4$-$5H_2O$, 0.048% and $CoCl_2$-$6H_2O$, 0.002%.

e. Thiamine -HCl, 0.5%, Riboflavin, 0.8%; Niacin, 4.0%; Pyridoxine, 0.5%; Ca - Pantothenate, 4.0%; Biotin, 0.04%; Folic Acid, 0.2%; Menadione, 0.5%; Cyanocobalanin (B_{12}), 0.003%; Inositol, 10.0%; p-Amino Benzoic Acid, 10.0%; Corn Starch, 65.36%; α-Tocopherol Succinate, 2.2%.

f. Vitamin Mix 2 same as Vitamin Mix 1 except Vitamin B_{12} is deleted.

Table 2 Composition of Cholesterol Supplemented Diet

	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
	%	%	%	%	%	%
Casein (devitaminized)[a]	11.8	29.4	11.8	29.4	11.8	29.4
Dextrose[b]	69.0	51.4	69.0	51.4	69.1	51.5
Coconut Oil[c]	8.0	8.0	8.0	8.0	8.0	8.0
Cellulose	5.0	5.0	5.0	5.0	5.0	5.0
Mineral Mix[d]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin Mix 1[e]	0.1	0.1	---	---	0.1	0.1
Vitamin Mix 2[f]	---	---	0.1	0.1	---	---
Choline Chloride	0.1	0.1	0.1	0.1	---	---
Cholesterol	1.0	1.0	1.0	1.0	1.0	1.0
	100.0	100.0	100.0	100.0	100.0	100.0

a - f. See footnotes Table 1.

Table 3 Composition of Cholesterol and Hydrogenated Coconut Oil Supplemented Diet

	10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
	%	%	%	%	%	%
Casein (devitaminized)[a]	11.8	29.4	11.8	29.4	11.8	29.4
Dextrose[b]	44.5	26.9	44.5	26.9	44.6	27.0
Coconut Oil[c]	19.6	19.6	19.6	19.6	19.6	19.6
Cellulose	17.9	17.9	17.9	17.9	17.9	17.9
Mineral Mix[d]	5.0	5.0	5.0	5.0	5.0	5.0
Vitamin Mix 1[e]	0.1	0.1	---	---	0.1	0.1
Vitamin Mix 2[f]	---	---	0.1	0.1	---	---
Choline Chloride	0.1	0.1	0.1	0.1	---	---
Cholesterol	1.0	1.0	1.0	1.0	1.0	1.0
	100.0	100.0	100.0	100.0	100.0	100.0

a - f. See footnotes Table 1.

The experiment proceeded as planned except for the low-protein choline deficient groups. During the first five days on test, the typical choline deficiency syndrome was produced. Ten rats died on the low-protein choline deficient diet. Of the ten, nine were males. These rats were replaced by the extra rats purchased. After two weeks on test, no rats were replaced.

Because of this deficiency syndrome, choline was added to the diets to reduce the mortality rate. When the death of the animals ceased and appearance became normal, the choline content of the diet was reduced gradually. However, choline was again added to the diet to assure completion of the experiment. The following schedule shows the dietary choline level at various times of the experiment for the low-protein choline deficient group:

Date	Choline Content of Diet (mg/gm)
June 24, 1963	0.00
June 29, 1963	1.00
July 13, 1963	0.50
September 30, 1963	0.25
November 19, 1963	0.00
March 9, 1964	0.25

Although the low-protein choline deficient diet contained a minimal amount of choline, it still remained deficient.

Some of the rats on March 9, 1964 became ill with a suspected viral pneumonia. Three animals were posted at the

Veterinary Diagnostic Laboratory and no bacterial organisms were cultured. However, the rats had a red, crusty nasal discharge characteristic of pneumonia. Six animals died between March 9, 1964 and April 4, 1964 with the previously described symptoms. In order to prevent secondary infection which might disrupt the entire experiment, chlortetracycline was given to all animals. An injection of five mg per rat was given as recommended by Haberman et al. (1963). The diet was also supplemented with tetracycline hydrochloride[1] (0.25 mg/gm diet) until April 1, 1964. The tetracycline hydrochloride was not needed during the remaining experimental period.

The drinking water was changed three times per week. The waterers were rinsed two to three times before being refilled. Every two to three weeks, all water bottles were scrubbed and disinfected with a quaternary ammonium compound.[2]

The animals were weighed every two weeks. The frequency of weighing increased the handling and allowed careful observation of each rat on test.

The animals were killed in a jar saturated with ether at the conclusion of the experiment. Just before death, the animals were removed from the jar and blood samples were drawn from the jugular vein or by cardiac puncture.

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[1]Polyotic-Tetracyline Hydrochloride, American Cyanamid Co., New York 20, New York.

[2]Quan-Sept, Fort Dodge Laboratories, Inc., Fort Dodge, Iowa.

Plasma Analyses

A lipid extract was obtained from the rat plasma. A methanol to chloroform mixture of 2 to 1 was used for the extraction procedure. Two ml of plasma were placed in a 50 ml volumetric flask and brought to volume with the methanol:chloroform mixture. Samples of the extract were used for duplicate analyses on each of the chemical determinations. If two ml of plasma were not available, 1 ml was used and diluted to 25 ml with the methanol:chloroform mixture and only a single analysis was conducted for each of the four chemical determinations.

Total cholesterol and cholesterol ester

Total cholesterol and cholesterol ester were determined according to the method described by Webster (1962). One ml and two ml of lipid extract were used for the total cholesterol and cholesterol ester determinations, respectively.

Total esterified fatty acids

Five ml of lipid extract were used for each determination of total esterified fatty acids as described by Connerty *et al*. (1961).

Lipid phosphorus

The procedure of Fiske and Subba-Row (1925) was used to determine the lipid phosphorus. The acid-soluble phosphorus was determined from eight ml of the lipid extract.

Blood Analyses

Packed cell volume

The packed cell volume was determined by the microhematocrit method. Whole blood was drawn into heparinized microhematocrit tubes and centrifuged for five minutes at approximately 11,500 rpm. The percentage of packed cells was determined by reading directly from a microhematocrit reader.

Hemoglobin

Hemoglobin values were obtained by the cyanmethemoglobin[1] method.

Total leukocyte count

Total leukocyte counts were made on whole blood with 0.1N HCl as diluting fluid. The cells were counted in a Neubauer Bright Line Counting Chamber. Differential leukocyte counts were made on Tetrachrome[2] stained whole blood smears.

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[1]Hycel Cyanmethemoglobin Determination Instructions. Hycel, Inc., Houston, Texas.

[2]Tetrachrome, Hartman-Leddon Co., Philadelphia, Pennsylvania.

Physiological Analyses

Blood pressure

The indirect blood pressure of the rat was determined with a Pneumatic Pulse Transducer[1] on the tail. The complete equipment is shown in Figure 2. The original equipment was designed to record on paper. However, the system was adapted to determine the systolic blood pressure with an oscilloscope.

The most critical adjustment in obtaining the blood pressure of the rat is to maintain the proper heating temperature of the holding cage pad. The maintenance of proper temperature is essential to enhance blood flow through the tail. Sobin (1945), Proskauer *et al.* (1945) and Olmsted *et al.* (1951) have demonstrated that heating is essential for maximum circulation and greater accuracy for repeated measurements of blood pressure. The desirable temperature is between 40° and 42°C. During the initial moments in the cage, excitement occurs which elevates the blood pressure. In these studies, four rat holding cages were used for the systolic blood pressure determinations. The rats were held in the holding cages for about six to twelve minutes prior to the determination of blood pressure.

By the third blood pressure measurement, the rats were trained and did not fear the holding cage. The blood pressure recording procedure was as follows:

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[1]E and M Instrument Company, Inc., Houston 21, Texas.

Figure 2 Indirect blood pressure measuring equipment for the rat

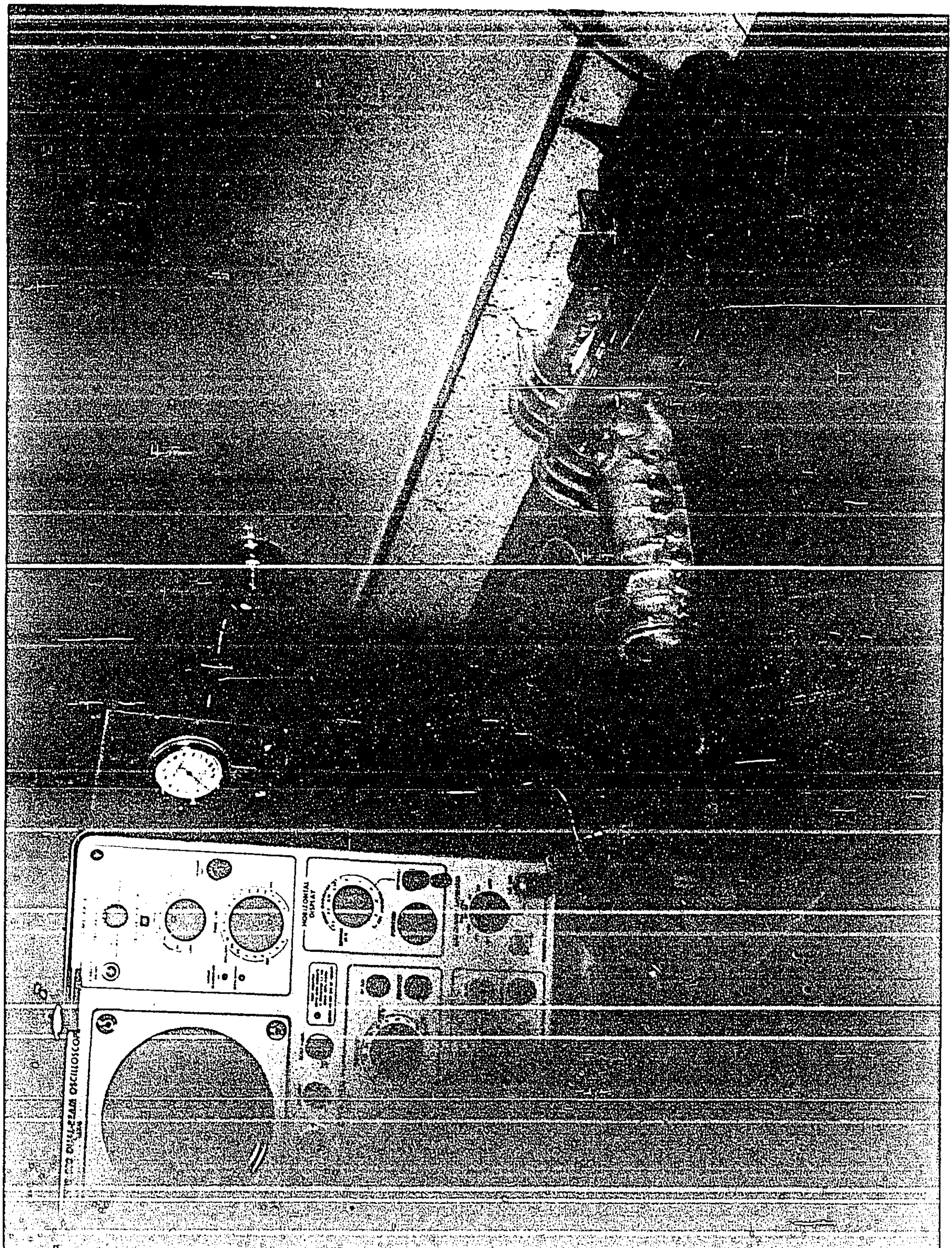
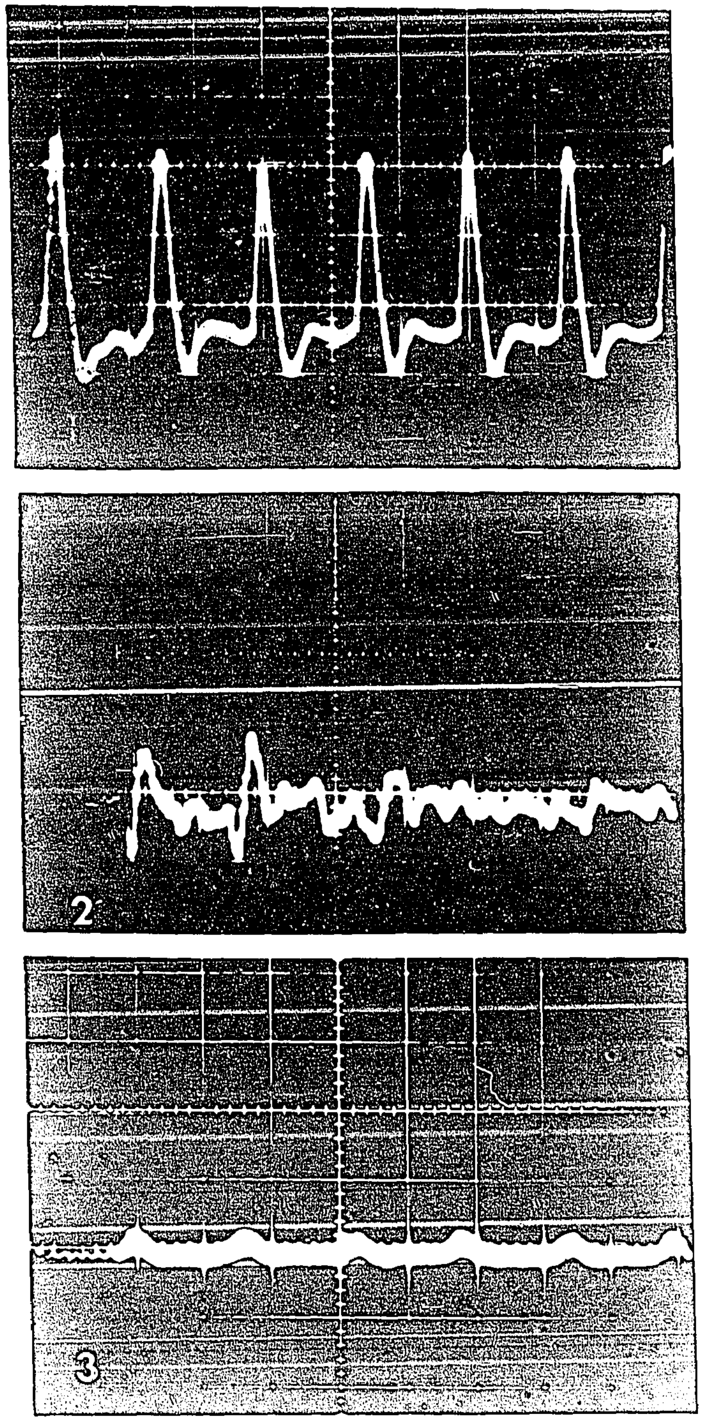

HORIZONTAL DISPLAY

Figure 3 Arterial pulse recordings

1. Arterial pulse recorded on the oscilloscope from the tail of a rat

2. Disappearance of arterial tail pulse with increased cuff pressure

3. Return of arterial tail pulse as cuff pressure decreased slowly



Sweep = 0.1 sec/cm

Sensitivity = 10.0 mv/cm

1. The pulse was picked up by the transducer and observed on the oscilloscope.

2. The tail cuff was occluded to stop blood flow. The pulse tracing on the oscilloscope disappeared and was replaced by either a straight line or random artifacts reflecting tail movements.

3. As the pressure was released from the cuff, the pulse reappeared on the screen of the oscilloscope.

4. The systolic pressure was recorded from the pressure gauge at the moment the pulse reappeared on the screen.

5. Two readings were taken in this same manner.

Figure 3 demonstrates the type of activity observed on the oscilloscope during the measurement of systolic pressure.

Liver fat and moisture

Total liver fat and moisture[1] were determined by procedures outlined in A. O. A. C. (1960).

Liver samples were frozen at the time of killing. Equal aliquots of liver (0.30 - 0.34 gm) were taken from each rat. The liver samples from both sexes in the same split-plot group were combined to form eighteen composite samples.

These samples represent all of the diets fed to the rats.

Histology

Tissue samples from the heart, aorta and liver were fixed in 10 per cent formalin. Paraffin tissue blocks and

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[1]Analyses were conducted by the Wisconsin Alumni Research Foundation, Madison, Wisconsin.

frozen tissue sections were prepared from the formalin-fixed samples. Frozen heart, aorta and liver sections were prepared from three or four randomly selected animals from each split-plot group. Hematoxylin and eosin slides were prepared from the same selected group of animals.

Photomicrographs of the fat stained tissues were taken on a Karl Zeiss Photomicroscope in the Department of Veterinary Pathology.

Hematoxylin and eosin stain

The hematoxylin and eosin staining technique as described in the Armed Forces Institute of Pathology (1957) was followed. However, ethyl eosin (1 per cent solution in 70 per cent alcohol) and 0.2 per cent glacial acetic acid were used in place of the eosin Y.

Sudan IV and Nile blue sulfate stains

The lipid components of the tissues were stained with Sudan IV and Nile blue sulfate after frozen sections had been prepared (Armed Forces Institute of Pathology, 1957).

The Sudan IV differentiated the lipids of the tissue by staining the fat orange to red and the nuclei blue. The Nile blue sulfate differentiated the lipids, although not too clearly, by staining the neutral fats pink, cholesterol red and the fatty acids blue to violet.

Method of evaluation

The slides stained for lipids were rated according to the intensity of staining. The rating scheme ranged from zero to four for the least to the greatest lipid staining components, respectively.

Computer Analyses

Data for this research project were analyzed statistically by an Analyses of Variance program with a computer.[1]

The variables studied are as follows: total cholesterol, cholesterol ester, total esterified fatty acids, lipid phosphorus, packed cell volume, hemoglobin, total leukocyte, body weight (end of experiment), and three blood pressure measurements. The F-test (Snedecor, 1956) was used for the evaluation of the results obtained from the computer.

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[1]Aardvark program, Computer Center. Iowa State University, Ames, Iowa.

RESULTS AND DISCUSSION

Plasma Analyses

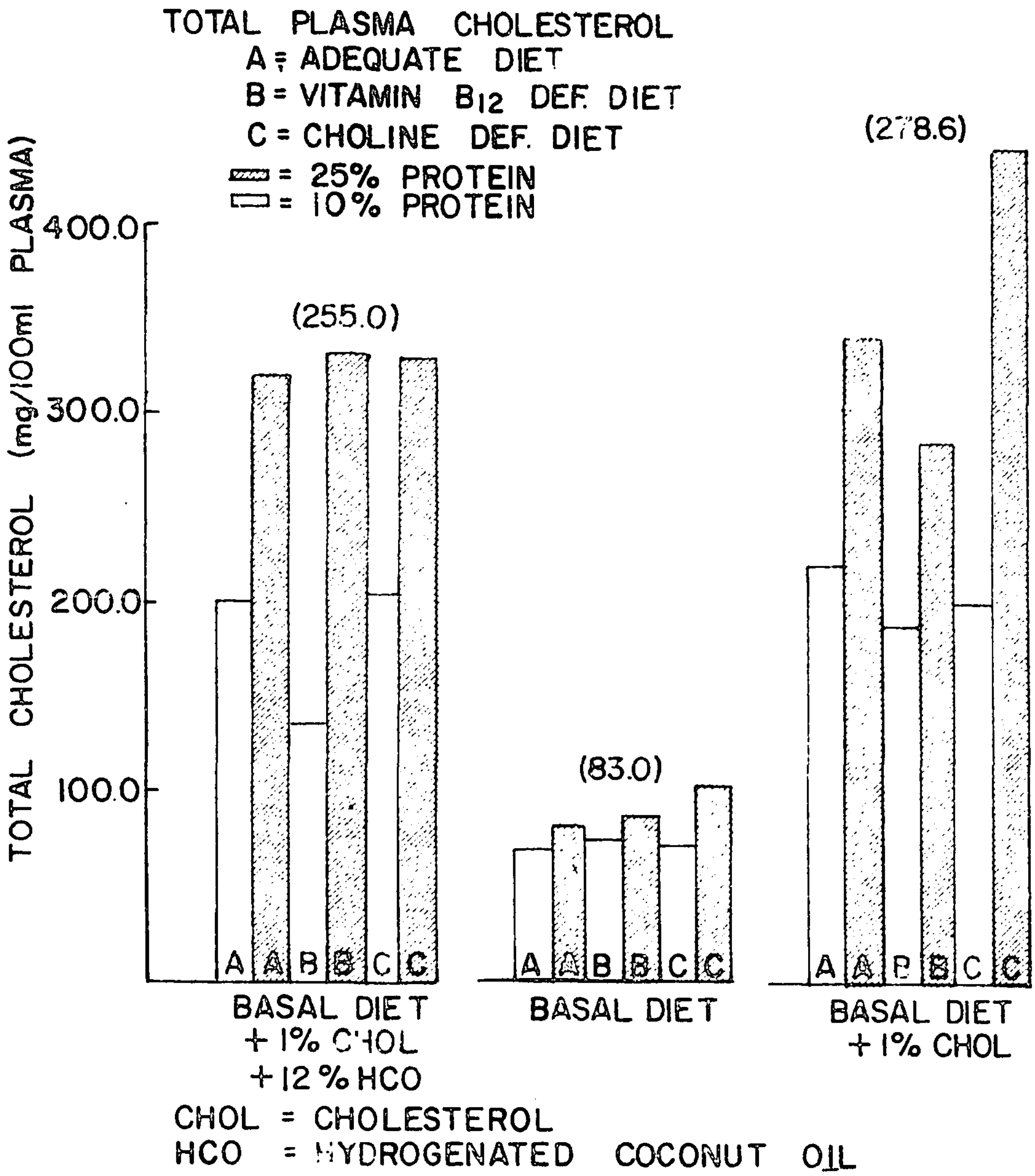
Total cholesterol

The analyses of variance and the F-test were conducted on blood cholesterol data. The hypercholesteremic response of the whole-plot supplemented with cholesterol and cholesterol plus hydrogenated coconut oil was significant at the one per cent level. These findings are in agreement with Nath (1958). As demonstrated in Figure 4, the mean whole-plot plasma cholesterol values are 83.2, 278.5 and 255.0 mg/100ml for the basal, cholesterol and the cholesterol plus hydrogenated coconut oil groups, respectively. Values for the males and females were averaged because of lack of interaction between sexes and whole-plot treatments.

The female rats were found to have significantly (1 per cent level) higher plasma cholesterol values than the males. By averaging the whole-plots and the split-plots, the female plasma cholesterol value was 231.1 mg/100ml and for the males 180.2. These sex differences are similar to the results reported by Aftergood *et al.* (1957), Okey *et al.* (1959) and Quackenbush and Pawlowski (1960).

In evaluating the split-plot influence on the plasma cholesterol, diets containing 25 per cent protein and supplemented with one per cent cholesterol and one per cent cholesterol plus hydrogenated coconut oil produced a

Figure 4 Total plasma cholesterol (mg/100ml) of rats on experimental diets


TOTAL PLASMA CHOLESTEROL
A = ADEQUATE DIET
B = VITAMIN B12 DEF. DIET
C = CHOLINE DEF. DIET
= 25% PROTEIN
= 10% PROTEIN
TOTAL CHOLESTEROL (mg/100ml PLASMA)
400.0
300.0
200.0
100.0
(255.0)
(83.0)
(278.6)
A A B B C C
A A B B C C
A A B B C C
BASAL DIET + 1% CHOL + 12% HCO
BASAL DIET
BASAL DIET + 1% CHOL
CHOL = CHOLESTEROL
HCO = HYDROGENATED COCONUT OIL

hypercholesteremia in both sexes. The hypercholesteremic response was significant at the one per cent level. These results agree with those by Best _et al_. (1936) and are similar to that of Jones and Huffman (1956) and Olson _et al_. (1958a), but contrary to findings of Nath et al. (1958), Fillios _et al_. (1956) and Moyer _et al_. (1956) who used atherogenic agents in the dietary regime.

There exists a protein to choline interaction in the females of the cholesterol supplemented group. The interaction is significant at the one per cent level and is represented by the following data:

Total Plasma Cholesterol
mg/100ml

	Choline	Choline Deficient	Difference
25% Protein	373.6	552.7	+179.1
10% Protein	236.6	211.2	- 25.4

Explanation of the interaction is not feasible. It is known that casein provides methyl groups in the synthesis of choline; however, one cannot explain why the hypercholesteremia occurred in the choline deficient group fed a 25 per cent protein while it did not occur in those supplemented with choline. Since the diets were identical in all nutrients except the protein level, it seems as though the amino acid content of the devitaminized casein may have directly caused this interaction. This interaction is also restricted to the females of the cholesterol supplemented group. Neither the males of the cholesterol supplemented group nor the females or males of the cholesterol plus hydrogenated coconut oil supplemented group exhibit a similar interaction. These results seem to indicate that the female hormones may also be involved in the regulation of the plasma cholesterol with the high-protein choline deficient diet.

Cholesterol ester

The whole-plot basal, cholesterol supplemented and cholesterol plus hydrogenated coconut oil supplemented groups have means of 70.5, 241.8 and 220.8 mg/100ml. The whole-plot differences as observed between the two supplemented whole-plots and the basal are significant at the one per cent level.

The plasma cholesterol ester is increased significantly at the one per cent level in the 25 per cent protein split-plot group by either cholesterol or cholesterol plus hydrogenated coconut oil supplementation. Table 4 demonstrates the cholesterol ester responses.

The females demonstrate an increase in the cholesterol ester which is significant at the one per cent level. The male and female averages are 155.6 and 199.7 mg/100ml, respectively. The plasma cholesterol ester responses are similar to the responses obtained for total plasma cholesterol.

Table 4 Plasma Cholesterol Ester (mg/100ml) of Experimental Rats[a]

	DIETS					
	10%	25%	10%	25%	10%	25%
Whole Plot Diets	Protein	Protein	Protein Vit. B_{12} Deficient	Protein Vit. B_{12} Deficient	Protein Choline Deficient	Protein Choline Deficient
Basal	62.8	70.6	64.4	73.3	66.0	85.5
Basal + Cholesterol	185.1	303.2	158.0	242.6	167.3	394.5
Basal + Cholesterol + Hydrogenated Coconut Oil	177.7	274.8	123.8	280.9	177.6	290.1

a. Values obtained by averaging both sexes.

Lipid phosphorus

The evidence, as demonstrated in Figure 5, shows that the responses to the whole-plot treatments produced values which are significantly different at the one per cent level. The plasma lipid phosphorus values for the basal, cholesterol and the cholesterol plus hydrogenated coconut oil groups are 4.8, 5.0 and 6.2 mg/100ml, respectively. The additional hydrogenated coconut oil caused a greater increase in lipid phosphorus than cholesterol supplementation alone.

Adams *et al*. (1963) have found that the extravascular subcutaneous plaque formation is not as severe if phospholipid is injected with cholesterol subcutaneously. The decreased ratio of phospholipid to cholesterol has been implicated as a cause of atherosclerosis (Moore and Williams, 1964a, and Kinsell and Micheals, 1955). When the phospholipid to cholesterol ratio is one or less, atherosclerosis has been shown to be more severe than when the ratio is greater than one. By multiplying the lipid phosphorus values obtained by 25, an approximation of the total phospholipid is obtained. Table 5 demonstrates the atherogenic capacities of the diets as indicated by the phospholipid:cholesterol ratio.

According to the phospholipid to cholesterol ratios, both the cholesterol and cholesterol plus hydrogenated coconut oil groups possess atherogenic capabilities. A further evaluation of these characteristics will be found in the histology section.

Figure 5 Plasma lipid phosphorus (mg/100ml) of the whole-plot and split-plot treatments

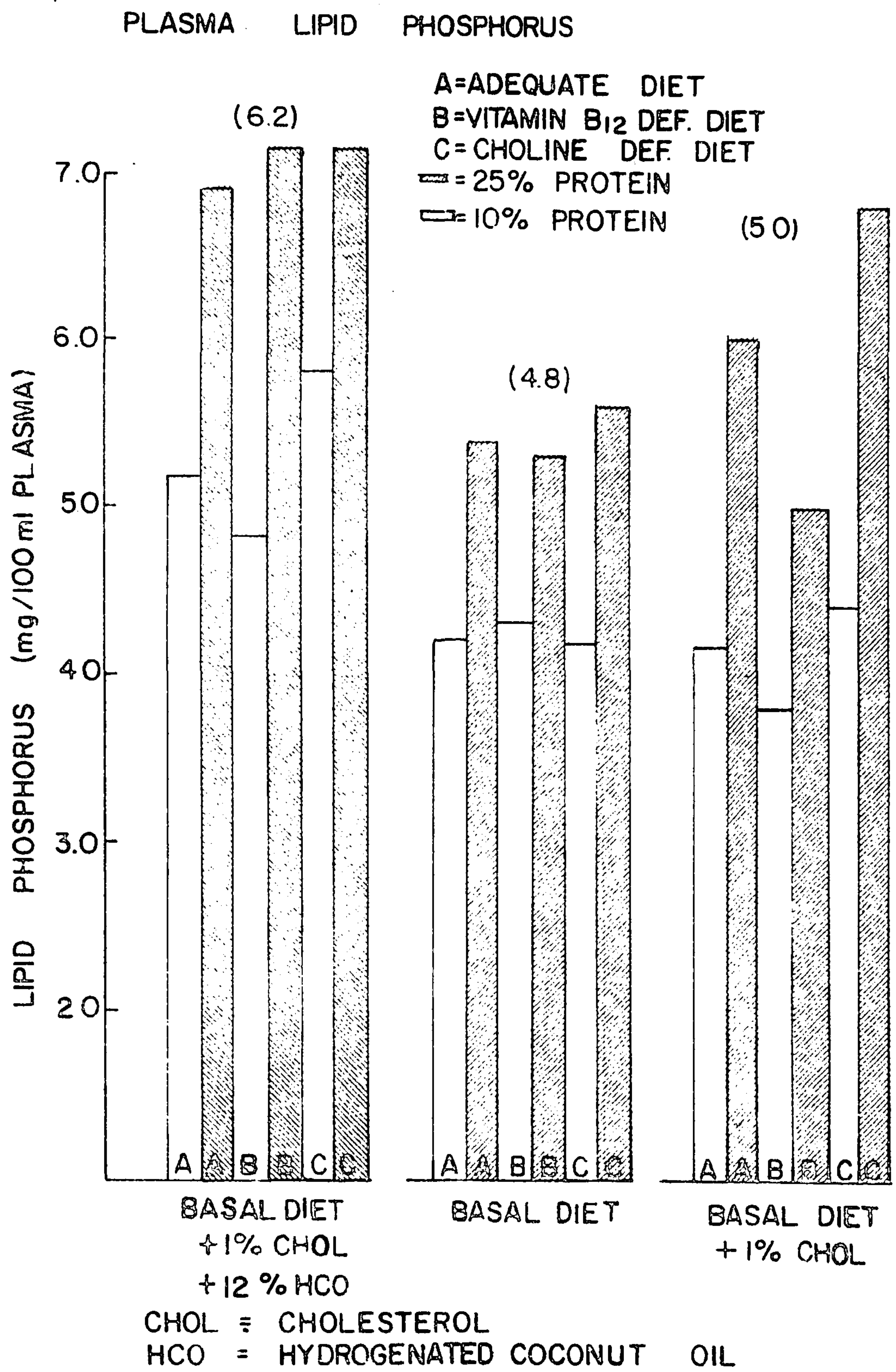

PLASMA LIPID PHOSPHORUS
A=ADEQUATE DIET
B=VITAMIN B12 DEF. DIET
C=CHOLINE DEF. DIET
= 25% PROTEIN
= 10% PROTEIN
(6.2)
(4.8)
(5.0)
7.0
6.0
5.0
4.0
3.0
2.0
LIPID PHOSPHORUS (mg/100 ml PLASMA)
A A B B C C
BASAL DIET + 1% CHOL + 12 % HCO
BASAL DIET
BASAL DIET + 1% CHOL
CHOL = CHOLESTEROL
HCO = HYDROGENATED COCONUT OIL

Table 5 Calculation of Phospholipid to Cholesterol Ratios and the Atherogenic Capacities of the Whole-Plot Treatments

Whole Plot Diets	Plasma Lipid Phosphorus mg/100ml	Phospholipid mg/100ml	Total Plasma Cholesterol mg/100ml	P:C Ratio	Atherogenic Capacity
Basal	4.8	120.0	83.3	1.44	-
Cholesterol	5.0	125.0	278.6	0.45	+
Cholesterol + Hydrogenated Coconut Oil	6.2	155.0	255.0	0.61	+

Table 6 Plasma Lipid Phosphorus (mg/100ml) of Experimental Rats

Whole Plot Diets[a]	Sex	10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	M	3.3	4.0	3.5	4.4	4.3	5.5
	F	5.1	6.9	5.2	6.4	4.2	5.8
B+C	M	3.4	5.4	2.9	3.9	4.5	6.3
	F	4.9	6.6	4.8	6.1	4.4	7.4
B+C+F	M	3.9	5.1	4.1	5.4	4.7	7.4
	F	6.6	8.8	5.6	8.8	6.9	6.8

(The six diet columns are printed under the spanning heading "DIETS".)

a. B = Basal
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

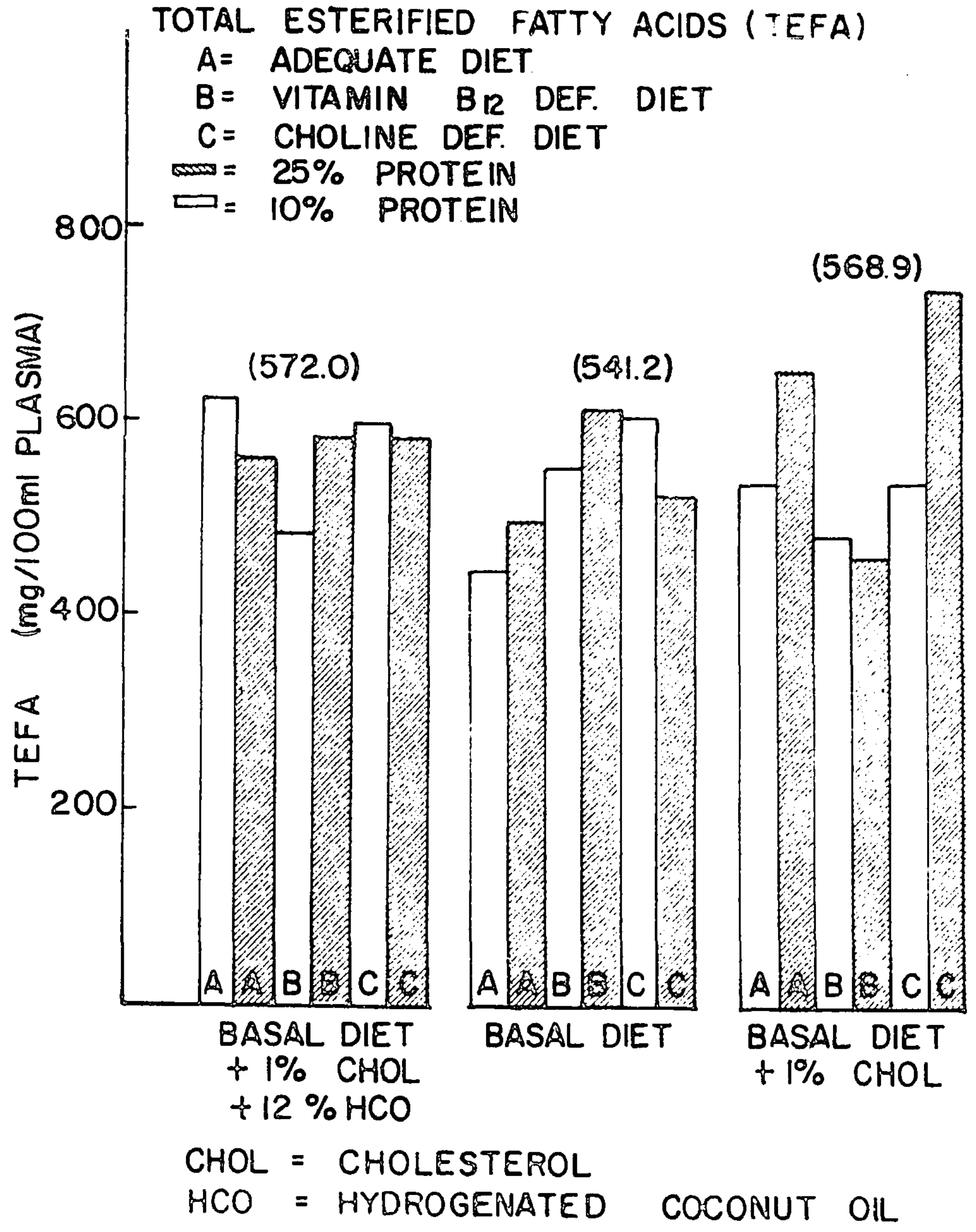
If the data are averaged according to sex (Table 6), the lipid phosphorus values obtained for the males and females are 4.6 and 6.2 mg/100ml, respectively. This difference is significant at the one per cent level. The plasma lipid phosphorus demonstrates a pattern very similar to that of total plasma cholesterol.

The split-plot treatment of 25 per cent protein increased significantly (1 per cent level) the lipid phosphorus in both sexes. Since choline is an integral part of the phospholipid molecule, the high casein diet may have provided sufficient methionine for the synthesis of choline. There is, however, another possibility which could explain the increase of the phospholipid content. The formation of cephalin, a phospholipid which does not contain choline, could have taken place. Since analyses for the different types of phospholipids were not conducted, it is impossible to determine the exact mechanism.

Total esterified fatty acids

With the sexes combined, the whole-plot total esterified fatty acid values for the basal, cholesterol and cholesterol plus hydrogenated coconut oil supplementation are 541.2, 568.9 and 572.0 mg/100ml, respectively (Figure 6). The observed responses were not significant which are similar to those reported by Libert and Rogg-Effront (1962).

Figure 6 Total plasma esterified fatty acids (mg/100ml) of the whole-plot and split-plot treatments with sexes combined


TOTAL ESTERIFIED FATTY ACIDS (TEFA)
A= ADEQUATE DIET
B= VITAMIN B12 DEF. DIET
C= CHOLINE DEF. DIET
= 25% PROTEIN
= 10% PROTEIN
800
600
400
200
TEFA (mg/100ml PLASMA)
(572.0)
(541.2)
(568.9)
A A B B C C
A A B B C C
A A B B C C
BASAL DIET + 1% CHOL + 12 % HCO
BASAL DIET
BASAL DIET + 1% CHOL
CHOL = CHOLESTEROL
HCO = HYDROGENATED COCONUT OIL

Within the cholesterol supplemented males, the 25 per cent level of protein significantly (1 per cent level) increased the total esterified fatty acids. However, in the cholesterol supplemented and cholesterol plus hydrogenated coconut oil supplemented males, an interaction between protein and vitamin B_{12} occurred. The interactions are, however, not identical but in opposite directions. The following data demonstrate the existing interactions:

Cholesterol Supplemented Males
total esterified fatty acids (mg/100ml)

	B_{12}	B_{12} Deficient	Difference
25% Protein	721.8	478.8	- 243.0
10% Protein	394.8	456.0	+ 61.2

Cholesterol Plus Hydrogenated Coconut Oil
Supplemented Males
total esterified fatty acids (mg/100ml)

	B_{12}	B_{12} Deficient	Difference
25% Protein	520.1	533.8	+ 13.7
10% Protein	645.9	412.0	- 233.9

The level of significance for the cholesterol supplemented males is 5 per cent while for the cholesterol plus hydrogenated coconut oil supplemented males it is one per cent.

These results indicated that the level of hydrogenated coconut oil may have caused the opposite interactions. However, more studies are needed to evaluate and verify this response.

With an exception of the total esterified fatty acids, the level of protein significantly affected the plasma total cholesterol, cholesterol ester and lipid phosphorus. The higher level of protein enhanced the plasma lipids by providing essential nutrients for the formation of the lipoproteins. The animals on low protein diet had values for plasma lipids which were much less than the high protein group. If hypercholesteremia is associated with atherosclerosis, then the level of dietary protein within the extremes of the normal limits does influence the plasma lipid. It is also evident that the hypercholesteremic response is greater in those diets supplemented with either additional hydrogenated coconut oil and cholesterol or cholesterol.

Blood Analyses

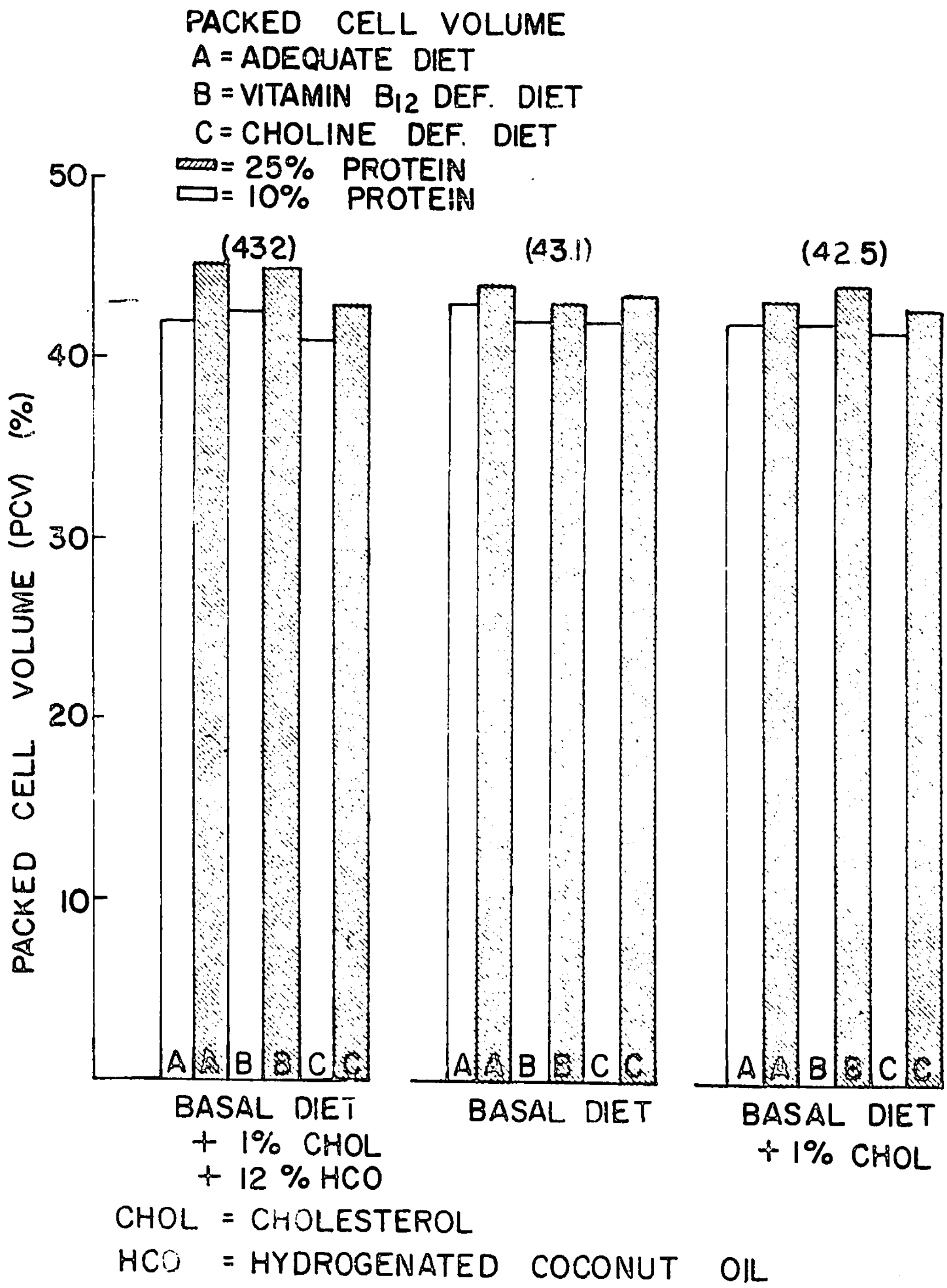
Packed cell volume

The packed cell volume averaged 43.1, 42.5 and 43.2 per cent for the basal, cholesterol supplemented, and cholesterol plus hydrogenated coconut oil supplemented groups, respectively (Figure 7). Although the whole-plot treatments did not affect the packed cell volume, a significant (1 per cent level) sex response was recorded. The males had a packed cell volume averaging 44.0 per cent and the females had an average of 41.9. There occurred within the split-plot groups a significant (1 per cent level) increase in the packed cell volume with increased levels of protein. Since interactions were not observed between the whole-plots and sex, whole-plots and split-plots, sex and whole-plots and sex and split-plots, it is possible to average the whole-plot treatments and sex to obtain six values representing the split-plot treatment. The following data represent the average split-plot response:

Split-Plot Group	Packed Cell Volume per cent
10% Protein	42.5
25% Protein	44.2
10% Protein - Vitamin B_{12} Deficient	42.2
25% Protein - Vitamin B_{12} Deficient	44.0
10% Protein - Choline Deficient	41.5
25% Protein - Choline Deficient	43.5

The data demonstrate that the packed cell volume values are normal for all the split-plot treatments.

Figure 7 Packed cell volume (per cent) of the whole-plot and split-plot treatments


PACKED CELL VOLUME
A = ADEQUATE DIET
B = VITAMIN B12 DEF. DIET
C = CHOLINE DEF. DIET
= 25% PROTEIN
= 10% PROTEIN
PACKED CELL VOLUME (PCV) (%)
50
40
30
20
10
(43.2)
(43.1)
(42.5)
A A B B C C
A A B B C C
A A B B C C
BASAL DIET
+ 1% CHOL
+ 12 % HCO
BASAL DIET
BASAL DIET
+ 1% CHOL
CHOL = CHOLESTEROL
HCO = HYDROGENATED COCONUT OIL

Hemoglobin

As found with the packed cell volume, the whole-plot treatments did not influence the hemoglobin values (Table 7). The statistical analyses revealed that both sex and split-plot treatments produced significant results. The mean hemoglobin value for the males was 14.9 gm/100ml and for the females 14.2. This sex response was significant at the one per cent level.

Since there were no interactions present, it is possible to average the hemoglobin values of the three whole-plots and of sex. The following averaged hemoglobin results represent the six split-plot treatments:

Split-Plot Group	Hemoglobin gm/100ml
10% Protein	14.1
25% Protein	14.8
10% Protein - Vitamin B_{12} Deficient	14.1
25% Protein - Vitamin B_{12} Deficient	15.0
10% Protein - Choline Deficient	14.1
25% Protein - Choline Deficient	15.0

The hemoglobin values obtained with the 25 per cent level of protein were significantly (1 per cent level) higher than those of the 10 per cent protein group. The hemoglobin values for all the split-plot treated groups were within the normal range. However, the higher protein supplementation probably provided adequate amino acids for both the body requirements and for the synthesis of hemoglobin. The data

Table 7 Hemoglobin Values (gm/100ml) of Whole-Plot Treatments

Whole Plot Diets[a]	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	14.4	15.0	14.2	14.9	14.3	15.3
B+C	14.0	14.7	14.1	15.0	14.1	14.7
B+C+F	13.9	14.9	14.2	15.1	13.8	15.1

a. B= Basal Diet
C= 1% Cholesterol
F= 12% Hydrogenated Coconut Oil.

also showed that the low protein provided sufficient amino acids for the synthesis of hemoglobin.

Leukocyte count

The whole-plot treatments demonstrated no influence on the total leukocyte counts. However, there are two responses which are significant. These responses are the effect of sex and the split-plot treatments of choline deficiency. With a significance level of one per cent, the males had a total leukocyte count of 7,837 per cmm which was higher than the count of 5,940 per cmm for the females.

The following data show these responses:

Split-Plot Diets	Total Leukocyte Count count/cmm		
	Males	Females	Avg.
10% Protein	8,162	6,418	7,290
25% Protein	8,588	6,123	7,356
10% Protein-Vitamin B_{12} Deficient	8,175	5,681	6,928
25% Protein-Vitamin B_{12} Deficient	8,075	6,040	7,056
10% Protein-Choline Deficient	6,716	5,730	6,223
25% Protein-Choline Deficient	7,310	5,650	6,480

Although the higher protein level appears to increase the leukocyte count, the increase is not significant. The reduction of the total leukocytes as demonstrated in the choline deficient group is significant at the one per cent level.

Physiological Analyses

Blood pressure

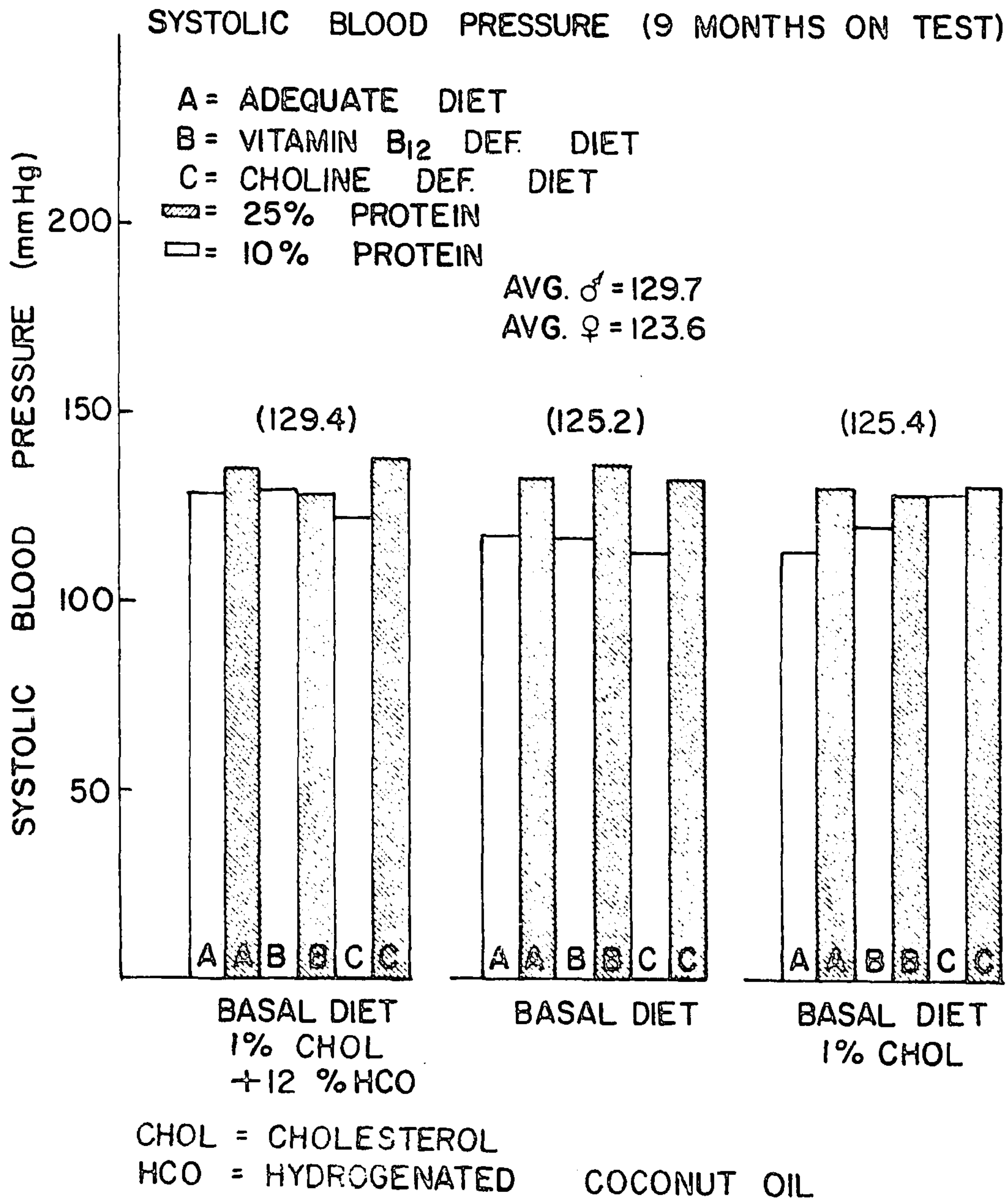
The most outstanding characteristic revealed by the statistical analyses was the influence of sex. In all three blood pressure measurements (9, 11 and 12 months) the males had a significantly (1 per cent level) higher systolic blood pressure than the females. The following data demonstrate the influence of sex:

Months on Test	Systolic Blood Pressure (mmHg)	
	Males	Females
9	129.7	123.6
11	132.8	124.9
12	129.9	123.9

Nine months on test In the blood pressure measurement taken after nine months on test (Figure 8) the higher level of protein significantly increased the blood pressure in all whole-plot groups.

An interaction (significant at 5 per cent level) was found to exist between the protein level and choline in the whole-plot treatment of cholesterol supplementation. The choline deficiency caused an elevated systolic blood pressure in the ten per cent protein, but not in the 25 per cent protein split-plot. The apparent cause of the elevated blood pressure in the low protein split-plot group can not be explained. However, it is possible that the lack of choline

Figure 8 Systolic blood pressures of rats on whole-plot and split-plot treatments after nine months on experiment


SYSTOLIC BLOOD PRESSURE (9 MONTHS ON TEST)
A = ADEQUATE DIET
B = VITAMIN B12 DEF. DIET
C = CHOLINE DEF. DIET
= 25% PROTEIN
= 10% PROTEIN
AVG. ♂ = 129.7
AVG. ♀ = 123.6
SYSTOLIC BLOOD PRESSURE (mm Hg)
200
150
100
50
(129.4)
(125.2)
(125.4)
A A B B C C
BASAL DIET 1% CHOL +12 % HCO
BASAL DIET
BASAL DIET 1% CHOL
CHOL = CHOLESTEROL
HCO = HYDROGENATED COCONUT OIL

resulted in a diminished acetylcholine production which allowed over stimulation of the vascular system by the sympathetic nervous system.

Also, within the whole-plot treatment of cholesterol supplementation, the protein and vitamin B_{12} produced an interaction which is significant at the five per cent level. The following data demonstrate the interaction:

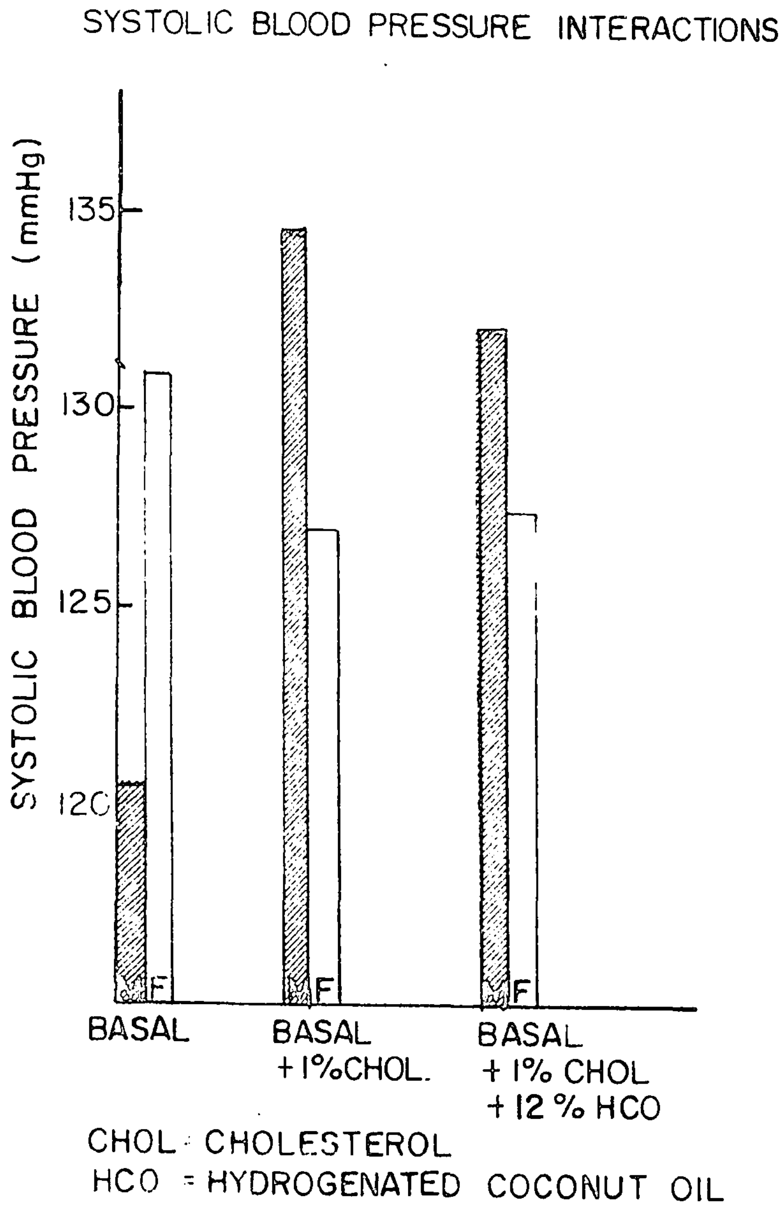
Systolic Blood Pressure (mmHg)

	B_{12}	B_{12} Deficiency	Difference
10% Protein	115.2	120.9	+ 5.7
25% Protein	130.4	127.4	- 3.0

An explanation of these results is impossible at this time. This interaction requires additional research to help explain the physiology involved.

Eleven months on test Of the three systolic blood pressure measurements, this measurement presented data which indicated that an interaction (1 per cent level of significance) exists between the whole-plot treatments and sex. Figure 9 shows the interaction between the whole-plot treatments and sex in the measurement taken after eleven months on test. The supplementation of the diets with either cholesterol or cholesterol plus hydrogenated coconut oil elevated the systolic pressure of the males and decreased the systolic pressure of the females.

Figure 9 Demonstration of the whole-plot and sex interaction of rats after eleven months on experiment


SYSTOLIC BLOOD PRESSURE INTERACTIONS
SYSTOLIC BLOOD PRESSURE (mmHg)
135
130
125
120
M
F
BASAL
BASAL
+1%CHOL.
BASAL
+1% CHOL
+12 % HCO
CHOL = CHOLESTEROL
HCO = HYDROGENATED COCONUT OIL

Although graphs of the same parameters for the first and last blood pressure measurements reveal similar characteristics, the results are not significant. The error term for both the first and last measurement is much higher than for the second.

Figure 10 demonstrates the relationship of sex to the split-plot treatment. The analyses of variance revealed that the 25 per cent protein diet significantly increased systolic pressure of the males but not the females. These findings indicate that a relationship exists between sex, diet and blood pressure.

As found in the first blood pressure measurement, the second measurement also demonstrated an interaction between protein and vitamin B_{12}. However, this interaction existed in the males and not the females. The following data reveal the interaction:

Systolic Blood Pressure (mmHg)

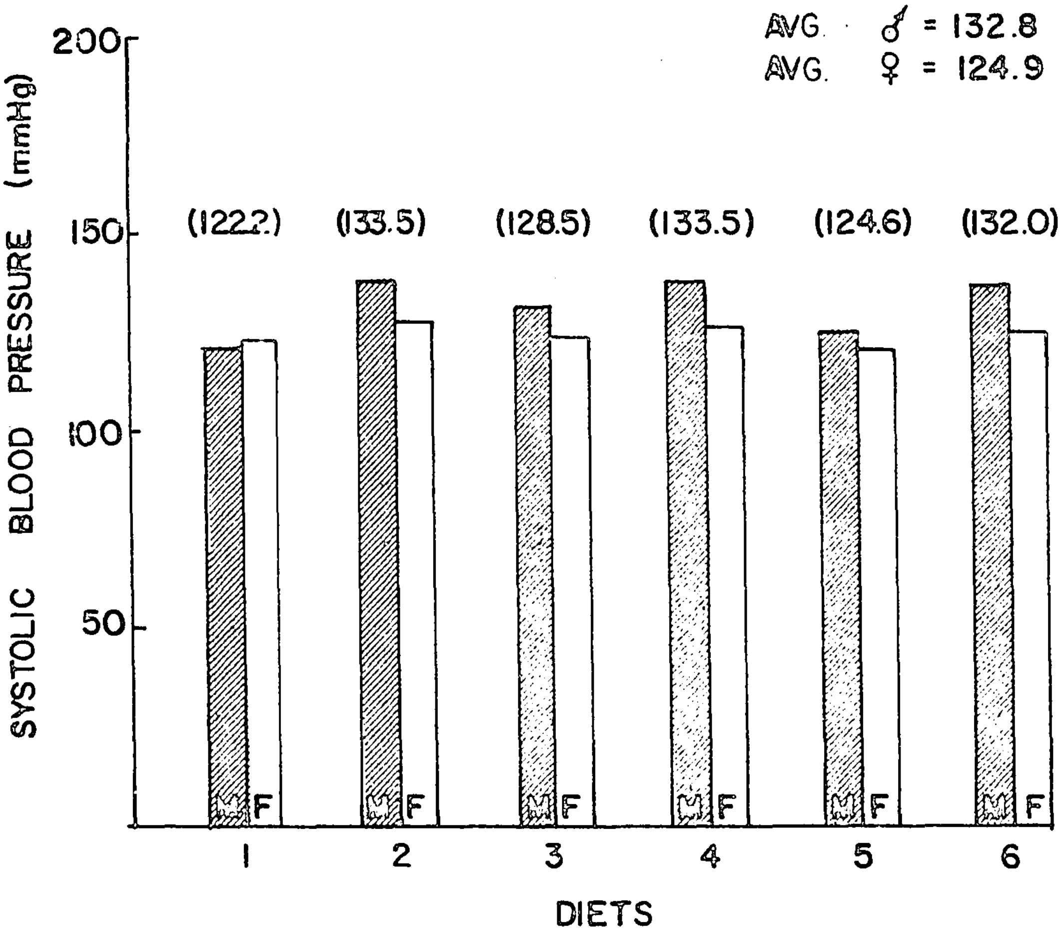
	B_{12}	B_{12} Deficiency	Difference
10% Protein	120.7	131.9	+ 11.2
25% Protein	139.2	139.0	- 0.2

The ten per cent protein-vitamin B_{12} deficient diet elevated the systolic pressure as compared with the 25 per cent level of protein. Since these data are consistent with the results obtained in the first blood pressure measurement, more work is required to clarify and help explain this interaction.

Interactions between vitamin B_{12} and protein were more pronounced in the blood pressure measurements than in the plasma analyses. These data suggest that deficiencies of vitamin B_{12} in low protein diets may have caused nervous system disorders before changes could be observed with the plasma analyses.

Figure 10 Systolic blood pressure of male and female rats on different split-plot treatments

SYSTOLIC BLOOD PRESSURE (11 MONTHS ON TEST)



1. 10% PROTEIN
2. 25% PROTEIN
3. 10% PROTEIN VITAMIN B_{12} DEFICIENT
4. 25% PROTEIN " " "
5. 10% PROTEIN CHOLINE DEFICIENT
6. 25% PROTEIN " "

Twelve months on test   The whole-plot treatments did not influence systolic blood pressure significantly. As demonstrated by the systolic blood pressures taken at eleven months on test, a sex and split-plot interaction was obtained. Testing within this interaction revealed that either a vitamin B_{12} or a choline deficiency produced elevated systolic blood pressures in females fed the ten per cent protein diet. The vitamin B_{12} interaction of the females is exactly opposite to that found in the males.

The protein to choline and protein to vitamin B_{12} interactions are demonstrated in the following data:

Protein to Choline Interaction
Female Systolic Blood Pressure (mmHg)

	Choline	Choline Deficiency	Difference
10% Protein	128.9	121.3	- 7.60
25% Protein	122.7	125.9	+ 3.20

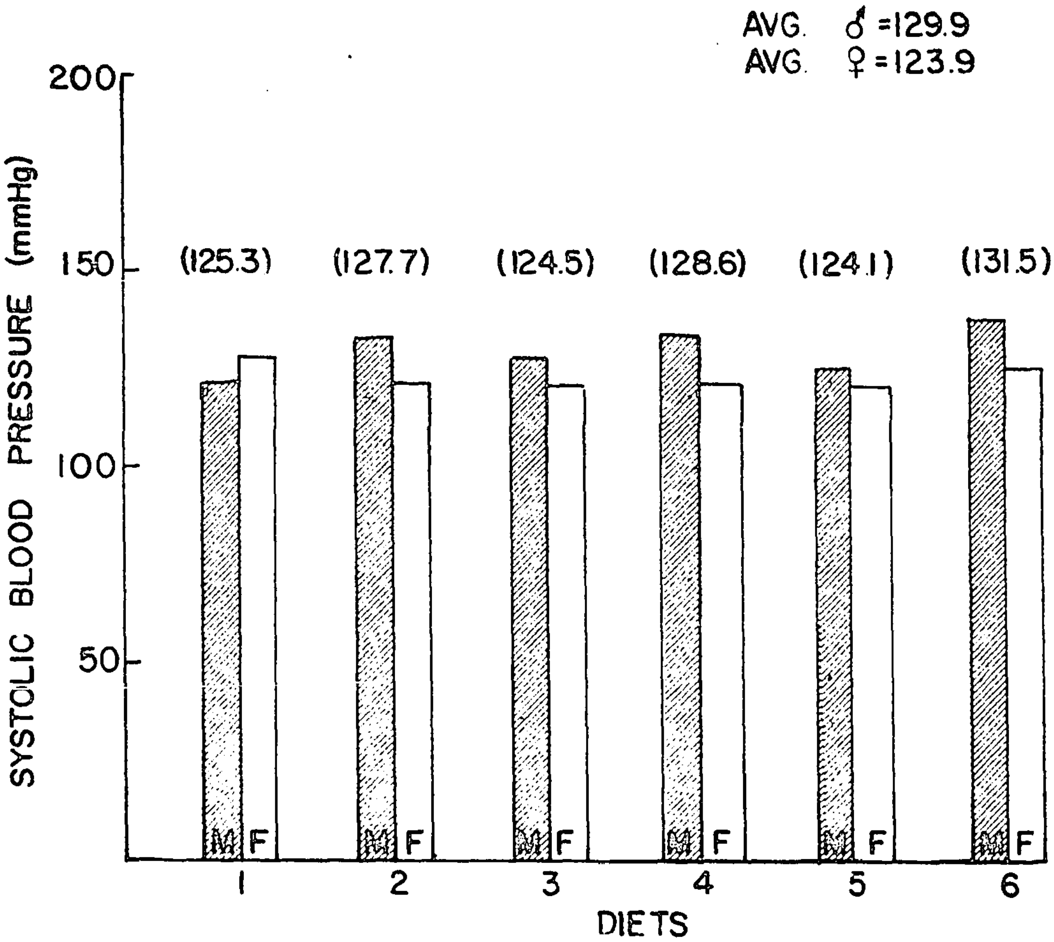
Protein to Vitamin B_{12} Interaction
Female Systolic Blood Pressure (mmHg)

	B_{12}	B_{12} Deficiency	Difference
10% Protein	128.9	121.1	- 7.8
25% Protein	122.7	123.9	+ 1.3

As demonstrated in Figure 11, the 25 per cent protein level produced significantly (1 per cent level) higher blood pressures in the males than females.

Figure 11 Systolic blood pressure of male and female rats in different split-plot groups

SYSTOLIC BLOOD PRESSURE (12 MONTHS ON TEST)



1. 10% PROTEIN
2. 25% PROTEIN
3. 10% PROTEIN VITAMIN B_{12} DEFICIENT
4. 25% PROTEIN " " "
5. 10% PROTEIN CHOLINE DEFICIENT
6. 25% PROTEIN " "

The level of protein was found to have some influence on the systolic blood pressures on the ninth, eleventh and twelfth month blood pressure measurements.

Final body weight

Figure 12 summarizes the final body weight of the rats. In all three whole-plot groups, the higher level of protein significantly (1 per cent level) increased the body weight. The whole-plot treatments averaged 275.3, 256.8 and 263.6 gms for the basal, cholesterol supplemented and the cholesterol plus hydrogenated coconut oil supplemented diets.

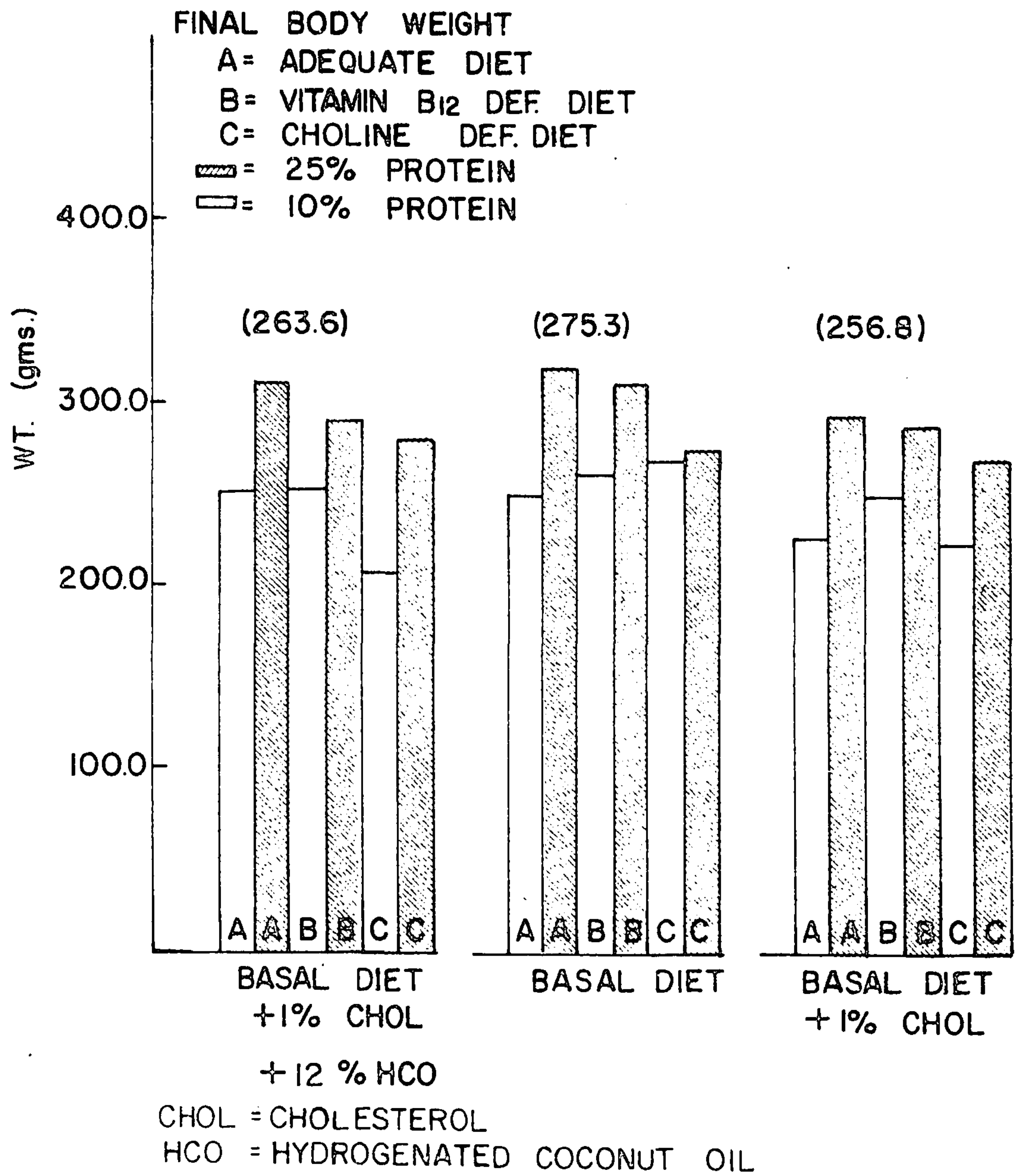
Within the cholesterol supplemented males and the cholesterol plus hydrogenated coconut oil females, an interaction existed between the protein and vitamin B_{12}. The following data demonstrate the interaction observed with the body weights:

Body Weight (gm)

		B_{12}	B_{12} Deficient	Difference
Cholesterol Supplemented (males)	25% Protein	382.2	335.0	- 47.2
	10% Protein	280.7	287.0	+ 6.3
Cholesterol + Hydrogenated Coconut Oil (females)	25% Protein	251.7	229.0	- 22.7
	10% Protein	196.7	217.0	+ 20.3

With the opposite sex of each of these groups, the vitamin B_{12} and protein interaction did not occur. In fact the final body weights of the vitamin B_{12} deficient group

Figure 12 Final body weights of rats of the whole-plot and split-plot treatments


FINAL BODY WEIGHT
A= ADEQUATE DIET
B= VITAMIN B12 DEF. DIET
C= CHOLINE DEF. DIET
= 25% PROTEIN
= 10% PROTEIN
WT. (gms.)
400.0
300.0
200.0
100.0
(263.6)
(275.3)
(256.8)
A A B B C C
A A B B C C
A A B B C C
BASAL DIET
+1% CHOL
+12 % HCO
BASAL DIET
BASAL DIET
+1% CHOL
CHOL = CHOLESTEROL
HCO = HYDROGENATED COCONUT OIL

were similar with those fed an adequate diet. Since two animals were housed per cage, the possibility exists that coprophagy between animals was of such magnitude that sufficient vitamin B_{12} may have been obtained to prevent a deficiency. No reason can be postulated for this sex and group specific interaction.

Liver fat and moisture

The 25 per cent protein diets in the split-plot treatment appeared to increase the total liver fat in the cholesterol and cholesterol plus hydrogenated coconut oil groups. The following data reflect the effect of dietary protein:

Liver Fat (Dry Matter Basis per cent)

Split-Plot Diets	Whole-Plot Treatments: Basal	Cholesterol	Cholesterol + Hydrogenated Coconut Oil
25% Protein	11.6	32.3	27.8
10% Protein	14.6	26.4	23.3

These data reveal also that cholesterol supplemented animals have liver fat accumulation equivalent to the animals supplemented with cholesterol and coconut oil.

Table 8 presents the liver analyses which were obtained from a composite sample from each split-plot group within each whole-plot. Portions of livers from both sexes were combined for these analyses.

Table 8 Moisture and Liver Fat on a Dry Matter Basis (%) of Experimental Rats

WHOLE PLOT DIETS[b]	BASAL DIETS[a] 1 M[c]	1 F-DM[c]	2 M	2 F-DM	3 M	3 F-DM	4 M	4 F-DM	5 M	5 F-DM	6 M	6 F-DM
B	70.3	13.8	70.6	12.9	70.1	16.4	71.0	12.1	70.9	13.7	70.0	10.0
B+C	69.4	23.5	67.0	30.3	67.5	25.8	66.5	30.4	67.1	29.8	64.4	36.2
B+C+F	69.0	22.5	66.5	31.3	70.5	22.4	68.1	25.4	65.9	25.2	66.1	26.8

a. 1 = 10% Protein
2 = 25% Protein
3 = 10% Protein - Vitamin B_{12} Deficient
4 = 25% Protein - Vitamin B_{12} Deficient
5 = 10% Protein - Choline Deficient
6 = 25% Protein - Choline Deficient.

b. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

c. M = Moisture
F-DM = Fat on Dry Matter Basis.

Histology

Heart

The Nile blue sulfate staining method did not demonstrate increased fat deposits in the heart. However, the Sudan IV stain revealed a slight increase in the amount of fat present in the heart muscle with cholesterol and cholesterol plus hydrogenated coconut oil supplementation. Data in Table 9 present the microscopic evaluation of the fat in the heart. The basal, cholesterol and cholesterol plus hydrogenated coconut oil groups had 5.1, 6.8 and 9.1 per cent of the animals with a number one rating. While there were no number two ratings in either the basal or cholesterol groups, the cholesterol plus hydrogenated coconut oil group had three per cent of the animals with the number two rating.

Although statistical analyses were not conducted on these data, the trend indicates that the cholesterol plus hydrogenated coconut oil supplemented group had a slight increase in the fat content of the heart.

Aorta

The evaluation data of the aorta (Table 9) indicate that the fat content of the intima of the aorta increases with either cholesterol or cholesterol plus hydrogenated coconut oil supplementation. The percentages of number one and two ratings are 8.3, 24.0 and 49.9 for the basal, cholesterol and

Table 9 Heart and Aorta Lipid Ratings (per cent) on Sudan IV Stained Histological Sections within Whole-Plot Treatments

Whole Plot Diets[a]	Rating[b]	Tissue Section Heart %	Aorta %
B	0-T	95.7	91.5
	1	5.1	5.5
	2	0.0	2.8
B+C	0-T	92.9	75.8
	1	6.8	17.2
	2	0.0	6.8
B+C+F	0-T	84.8	50.0
	1	9.1	43.7
	2	3.0	6.2

a. B = Basal
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. 0-T = Zero to trace increase of fat stained material
1 = Small amount of fat stained material
2 = Moderate amount of fat stained material.

cholesterol plus hydrogenated coconut oil supplementation, respectively. The severity of lipid stained aortas, as demonstrated with microscopic evaluation, demonstrates the dietary influence. Figures 13, 14 and 15 are examples of numerical ratings and demonstrations of aortas with lipid deposits.

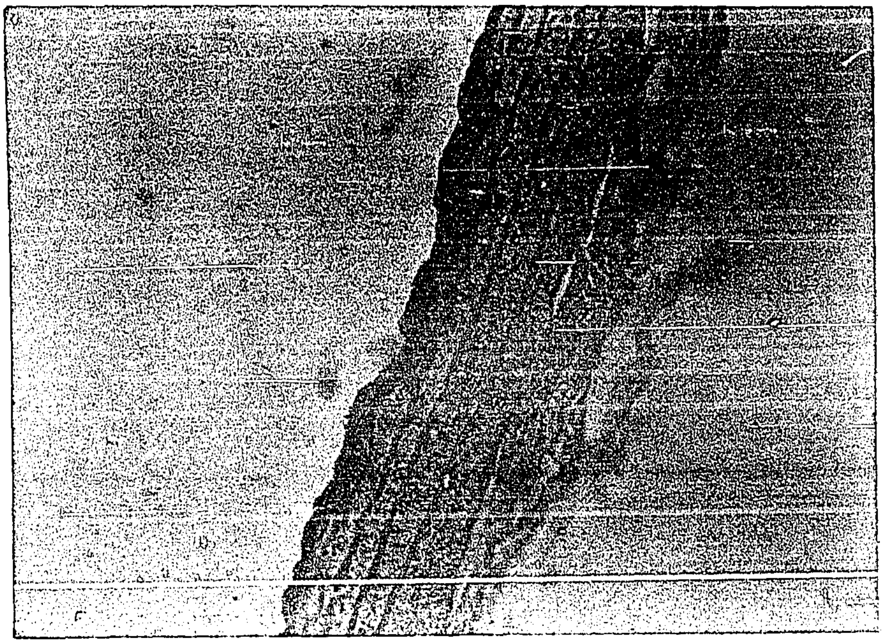
The heart appears to be more resistant than the aorta to lipid changes with the dietary supplementation of cholesterol or cholesterol plus hydrogenated coconut oil. The high

Figure 13 Aorta of rat 13 fed a 25% protein-basal diet supplemented with cholesterol and hydrogenated coconut oil. Sudan IV. 170x. (Microscopic evaluation - 2)

Note the red stained fat in the intima.

Figure 14 Aorta of rat 248 on the 10% protein-basal diet supplemented with cholesterol. Sudan IV. 170x. (Microscopic evaluation - T)

Note fat deposits in the media and just beneath the intima.



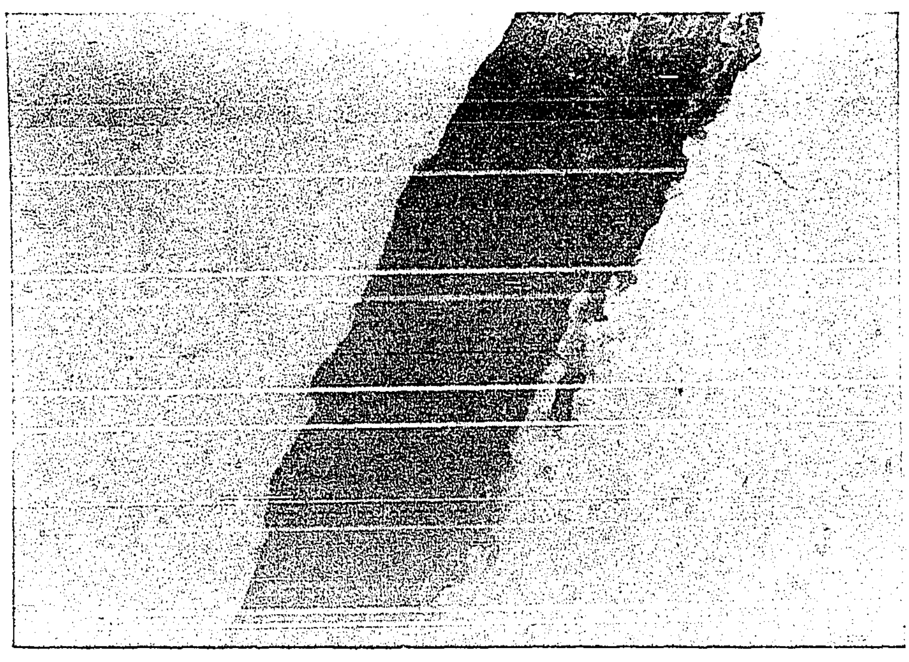
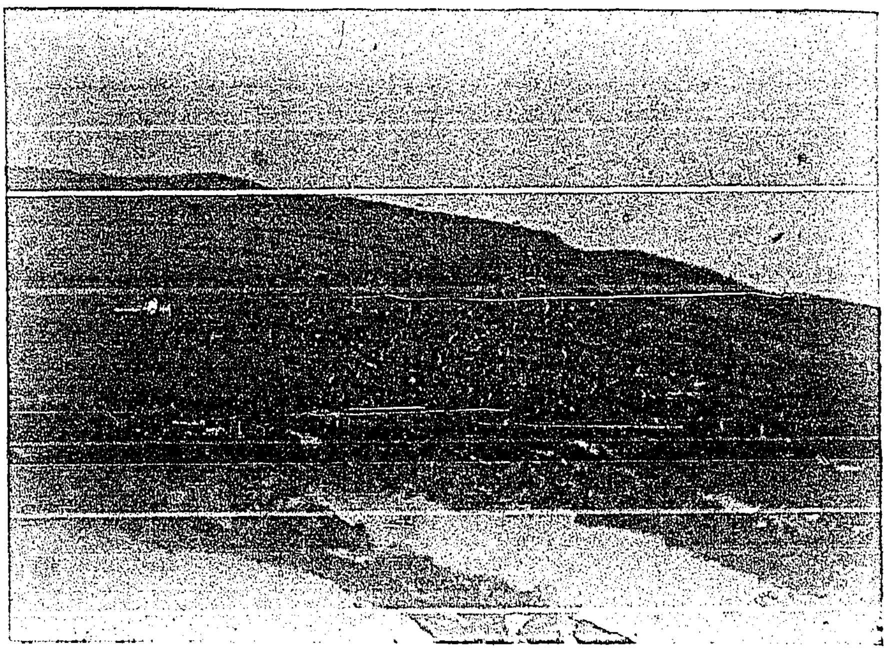


Figure 15 Aorta of rat 276 fed a 25% protein-vitamin B_{12} deficient diet supplemented with cholesterol Sudan IV. 170x. (Microscopic evaluation - 1) Note the presence of lipid stained material in the intima.



activity of the beating heart and the metabolic rate are possible factors contributing to the low fat content in cardiac muscle. On the other hand, the overall activity and metabolic rate of the aorta are probably much less than in the heart.

The phospholipid to cholesterol ratio, as previously discussed (Table 5), indicated that the two whole-plot treatments of cholesterol and cholesterol plus hydrogenated coconut oil had an atherogenic potential.

The following data combine Tables 5 and 9 to show the relationship of the phospholipid to cholesterol ratio and the microscopic evaluations of lipid deposits in the aorta:

Whole Plot Diets	Phospholipid: Cholesterol Ratio	Microscopic Evaluations		
		0-T	1	2
Basal	1.44	91.5%	5.5%	2.8%
Basal + Cholesterol	0.45	75.8%	17.2%	6.8%
Basal + Cholesterol + Hydrogenated Coconut Oil	0.61	50.0%	43.7%	6.2%

It is evident that some relationship may occur between the aortic lipid deposition and the phospholipid to cholesterol ratio. The data show that either cholesterol supplementation or cholesterol plus hydrogenated coconut oil supplementation increase the incidence of lipid deposition in the aorta.

Liver

The Nile blue sulfate stain did not provide clear-cut differential staining of the liver lipids. However, after summarizing the evaluation in Table 10, two characteristics are outstanding. The males of both the cholesterol and the cholesterol plus hydrogenated coconut oil groups demonstrate the greatest intensity of staining the neutral fats and cholesterol. The females in the cholesterol supplemented group demonstrate an increase in neutral fat and cholesterol as compared with the females in the two remaining whole-plot groups.

According to the data obtained from the Nile blue sulfate stain evaluation, the males responded differently than the females. The females of the whole-plot treatment of cholesterol plus hydrogenated coconut oil supplementation were resistant to the deposition of neutral fat and cholesterol (Nile blue sulfate). These results indicate that a hormonal influence may have contributed to the deposition of liver lipids especially in the high coconut oil group. However, as shown in the Sudan IV sections, the females had a high concentration of total lipid. It seems quite possible to conclude from these microscopic evaluations that either hormones or a metabolic peculiarity has inhibited the deposition of neutral fat and cholesterol when the diet contained additional coconut oil.

Table 10 Ratings[a] of Nile Blue Sulfate Stained Liver Sections of Experimental Rats

Whole Plot Diets[b]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient	Total
B	Male	0.1	0.0	0.0	0.0	0.0	0.0	0.1
	Female	0.0	0.0	0.0	0.0	0.0	0.0	0.0
B+C	Male	0.3	2.3	2.2	1.5	1.8	1.4	9.7
	Female	1.5	0.0	0.1	0.6	1.3	3.5	7.0
B+C+F	Male	0.1	1.2	2.0	1.8	1.8	2.0	8.9
	Female	0.0	0.0	0.0	0.0	0.0	0.0	0.0

a. Tissue sections were rated from zero for least fat stained to four for the greatest amount of fat stained:

0 = no increase of fat stained material
1 = small amount of fat stained material
2 = moderate amount of fat stained material
3 = marked amount of fat stained material
4 = abundant amount of fat stained material.

b. B = Basal
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

The Sudan IV, which stains total lipids, demonstrates the influence of the different dietary regimes. The microscopic evaluation closely parallels the data received with the liver fat analyses.

If the data were summed over the split-plot groups according to sex, a number is obtained which indicates the sex response to the whole-plot treatment. As demonstrated in Table 11, the females of the basal and the cholesterol plus hydrogenated coconut oil whole-plot treatments have decreased lipid staining intensity as compared with the males. However, in the cholesterol supplemented group the sex difference is not observed. There appears to be some hormonal relationship to the development of fat in the liver. Figures 16-23, selected as representative sections, demonstrate the liver fat staining and examples of the rating method.

With the exception of the 10 and 25 per cent protein-basal groups and the 10 and 25 per cent protein-vitamin B_{12} deficient groups supplemented with cholesterol, the microscopic evaluations parallel the total liver lipid analyses. As shown by both procedures, dietary cholesterol with or without hydrogenated coconut oil elevated the total liver lipid.

Because blood from the viscera and systemic circulation enters the liver in the portal canal area, nutrients of high concentration such as fat and cholesterol would be dispersed in the immediate area surrounding the portal canal. In the

Table 11 Ratings[a] of Sudan IV Stained Liver Sections of Experimental Rats

Whole Plot Diets[b]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient	Total
B	Male	2.3	2.8	2.2	0.8	1.3	0.5	9.9
	Female	0.5	0.3	0.8	0.5	1.6	0.5	4.2
B+C	Male	3.3	3.7	4.0	3.3	3.3	4.0	21.6
	Female	3.3	3.3	3.7	3.5	3.8	4.0	21.6
B+C+F	Male	3.3	3.7	3.5	4.0	3.7	3.5	21.7
	Female	2.7	3.3	2.0	4.0	2.3	3.0	17.3

a. Tissue sections were rated from zero for least fat stained to four for the greatest fat stained:

0 = no increase of fat stained material
1 = small amount of fat stained material
2 = moderate amount of fat stained material
3 = marked amount of fat stained material
4 = abundant amount of fat stained material.

b. B = Basal Diets
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

portal canal area, the hepatic artery, bile ducts and portal vein are adjacent to each other.

Since the basal whole-plot treated animals exhibited very low microscopic evaluations of liver lipids as compared with the other whole-plot groups, the low lipid concentration from the portal vein is probably the main factor in controlling liver fat concentration. It is possible, however, that the metabolic activity of hepatic cells adjacent to the central vein have been able to remove these lipids thus preventing accumulation.

Figures 16, 17 and 18 from control rats demonstrate the lack of lipid stained material near the portal vein and in adjacent hepatic cells. In contrast to the basal whole-plot diet, the cholesterol plus hydrogenated coconut oil supplemented animals show a high concentration of lipid around the portal canal and adjacent hepatic cells (Figures 19 and 20). In most of the sections, the concentration of lipid material was so high that nearly all of the hepatic cells between the portal vein and the central vein contained lipid material. The metabolic activity of this whole-plot treated group is not capable of removing the high lipid concentration brought by the portal vein from the small intestine.

The cholesterol supplemented group demonstrated characteristics similar to those of the cholesterol plus hydrogenated coconut oil supplemented group.

The elevated concentration of blood lipids including cholesterol has surpassed the normal limits of storage and metabolic utilization in the hepatic cells near the portal canal. The deposition of lipid material is similar to that previously described because the first deposition occurs near the portal vein and moves into adjacent hepatic cells, towards the central vein. As observed in Figures 21, 22 and 23, the lipid deposition appears granular as compared with the deposits of Figures 19 and 20. These may be cholesterol deposits, but individual analyses for the various lipid components would have to be conducted to verify the actual composition.

In most of the tissues sectioned, stained and evaluated, the hepatic cells adjacent to the central vein appear to be functioning normally. The deposition of lipid material in hepatic cells around the central vein in excess of that near the portal canal was not present. This indicates that the nutrient supply, including oxygen, to that area was sufficient to maintain normal cellular activity.

Mortality

During the course of the experiment, 21 rats died. The number of deaths totaled 5.9 per cent. A low grade viral pneumonia was diagnosed in the rats autopsied by the Iowa State Veterinary Diagnostic Laboratory. Table 12 gives the distribution of deaths in their respective groups. It is

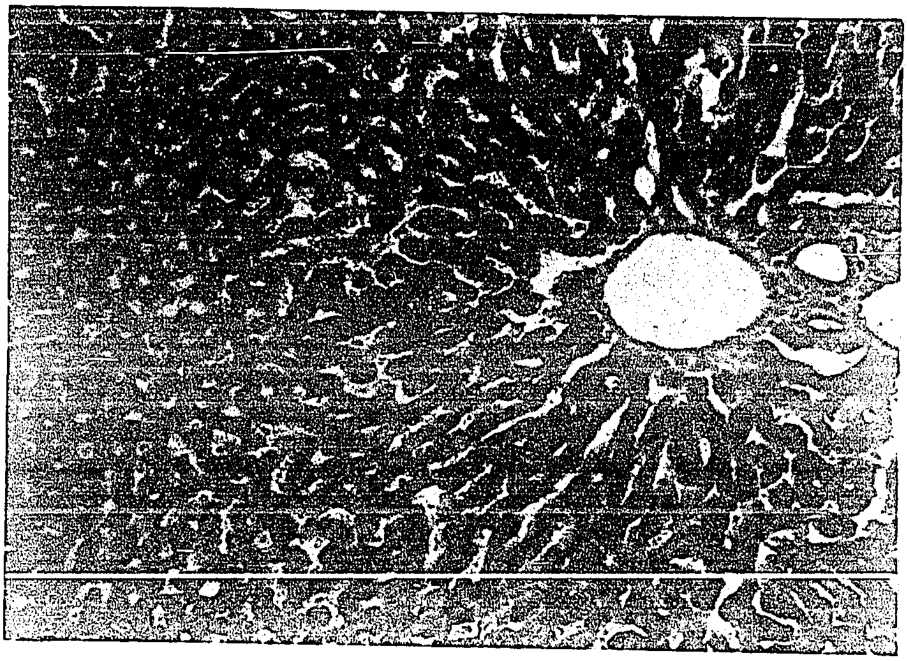
Figure 16 Liver of male rat 136 fed a 25% protein-basal diet

Sudan IV. 170x (Microscopic evaluation - 1)

Note the presence of the red stained lipid material near the portal canal. However, the lipid concentration decreases rapidly at a short distance from the portal canal.

Figure 17 Liver of male rat 168 fed a 10% protein-choline deficient diet. Sudan IV. 170x (microscopic evaluation - 0)

Note the absence of lipid stained material near the portal canal or in the microscopic field. There appears to be one small focal area of lipid concentration.



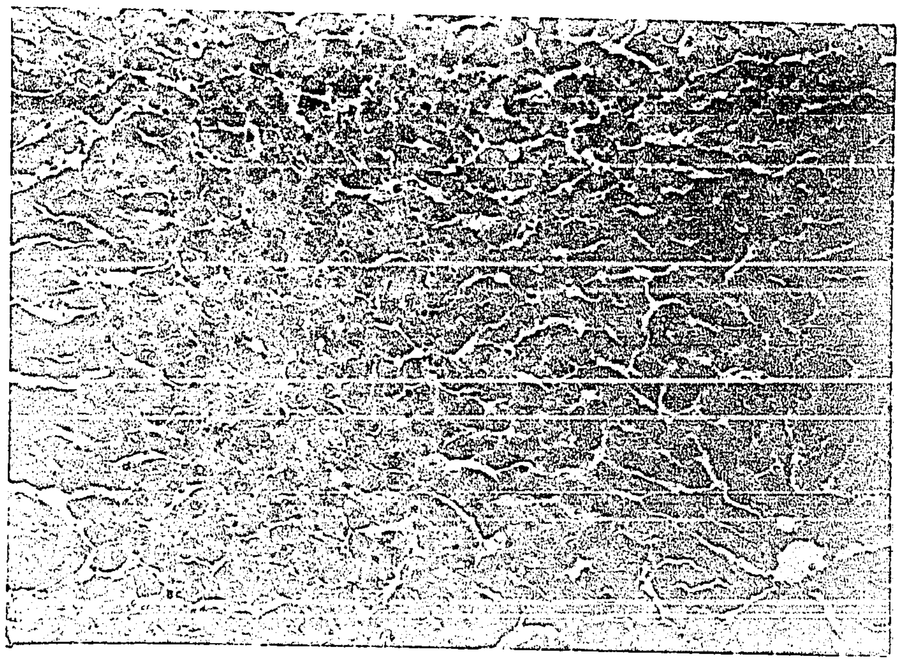
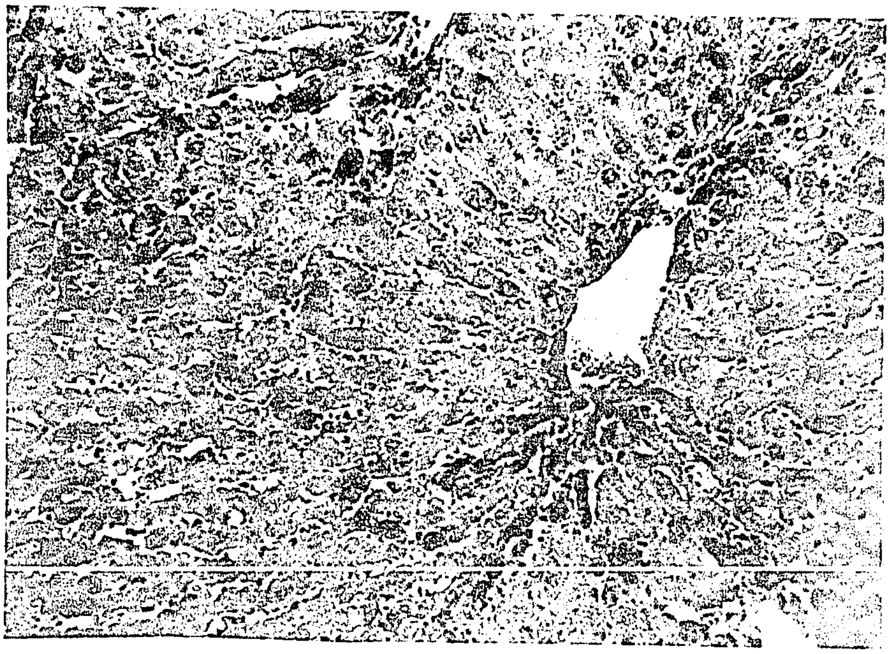


Figure 18 Liver of female rat 228 fed a 10% protein-choline deficient diet. Sudan IV. 170x (Microscopic evaluation - 0)

Note there are no large lipid concentrations visible. The central vein area is completely cleared of lipid.

Figure 19 Liver of male rat 41 fed a 25% protein-vitamin B_{12} deficient diet supplemented with cholesterol and hydrogenated coconut oil. Sudan IV. 170x (Microscopic evaluation - 4)

Note the increased lipid concentration near the portal canal. A rating of 4, such as this, indicates that the staining density was high in surrounding hepatic cells extending to the central vein. Note clear spaces where large fat globules were present.



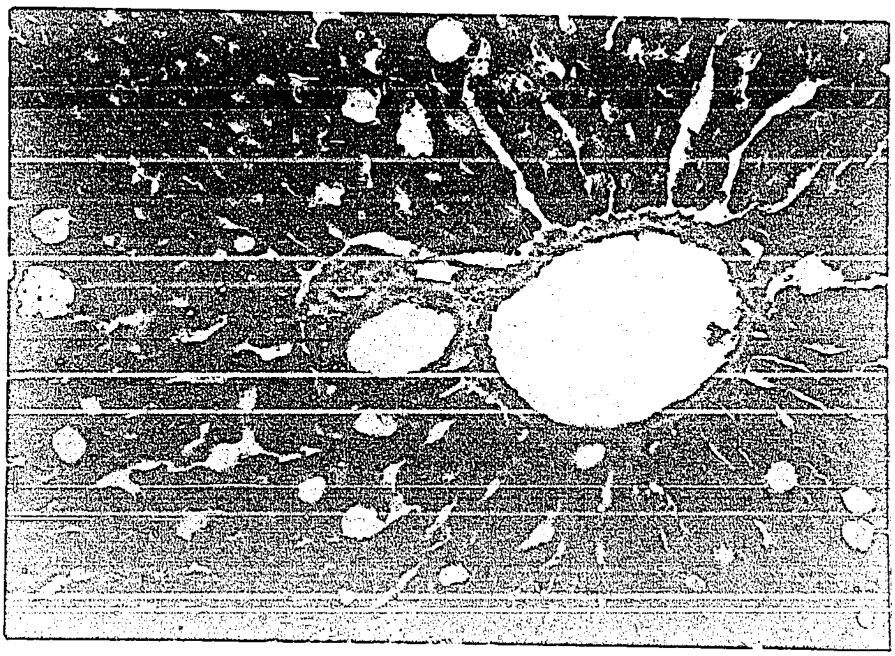
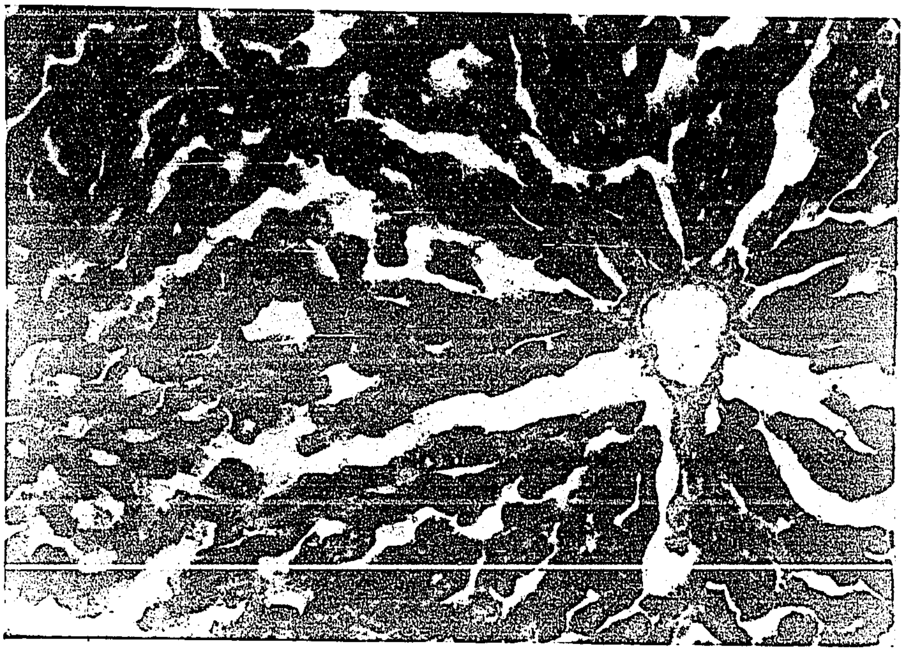


Figure 20 Liver of male rat 60 fed a 25% protein-choline deficient diet supplemented with cholesterol and hydrogenated coconut oil. Sudan IV. 170x (Microscopic evaluation - 4)

Note the density of the lipid staining around the portal canal and hepatic cells. The enlarged sinusoids were quite characteristic of the choline deficient group. A close examination reveals numerous small lipid globules.

Figure 21 Liver of male rat 248 fed a 10% protein-basal diet supplemented with cholesterol. Sudan IV 170x (Microscopic evaluation - 2)

Note the deposition of a slightly darker lipid with a more defined granulation than observed in previous figures 19 and 20. The stained lipids do not extend far from the portal canal. There are areas between the portal canal and the central vein where tissue appears normal.



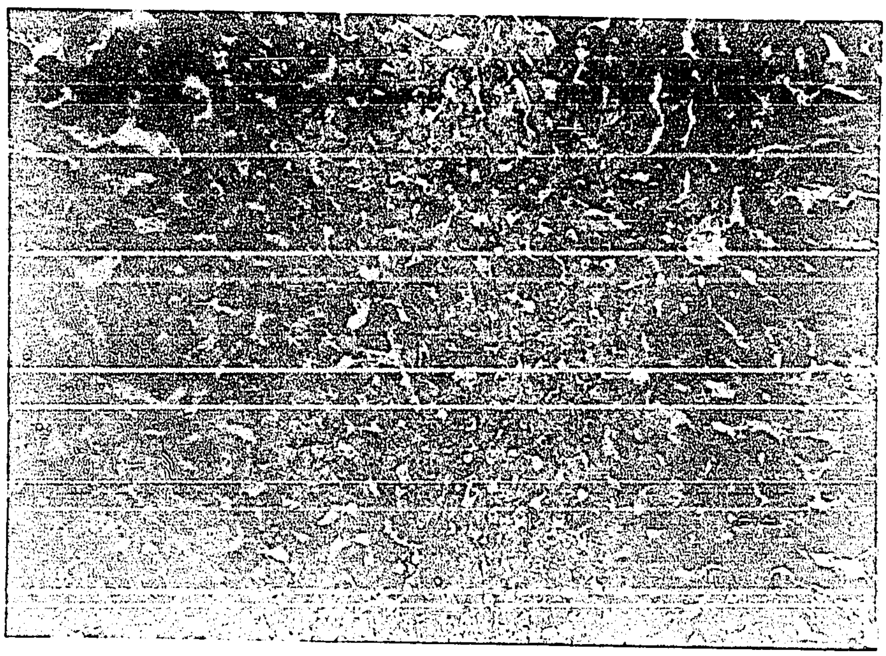
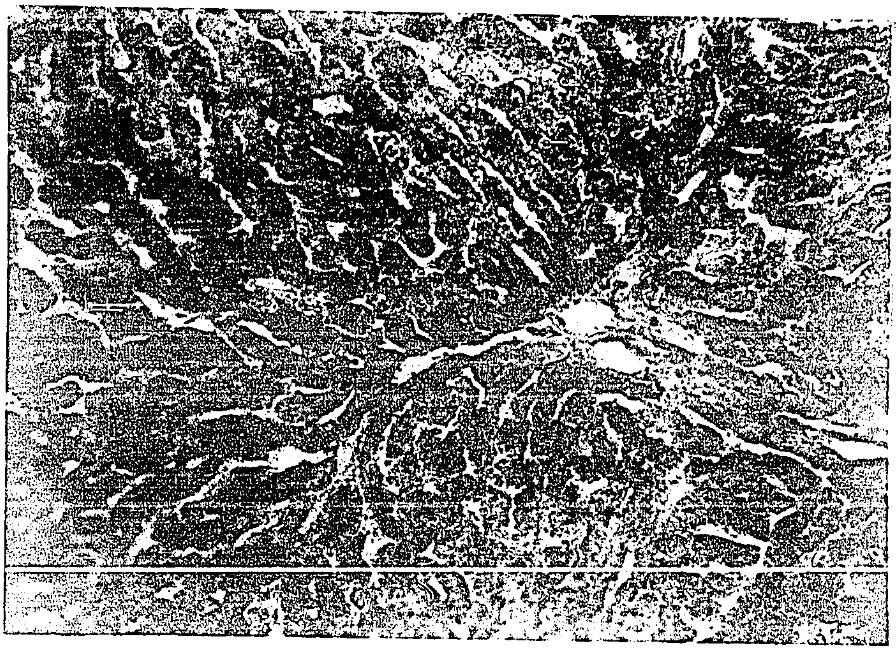


Figure 22 Liver of male rat 276 fed a 25% protein-vitamin B_{12} deficient diet supplemented with cholesterol. Sudan IV. 170x (Microscopic evaluation - 3)

Note the staining characteristics of this liver are quite similar to figure 21. However, the stained lipids extend almost from the portal canal to the central vein. The intensity is much darker than previous figure.

Figure 23 Liver of male rat 298 fed a 25% protein - choline deficient diet supplemented with cholesterol. Sudan IV. 170x (microscopic evaluation - 4)

Note that the fat stained material, as revealed by color intensity, decreases from a high lipid concentration at the portal canal area to a moderate concentration near the central vein.



interesting to note that of the 21 rats which died 15 were males. Also, the choline deficient groups had the lowest mortality of all the split-plot treatments.

Table 12 Mortality of Rats According to Sex, Whole-Plot and Split-Plot Groups

Split-Plot Groups	Basal + 1% Cholesterol		Basal		Basal + 1% Cholesterol + 12% Hydrogenated Coconut Oil		Total
	Male	Female	Male	Female	Male	Female	
10% Protein	1	0	2[a]	0	0	0	3
25% Protein	0	1	3[b]	1	0	0	5
10% Protein Vit. B_{12} Deficient	1	0	2[a]	0	0	2	5
25% Protein Vit. B_{12} Deficient	2	0	2	1	1	0	6
10% Protein Choline Deficient	0	0	0	0	1	0	1
25% Protein Choline Deficient	0	0	0	1	0	0	1
Total	4	1	9	3	2	2	21

a. Both animals were from the same cage.

b. One rat was killed accidently.

SUMMARY AND CONCLUSIONS

The dietary parameters of protein, vitamin B_{12} and choline were evaluated in the rat as to their influence on biochemical and physiological responses when diets were supplemented with cholesterol and cholesterol plus hydrogenated coconut oil.

Chemical analyses were conducted on the plasma for total cholesterol, cholesterol ester, lipid phosphorus and total esterified fatty acids. Packed cell volumes, hemoglobin values and total leukocyte counts were determined on the whole blood. Indirect systolic blood pressure measurements were taken at the ninth, eleventh and twelfth month on test. Histological evaluations were made on frozen sections stained with Sudan IV and with Nile blue sulfate.

The whole-plot treatments of cholesterol and cholesterol plus hydrogenated coconut oil significantly increased the total cholesterol, cholesterol ester and lipid phosphorus while the total esterified fatty acids were unaffected.

The split-plot treatment of 25 per cent protein elevated the plasma total cholesterol, cholesterol ester and lipid phosphorus of the cholesterol and the cholesterol plus hydrogenated coconut oil groups. Although similar elevations were observed in the basal whole-plot, the results were not significant.

Sex of the animal was found to affect the total cholesterol, cholesterol ester, and lipid phosphorus. The females had higher values for these parameters than the males.

Plasma phospholipid to cholesterol ratio demonstrated that the diets with cholesterol and cholesterol plus hydrogenated coconut oil supplementation were atherogenic.

The whole-plot treatments did not affect the packed cell volume, hemoglobin or total leukocyte count. The packed cell volumes and hemoglobin values were increased by the 25 per cent protein diet. Although similar responses were observed in the total leukocyte count, the results were nonsignificant. The males had higher packed cell volumes, hemoglobin values and total leukocyte counts than the females. The total leukocyte count in both sexes was reduced significantly by a choline deficiency.

Blood pressure measurements, which were taken at the ninth, eleventh and twelfth month of test, demonstrate that the males had higher systolic pressures than the females.

The split-plot treatment of 25 per cent protein was found to increase the systolic pressure in the three time measurements. In the ninth and twelfth month blood pressure measurements, the whole plots did not influence the systolic pressure. However, a significant whole-plot and sex interaction occurred in the cholesterol and cholesterol plus hydrogenated coconut oil supplemented groups. As indicated in this interaction, the systolic pressure of the males increased while in the females it decreased.

The interactions observed in this experiment will require additional study to determine their physiological, nutritional and biochemical significance.

Both liver lipid analyses and histological evaluation showed the development of fatty livers in animals fed either the cholesterol or cholesterol plus hydrogenated coconut oil supplemented diets. The lipid concentration in heart muscle was not affected by either the split-plot or whole-plot treatments. However, the aortas of the cholesterol plus hydrogenated coconut oil whole plot group showed evidence of a greater lipid concentration than other whole plot groups.

The animals on these diets, which were low in essential fatty acids, did not show signs of this deficiency. The whole-plot treatment of cholesterol did not sufficiently stress the dietary level of essential fatty acids to enhance the deficiency syndrome.

The long duration of the experiment has proved that animals can adapt to a diet low or deficient in choline. Diets which are deficient in choline have been previously shown to produce fatty livers. It was quite evident that the choline deficient diets when not supplemented with cholesterol with or without additional fat did not produce fatty livers on the basal diet. Hence, the animal has been able to metabolically adjust for this deficiency. Also, the levels of dietary casein may have provided sufficient methionine to allow complete synthesis of metabolic choline. The hypercholesteremic response observed in the choline deficient group was nearly identical to the response of the adequate dietary choline. Hence, it seems that experiments of long duration are necessary for the evaluation of dietary factors.

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APPENDIX

Table 13 Total Cholesterol in Blood Plasma (mg/100ml) of Female Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	94.9	86.2	75.9	65.3	116.6	74.2[b]
Rat 2	99.3	77.0	56.1	94.3	78.3	--
Cage Average	97.1	81.6	66.0	79.8	97.5	74.2
Rat 3	71.7	126.4	76.5	106.3	46.5	75.4
Rat 4	69.5	72.9	56.8	98.9	--[a]	117.0
Cage Average	70.6	99.7	66.7	102.6	46.5	96.2
Rat 5	109.8	140.3	80.0	101.5	72.6	93.7
Rat 6	59.7	113.5	95.1	85.5	66.0	65.2
Cage Average	84.8	126.9	87.6	93.5	69.3	79.5
Rat 7	80.0	56.3	99.3	71.1	62.5	97.8
Rat 8	60.0	74.1	80.0	148.0	81.8	186.7
Cage Average	70.0	65.2	89.7	109.6	72.2	142.3
Rat 9	67.7	110.8	127.1	--	70.0	140.8
Rat 10	82.4	--	239.4	120.0	72.9	--
Cage Average	75.0	110.8	183.3	120.0	71.5	140.8
Split-Plot Average	79.5	96.8	98.7	101.1	71.4	106.6

a. Dash indicates death of animal or quantity not sufficient for analysis.
b. Combined the two blood samples for analyses.

Table 14 Total Cholesterol in Blood Plasma (mg/100ml) of Male Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	57.2	51.2	42.5	67.4	79.3	116.7
Rat 2	67.6	54.7	71.3	--[a]	58.3	76.7
Cage Average	62.4	52.9	56.9	67.4	68.8	96.7
Rat 3	--	--	55.6	63.2	67.7	77.0
Rat 4	--	--	83.2	--	80.5	105.7
Cage Average	(59.8)[b]	(68.4)	69.4	63.2	74.1	91.4
Rat 5	54.5	68.4	32.0	75.6	99.2	135.6
Rat 6	60.3	60.5	61.2	77.9	91.1	190.6
Cage Average	57.4	64.5	46.6	76.8	95.2	163.1
Rat 7	55.2	88.1	--	73.8	93.0	56.1
Rat 8	67.1	--	--	68.5	128.1	62.4
Cage Average	61.1	88.1	(56.7)	71.2	110.6	59.3
Rat 9	62.1	64.9	54.2	88.4	65.4	102.2
Rat 10	54.7	71.3	54.0	72.1	58.5	134.7
Cage Average	58.4	68.1	53.9	80.3	61.9	118.5
Split-Plot Average	59.8	68.4	56.7	71.8	82.1	105.8

a. Dash indicates death of animal or quantity not sufficient for analyses.

b. Average values were determined from other animals in split-plot group for computer analyses.

Table 15 Total Cholesterol in Blood Plasma (mg/100ml) of Female Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	183.9	--[a]	85.3	307.9	74.0	731.3
Rat 2	184.0	137.2	--	289.4	280.0	270.8
Cage Average	183.9	137.2	85.3	298.7	177.0	501.1
Rat 3	239.0	290.7	250.0	329.8	420.7	294.1
Rat 4	189.8	597.6	516.1	315.3	195.6	282.9
Cage Average	214.4	444.2	383.1	322.6	308.2	288.5
Rat 5	126.9	265.0	145.1	191.8	46.1	667.7
Rat 6	381.0	615.7	146.3	305.8	178.7	765.9
Cage Average	253.9	440.4	145.7	248.8	112.4	716.8
Rat 7	143.9	200.9	186.6	298.8	78.7	321.9
Rat 8	227.8	342.7	95.1	300.0	504.9	226.8
Cage Average	185.9	271.8	140.9	299.4	291.8	274.4
Rat 9	140.2	501.0	358.6	275.0	268.3	1027.5
Rat 10	549.1	647.8	332.9	330.0	64.5	937.6
Cage Average	344.7	574.4	345.7	302.5	166.4	982.6
Split-Plot Average	236.6	373.6	200.1	294.4	211.2	552.7

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 16 Total Cholesterol in Blood Plasma (mg/100ml) of Male Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	246.0	--	329.0	161.9	463.4
Rat 2	294.8	311.9	81.5	237.8	272.5	222.6
Cage Average	294.8	278.9	81.5	283.4	217.2	343.0
Rat 3	295.1	200.7	103.7	221.4	218.4	276.3
Rat 4	93.7	178.6	171.2	375.6	105.3	328.9
Cage Average	194.4	189.6	137.5	298.5	161.9	302.6
Rat 5	185.0	397.4	190.5	--	216.5	254.2
Rat 6	170.3	274.5	142.1	244.4	169.7	388.0
Cage Average	177.7	335.9	166.3	244.4	193.1	321.1
Rat 7	103.0	339.5	178.4	85.4	230.6	298.1
Rat 8	355.3	260.0	113.2	239.5	124.4	476.3
Cage Average	229.2	299.7	145.8	162.5	177.5	387.2
Rat 9	175.6	360.5	468.4	402.4	240.8	314.0
Rat 10	74.6	485.7	173.0	357.3	126.4	319.0
Cage Average	125.1	423.1	320.5	379.8	183.6	316.5
Split-Plot Average	204.2	305.4	170.3	273.2	186.7	334.1

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 17 Total Cholesterol in Blood Plasma (mg/100ml) of Female Rats Fed Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	393.7	318.3	106.3	--[a]	400.0	229.2
Rat 2	70.3	640.0	88.6	710.6	72.4	467.1
Cage Average	232.0	479.1	97.4	710.6	236.2	348.2
Rat 3	106.8	304.6	73.5	164.0	384.6	87.2
Rat 4	284.9	295.0	239.1	280.5	167.8	419.2
Cage Average	195.9	299.8	156.3	222.3	276.2	253.2
Rat 5	654.2	278.2	75.5	--	211.8	364.1
Rat 6	125.3	261.6	358.7	430.4	239.4	87.1
Cage Average	389.7	269.9	217.1	430.4	225.6	225.6
Rat 7	173.7	626.3	222.1	195.7	118.8	227.8
Rat 8	217.5	315.8	341.9	307.5	257.8	365.8
Cage Average	195.6	471.1	282.0	251.6	188.3	296.8
Rat 9	104.1	738.1	75.4	260.7	161.5	402.5
Rat 10	319.5	203.9	217.2	454.8	230.2	295.9
Cage Average	211.8	471.0	146.3	357.7	195.9	349.2
Split-Plot Average	245.0	398.2	179.8	394.5	224.4	294.6

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 18 Total Cholesterol in Blood Plasma (mg/100ml) of Male Rats Fed a Cholesterol and Fat Supplemented Diet

	DIETS					
ANIMALS	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	118.3	--[a]	150.0	408.7	151.6	263.8
Rat 2	78.5	144.3	120.5	245.7	--	497.4
Cage Average	98.3	144.3	135.2	327.2	151.6	380.6
Rat 3	279.3	284.2	63.9	269.8	109.3	437.5
Rat 4	194.1	328.2	103.6	298.8	113.1	520.8
Cage Average	236.7	306.2	83.8	284.3	111.2	479.1
Rat 5	60.9	317.1	90.2	--	269.5	373.2
Rat 6	304.2	286.1	96.7	202.5	181.4	287.7
Cage Average	182.6	301.6	93.5	202.5	225.5	330.5
Rat 7	88.2	224.3	93.2	442.1	221.3	406.8
Rat 8	210.0	268.3	--	198.2	238.4	273.7
Cage Average	149.1	246.3	93.2	320.2	229.8	340.3
Rat 9	127.6	287.5	--	297.3	264.1	441.8
Rat 10	105.3	193.6	72.0	103.3	160.6	207.5
Cage Average	116.5	240.6	72.0	200.3	212.4	324.7
Split-Plot Average	156.6	247.8	95.5	266.9	186.1	371.0

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 19 Cholesterol Ester in Blood Plasma (mg/100ml) of Female Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	76.7	83.0	61.9	53.2	90.9	59.5[b]
Rat 2	81.0	74.0	42.2	81.9	71.4	--
Cage Average	78.8	78.5	52.1	67.5	81.1	59.5
Rat 3	66.7	119.0	47.7	105.5	42.7	54.4
Rat 4	60.8	49.3	53.5	70.5	---[a]	106.6
Cage Average	63.8	84.2	50.6	88.0	42.7	80.5
Rat 5	100.0	104.5	69.8	88.0	71.4	59.5
Rat 6	59.3	89.3	84.2	40.9	40.3	56.7
Cage Average	79.7	96.9	77.0	64.5	55.9	58.1
Rat 7	66.2	37.6	80.5	53.9	61.4	77.9
Rat 8	47.1	68.9	31.9	131.6	71.4	143.7
Cage Average	56.7	53.3	56.2	92.8	66.4	110.8
Rat 9	59.1	87.5	105.1	--	66.7	103.8
Rat 10	66.7	--	220.3	115.9	72.0	--
Cage Average	62.9	87.5	162.7	115.9	69.4	103.8
Split-Plot Average	68.4	80.1	79.7	85.7	63.1	82.5

a. Dash indicates death of animal or quantity not sufficient for analyses.
b. Combined the two blood samples for analyses.

Table 20 Cholesterol Ester in Blood Plasma (mg/100ml) of Male Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	51.6	42.2	42.0	61.6	58.2	110.4
Rat 2	62.5	46.2	56.4	--[a]	54.5	70.5
Cage Average	57.1	44.2	49.2	61.6	68.8	90.5
Rat 3	--	--	51.1	50.5	67.7	60.5
Rat 4	--	--	59.5	--	80.5	72.0
Cage Average	(57.3)[b]	(61.1)	55.3	50.5	74.1	66.3
Rat 5	51.7	64.7	32.1	71.5	99.2	125.4
Rat 6	55.0	52.5	53.2	50.8	91.1	173.2
Cage Average	53.4	58.6	42.7	61.2	95.2	149.3
Rat 7	55.0	78.4	--	65.5	93.0	53.4
Rat 8	66.9	--	--	67.9	128.1	58.4
Cage Average	60.9	78.4	(49.4)	66.7	110.6	55.9
Rat 9	61.7	58.6	53.7	79.3	65.4	83.3
Rat 10	53.8	67.4	47.0	48.9	58.5	77.5
Cage Average	57.8	63.0	50.5	64.1	61.9	80.4
Split-Plot Average	57.3	61.1	49.2	60.8	68.9	88.5

a. Dash indicates death of animal or quantity not sufficient for analyses.

b. Average values were determined from other animals in split-plot group for computer analyses.

Table 21 Cholesterol Ester in Blood Plasma (mg/100ml) of Female Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	170.2	--[a]	57.8	255.2	71.3	665.0
Rat 2	148.9	136.4	--	286.6	275.9	266.9
Cage Average	159.5	136.4	57.8	270.9	173.6	465.9
Rat 3	208.4	255.0	227.8	306.5	288.7	286.0
Rat 4	159.3	520.5	457.5	307.6	106.6	260.5
Cage Average	183.8	387.8	342.7	307.1	197.7	273.3
Rat 5	114.5	243.4	125.0	181.1	28.2	552.3
Rat 6	363.1	531.0	143.7	266.7	147.2	631.0
Cage Average	238.9	382.7	134.3	223.9	87.7	591.5
Rat 7	56.8	142.8	167.8	223.4	76.3	250.0
Rat 8	125.6	297.7	79.3	263.0	457.8	219.5
Cage Average	91.2	220.3	123.6	243.2	267.1	234.8
Rat 9	85.7	471.9	282.6	266.7	253.5	910.6
Rat 10	494.3	626.9	287.6	321.8	48.8	831.2
Cage Average	290.0	549.4	285.1	294.3	151.2	870.9
Split-Plot Average	192.7	335.2	168.7	267.9	175.5	487.3

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 22 Cholesterol Ester in Blood Plasma (mg/100ml) of Male Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	217.5	--	244.9	158.2	369.1
Rat 2	247.7	309.5	74.3	180.8	246.5	185.8
Cage Average	247.7	263.5	74.3	212.9	202.4	277.5
Rat 3	295.5	172.7	95.5	195.5	187.7	255.3
Rat 4	73.8	130.9	127.0	321.8	80.5	308.7
Cage Average	184.2	151.8	111.3	258.6	134.1	282.0
Rat 5	178.3	336.2	183.0	--	189.4	224.5
Rat 6	137.5	234.9	116.6	200.9	157.0	375.0
Cage Average	157.9	285.6	149.8	200.9	173.2	299.7
Rat 7	83.3	304.8	129.4	83.2	163.3	276.0
Rat 8	298.2	208.0	96.8	172.3	102.6	423.3
Cage Average	190.7	256.4	113.1	127.8	132.9	349.6
Rat 9	150.0	351.8	404.7	323.7	211.8	281.8
Rat 10	65.0	443.9	171.6	249.8	94.1	317.0
Cage Average	107.5	397.8	288.7	286.8	152.9	299.4
Split-Plot Average	177.6	271.0	147.3	217.4	159.1	301.6

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 23 Cholesterol Ester in Blood Plasma (mg/100ml) of Female Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS					
	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	319.5	234.7	81.9	--[a]	319.0	210.0
Rat 2	68.8	608.8	77.2	620.5	63.1	450.0
Cage Average	194.1	421.8	79.6	620.5	191.1	330.0
Rat 3	101.2	292.5	71.5	129.6	350.9	85.1
Rat 4	279.6	293.1	213.5	273.5	148.8	389.9
Cage Average	190.4	292.8	142.5	201.6	249.9	237.5
Rat 5	610.1	272.6	51.5	--	177.8	312.5
Rat 6	106.9	239.0	360.0	334.1	195.6	79.1
Cage Average	358.9	255.8	205.8	334.1	186.7	195.8
Rat 7	92.3	478.2	217.2	161.9	109.4	206.6
Rat 8	208.2	236.1	274.5	217.5	212.8	255.9
Cage Average	150.3	357.2	245.9	189.7	161.1	231.3
Rat 9	104.1	574.7	71.8	248.0	129.9	333.4
Rat 10	264.1	190.1	195.6	440.0	218.1	266.5
Cage Average	184.1	382.1	133.7	344.0	174.0	299.9
Split-Plot Average	215.6	341.9	161.5	337.9	192.6	258.9

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 24 Cholesterol Ester in Blood Plasma (mg/100ml) of Male Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	89.5	--[a]	145.5	380.0	105.6	217.3
Rat 2	60.7	123.7	118.3	231.1	--	378.0
Cage Average	75.1	123.7	131.9	305.6	105.6	297.7
Rat 3	276.3	218.4	47.7	236.8	98.2	381.6
Rat 4	192.1	287.5	101.2	239.6	97.0	436.7
Cage Average	234.2	253.0	74.5	238.2	97.6	409.2
Rat 5	45.5	276.2	88.1	--	242.2	366.7
Rat 6	285.4	228.2	85.8	144.8	167.3	257.9
Cage Average	165.5	252.2	86.9	144.8	204.8	312.3
Rat 7	81.4	222.8	76.4	328.6	219.2	260.6
Rat 8	163.0	219.0	--	186.4	231.7	269.8
Cage Average	122.2	220.9	76.4	257.5	225.5	265.2
Rat 9	104.4	210.0	--	247.1	229.6	437.5
Rat 10	99.2	166.4	60.5	99.5	130.4	206.8
Cage Average	101.8	188.2	60.5	173.3	180.0	322.1
Split-Plot Average	139.8	207.6	86.0	223.9	162.7	321.3

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 25 Free Cholesterol in Blood Plasma (mg/100ml) of Female Rats Fed Basal Diet

ANIMALS	DIETS: 10% Protein	25% Protein	10% Protein Vit.B12 Deficient	25% Protein Vit.B12 Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	18.2	3.2	14.0	12.1	25.7	14.7[b]
Rat 2	18.3	3.0	13.9	12.4	6.9	
Cage Average	18.3	3.1	13.9	12.3	16.4	14.7
Rat 3	5.0	7.4	28.8	0.8	3.8	21.0
Rat 4	8.7	23.6	3.3	28.4	--[a]	10.4
Cage Average	6.9	15.5	16.1	14.6	3.8	15.7
Rat 5	9.9	35.8	10.2	13.5	1.2	34.2
Rat 6	0.4	24.2	10.9	44.6	25.6	8.5
Cage Average	5.2	30.0	10.5	29.0	13.4	21.4
Rat 7	13.8	18.7	18.8	17.2	1.1	19.9
Rat 8	12.9	5.2	48.1	16.4	10.4	43.0
Cage Average	13.4	12.0	33.5	16.8	5.8	31.5
Rat 9	8.6	23.3	22.0	--	3.3	37.0
Rat 10	15.7	--	19.1	4.1	0.9	--
Cage Average	12.2	23.3	20.6	4.1	2.1	37.0
Split-Plot Average	11.2	16.8	18.9	15.4	8.3	24.1

a. Dash indicates death of animal or quantity not sufficient for analyses.

b. Combined the two blood samples for analyses.

Table 26 Free Cholesterol in Blood Plasma (mg/100ml) of Male Rats Fed Basal Diet

ANIMALS	DIETS					
	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	5.6	9.0	0.5	5.8	21.1	6.3
Rat 2	5.1	8.5	14.9	--[a]	3.8	6.2
Cage Average	5.4	8.8	7.7	5.8	12.5	6.3
Rat 3	--	--	4.5	12.7	14.3	16.5
Rat 4	--	--	23.7	--	9.0	33.7
Cage Average	(2.6)[b]	(7.3)	14.1	12.7	11.7	25.1
Rat 5	2.8	3.7	0.1	4.1	32.0	10.2
Rat 6	5.3	8.0	8.0	27.1	12.4	17.4
Cage Average	4.0	5.6	4.1	15.6	22.2	13.8
Rat 7	0.2	9.7	--	8.3	8.9	2.7
Rat 8	0.2	--	--	0.6	12.4	4.0
Cage Average	0.2	9.7	(7.4)	4.5	10.7	3.4
Rat 9	0.4	6.3	0.5	9.1	9.2	18.9
Rat 10	0.9	3.9	7.0	23.2	8.7	57.2
Cage Average	0.7	5.1	3.8	16.2	8.9	38.1
Split-Plot Average	2.5	7.3	7.4	10.9	13.2	17.3

a. Dash indicates death of animal or quantity not sufficient for analyses.

b. Average values were determined from other animals in split-plot group for computer analyses.

Table 27 Free Cholesterol in Blood Plasma (mg/100ml) of Female Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	13.7	--[a]	28.0	52.7	2.7	66.3
Rat 2	35.1	0.8	--	2.8	4.1	3.4
Cage Average	24.4	0.8	28.0	27.8	3.4	31.2
Rat 3	30.6	35.7	22.2	23.3	132.0	8.1
Rat 4	30.5	77.1	58.6	7.7	89.0	22.4
Cage Average	30.6	56.4	40.4	15.5	110.5	15.2
Rat 5	12.4	22.1	20.1	10.7	17.9	115.4
Rat 6	17.9	84.7	2.6	39.1	31.5	134.9
Cage Average	15.1	57.7	11.4	24.9	24.7	125.3
Rat 7	87.1	58.1	18.8	75.5	2.4	71.9
Rat 8	102.2	45.0	15.8	37.0	47.1	7.3
Cage Average	94.7	51.5	17.3	56.2	24.7	39.6
Rat 9	54.5	29.1	76.0	8.3	14.8	116.9
Rat 10	54.8	20.9	45.3	8.2	15.7	106.4
Cage Average	54.7	25.0	60.6	8.2	15.2	111.7
Split-Plot Average	43.9	38.3	31.5	26.5	35.7	64.6

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 28 Free Cholesterol in Blood Plasma (mg/100ml) of Male Rats Fed Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	28.5	--	84.1	3.7	94.3
Rat 2	47.1	2.4	7.2	57.0	25.9	36.8
Cage Average	47.1	15.4	7.2	70.5	14.8	65.5
Rat 3	0.6	28.0	8.2	25.9	30.7	21.0
Rat 4	19.9	47.7	44.2	53.8	24.8	20.2
Cage Average	10.2	37.8	26.2	39.9	27.8	20.6
Rat 5	6.7	61.2	7.5	--	27.1	29.7
Rat 6	32.8	39.9	25.5	73.4	12.7	13.0
Cage Average	19.8	50.4	16.5	73.4	19.9	21.4
Rat 7	19.7	34.7	49.0	2.2	67.3	22.1
Rat 8	57.1	52.0	16.4	67.2	21.8	53.0
Cage Average	38.4	43.4	32.7	34.7	44.5	37.6
Rat 9	25.6	8.7	63.7	78.7	29.0	32.2
Rat 10	9.6	41.8	1.4	107.5	32.3	2.0
Cage Average	17.6	25.3	32.3	93.0	30.6	17.1
Split-Plot Average	26.2	34.5	22.9	62.3	27.5	32.4

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 29 Free Cholesterol in Blood Plasma (mg/100ml) of Female Rats Fed Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	74.2	83.6	24.2	--[a]	81.0	19.2
Rat 2	1.5	31.2	11.4	90.1	9.3	17.1
Cage Average	37.9	57.4	17.8	90.1	45.2	18.2
Rat 3	5.6	12.1	2.0	34.4	33.7	2.1
Rat 4	5.4	1.9	25.6	7.0	19.0	29.3
Cage Average	5.5	7.0	13.8	20.7	26.4	15.7
Rat 5	44.1	5.6	24.0	--	34.0	51.6
Rat 6	18.4	22.6	1.3	91.3	43.8	8.0
Cage Average	31.3	14.1	12.6	91.3	38.9	29.8
Rat 7	81.4	148.1	4.9	33.8	9.4	21.2
Rat 8	9.3	79.7	67.4	90.0	45.0	109.9
Cage Average	45.4	113.9	36.2	61.9	27.2	65.5
Rat 9	0.0	163.4	3.6	12.7	31.6	69.1
Rat 10	55.4	13.8	21.6	14.8	12.1	29.4
Cage Average	27.7	88.6	12.6	13.7	21.9	49.3
Split-Plot Average	29.6	56.2	18.6	55.5	31.9	35.7

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 30 Free Cholesterol in Blood Plasma (mg/100ml) of Male Rats Fed Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	28.8	--[a]	4.5	28.7	46.0	46.5
Rat 2	17.8	20.6	2.2	14.6	--	119.4
Cage Average	23.3	20.6	3.3	21.6	46.0	82.9
Rat 3	3.0	65.8	16.2	33.0	11.1	55.9
Rat 4	2.0	40.7	2.4	59.2	16.1	84.1
Cage Average	2.5	53.2	9.3	46.1	13.6	69.9
Rat 5	15.4	40.9	2.1	--	27.3	6.5
Rat 6	18.8	57.9	10.9	57.7	14.1	29.8
Cage Average	17.1	49.4	6.6	57.7	20.7	18.2
Rat 7	6.8	1.5	16.8	113.5	2.1	146.2
Rat 8	47.0	49.2	--	11.8	6.7	3.9
Cage Average	26.9	25.4	16.8	62.7	4.3	75.1
Rat 9	23.2	77.5	--	50.2	34.5	3.9
Rat 10	6.1	37.2	11.5	3.8	30.2	0.7
Cage Average	14.7	52.4	11.5	27.0	32.4	1.3
Split-Plot Average	16.9	40.2	9.5	43.0	23.4	49.4

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 31 Total Esterified Fatty Acids in Blood Plasma (mg/100ml) of Female Rats Fed Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	520.9	509.6	611.7	675.0	951.0	583.3[b]
Rat 2	700.0	409.6	666.7	683.3	392.9	
Cage Average	610.5	459.6	639.2	679.1	671.9	583.3
Rat 3	315.0	633.3	648.2	438.1	423.1	529.1
Rat 4	495.9	662.5	424.0	495.2	---[a]	564.7
Cage Average	405.5	647.9	536.1	466.7	423.1	546.9
Rat 5	295.0	361.6	440.0	504.3	1128.6	480.0
Rat 6	466.7	461.5	1178.6	428.6	781.0	416.0
Cage Average	380.9	411.6	809.3	466.4	954.8	448.0
Rat 7	476.9	426.9	666.7	808.4	558.3	420.0
Rat 8	330.8	492.3	600.0	833.4	516.3	536.0
Cage Average	403.8	459.6	633.3	820.9	537.3	478.0
Rat 9	476.9	415.6	808.9	--	461.5	758.2
Rat 10	361.5	--	433.4	528.0	361.5	--
Cage Average	419.2	415.6	621.2	528.0	411.5	758.2
Split-Plot Average	443.9	478.6	647.8	592.2	599.7	562.9

a. Dash indicates death of animal or quantity not sufficient for analyses.
b. Combined the two blood samples for analyses.

Table 32 Total Esterified Fatty Acids in Blood Plasma (mg/100ml) of Male Rats Fed Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	363.0	500.0	466.7	533.4	584.0	753.2
Rat 2	600.0	592.3	565.0	--[a]	827.0	459.7
Cage Average	481.5	546.2	515.8	533.4	705.5	606.5
Rat 3	--	--	695.0	787.9	295.7	295.7
Rat 4	--	--	322.3	--	751.6	457.1
Cage Average	(453.3)[b]	(511.2)	508.6	787.9	523.7	376.4
Rat 5	457.1	825.0	387.2	600.0	728.6	486.3
Rat 6	466.7	414.1	384.7	641.7	576.5	514.8
Cage Average	461.9	619.6	385.9	620.8	652.6	500.6
Rat 7	307.7	463.0	--	495.9	376.5	388.5
Rat 8	564.7	--	--	840.9	421.5	340.0
Cage Average	436.2	463.0	(468.5)	668.4	399.0	364.4
Rat 9	435.3	404.0	420.0	734.7	773.4	600.0
Rat 10	431.4	428.0	508.0	428.6	786.1	558.3
Cage Average	433.4	416.0	404.0	581.7	779.8	579.3
Split-Plot Average	453.3	511.2	468.6	638.4	612.1	485.2

a. Dash indicates death of animal or quantity not sufficient for analyses.
b. Average values were determined from other animals in split-plot group for computer analyses.

Table 33 Total Esterified Fatty Acids in Blood Plasma (mg/100ml) of Female Rats Fed Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	633.3	--[a]	343.9	437.5	457.1	737.1
Rat 2	650.0	484.2	--	287.8	419.0	820.0
Cage Average	641.5	484.2	343.9	362.7	438.0	778.5
Rat 3	504.8	417.4	425.7	500.0	804.1	484.0
Rat 4	641.2	726.6	410.3	480.0	365.0	504.0
Cage Average	573.0	572.0	418.0	490.0	584.6	494.0
Rat 5	696.3	382.5	785.0	442.3	680.0	791.1
Rat 6	840.9	622.9	635.0	400.0	554.2	848.5
Cage Average	768.6	502.7	710.0	421.2	617.1	819.8
Rat 7	808.3	752.4	456.5	429.2	448.9	781.4
Rat 8	691.7	734.7	425.7	692.6	643.7	840.9
Cage Average	750.0	743.6	441.1	560.9	566.3	811.2
Rat 9	666.7	560.0	691.7	442.6	468.0	1185.7
Rat 10	725.0	694.1	575.0	428.6	460.0	857.1
Cage Average	695.8	627.1	633.4	435.6	464.0	1021.4
Split-Plot Average	685.8	585.9	509.3	454.1	534.0	784.9

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 34 Total Esterified Fatty Acids in Blood Plasma (mg/100ml) of Male Rats Fed Cholesterol Supplemented Diet

ANIMALS	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	508.0	--	750.0	368.0	466.7
Rat 2	372.7	902.1	294.8	315.8	544.0	485.2
Cage Average	372.7	705.1	294.8	532.9	456.0	476.0
Rat 3	610.0	486.3	444.0	706.1	361.0	1071.5
Rat 4	288.3	1162.8	448.0	350.9	466.7	400.2
Cage Average	449.3	824.5	446.0	528.5	413.9	738.9
Rat 5	305.3	791.7	606.1	--	758.4	788.4
Rat 6	298.3	863.6	878.8	333.3	733.1	842.1
Cage Average	301.8	827.7	742.5	333.3	745.7	815.3
Rat 7	596.4	525.5	287.8	477.8	633.3	750.1
Rat 8	375.0	513.7	353.8	343.9	575.0	800.0
Cage Average	485.7	519.6	320.8	410.9	604.2	775.0
Rat 9	234.8	600.8	432.0	496.3	486.3	656.8
Rat 10	490.0	863.2	520.0	680.0	477.2	552.0
Cage Average	362.4	732.0	476.0	588.2	481.8	604.4
Split-Plot Average	394.4	721.8	456.0	478.8	540.3	681.9

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 35 Total Esterified Fatty Acids in Blood Plasma (mg/100ml) of Female Rats Fed Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS					
	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	531.0	300.0	424.0	--[a]	703.7	795.6
Rat 2	560.0	827.9	352.0	740.0	451.0	611.8
Cage Average	545.5	563.9	388.0	740.0	577.3	703.7
Rat 3	432.2	466.7	376.9	252.2	662.5	768.1
Rat 4	323.1	409.5	400.0	541.7	633.3	553.0
Cage Average	377.7	438.1	388.0	396.9	647.9	674.0
Rat 5	808.9	708.3	860.6	--	407.7	507.1
Rat 6	824.3	500.0	415.4	812.2	361.5	538.5
Cage Average	816.6	604.2	638.0	812.2	384.6	522.8
Rat 7	1164.3	691.7	437.5	500.0	793.8	323.2
Rat 8	435.1	662.5	729.6	707.4	334.8	416.7
Cage Average	799.7	677.1	583.6	603.7	564.3	369.9
Rat 9	410.5	716.7	669.4	654.5	425.0	420.9
Rat 10	493.9	725.0	829.5	630.3	916.0	486.3
Cage Average	452.2	720.9	749.4	642.4	670.5	453.6
Split-Plot Average	598.3	600.8	549.4	639.0	568.9	544.8

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 36 Total Esterified Fatty Acids in Blood Plasma (mg/100ml) of Male Rats Fed Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	495.8	--[a]	435.1	410.3	733.3	819.1
Rat 2	800.0	368.0	504.0	570.0	--	319.3
Cage Average	647.9	368.0	469.6	490.2	733.3	569.3
Rat 3	466.7	581.8	350.0	915.8	933.4	650.0
Rat 4	576.5	685.2	385.7	693.9	800.0	737.8
Cage Average	521.6	633.5	367.8	804.8	866.7	693.9
Rat 5	812.2	583.4	533.4	--	578.3	857.2
Rat 6	751.1	334.8	435.1	454.9	484.3	486.3
Cage Average	781.7	459.1	484.3	454.9	531.3	671.7
Rat 7	307.7	647.1	338.5	487.5	449.1	589.2
Rat 8	828.6	762.8	--	407.4	475.0	604.0
Cage Average	568.2	705.0	338.5	447.5	462.1	596.6
Rat 9	820.3	700.0	--	632.8	620.4	712.0
Rat 10	600.2	169.6	400.0	310.2	500.0	435.1
Cage Average	710.3	434.8	400.0	471.5	560.2	573.6
Split-Plot Average	645.9	520.1	412.0	533.8	630.7	621.0

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 37 Lipid Phosphorus in Blood Plasma (mg/100ml) of Female Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	5.13	5.95	6.12	3.81	3.13	4.26[b]
Rat 2	5.99	3.76	4.26	6.66	4.37	---
Cage Average	5.56	4.86	5.19	5.24	3.75	4.26
Rat 3	5.33	2.00	5.50	6.89	3.60	3.52
Rat 4	5.27	18.94	3.42	8.35	---[a]	7.04
Cage Average	5.30	10.47	4.46	7.62	3.60	5.28
Rat 5	5.13	6.19	5.10	5.95	5.04	5.51
Rat 6	5.23	6.66	5.47	2.50	2.75	7.89
Cage Average	5.18	6.43	5.29	4.23	3.89	6.70
Rat 7	5.06	4.00	5.19	3.95	3.86	7.26
Rat 8	4.40	5.73	3.86	12.52	5.33	9.01
Cage Average	4.73	4.87	4.53	8.24	4.59	8.14
Rat 9	4.00	7.66	4.73	---	4.53	4.43
Rat 10	5.46	---	8.25	6.54	5.59	---
Cage Average	4.73	7.66	6.49	6.54	5.06	4.43
Split-Plot Average	5.10	6.86	5.19	6.37	4.18	5.76

a. Dash indicates death of animal or quantity not sufficient for analyses.
b. Combined the two blood samples for analyses.

Table 38 Lipid Phosphorus in Blood Plasma (mg/100ml) of Male Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	2.81	2.93	2.50	4.27	3.97	4.30
Rat 2	3.87	5.86	2.75	--[a]	1.88	5.14
Cage Average	3.34	4.39	2.63	4.27	2.93	4.72
Rat 3	--	--	4.28	4.64	6.12	4.75
Rat 4	--	--	3.78	--	4.20	6.68
Cage Average	(3.38)[b]	(4.07)	4.03	4.64	5.16	5.72
Rat 5	3.34	3.97	4.96	8.94	2.38	6.84
Rat 6	3.13	5.46	3.77	2.50	3.59	9.52
Cage Average	3.24	4.72	4.37	5.72	2.99	8.18
Rat 7	3.75	4.37	--	4.34	4.96	2.29
Rat 8	3.00	--	--	3.29	8.02	4.29
Cage Average	3.38	4.37	(3.56)	3.82	6.49	3.29
Rat 9	4.20	2.85	2.75	4.27	3.99	4.53
Rat 10	2.91	2.71	3.63	2.66	3.82	6.46
Cage Average	3.56	2.78	3.19	3.47	3.91	5.49
Split-Plot Average	3.38	4.07	3.56	4.38	4.30	5.48

a. Dash indicates death of animal or quantity not sufficient for analyses.

b. Average values were determined from other animals in split-plot group for computer analyses.

Table 39 Lipid Phosphorus in Blood Plasma (mg/100ml) of Female Rats Fed a Cholesterol Supplemented Diet

ANIMALS	10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	3.21	--[a]	2.81	4.86	1.75	9.47
Rat 2	3.19	6.90	--	4.17	6.68	7.10
Cage Average	3.20	6.90	2.81	4.52	4.22	8.29
Rat 3	7.51	6.98	3.02	5.26	5.85	3.27
Rat 4	5.42	8.25	5.97	6.51	4.21	4.91
Cage Average	6.47	7.62	4.49	5.89	5.03	4.09
Rat 5	4.43	6.98	7.71	5.73	4.22	7.86
Rat 6	5.01	8.02	5.19	7.99	2.82	11.65
Cage Average	4.72	7.50	6.45	6.86	3.52	9.76
Rat 7	3.50	3.83	4.73	5.33	4.77	4.95
Rat 8	3.33	4.40	5.73	6.80	5.35	6.74
Cage Average	3.42	4.12	5.23	6.07	5.06	5.85
Rat 9	5.33	7.10	5.93	6.47	3.13	8.64
Rat 10	7.59	6.65	4.26	7.84	4.98	9.39
Cage Average	6.46	6.88	5.09	7.16	4.06	9.02
Split-Plot Average	4.85	6.60	4.81	6.10	4.38	7.40

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 40 Lipid Phosphorus in Blood Plasma (mg/100ml) of Male Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	6.40	--	6.11	2.75	4.70
Rat 2	5.54	4.07	2.45	3.17	8.24	4.24
Cage Average	5.54	5.24	2.45	4.64	5.49	4.47
Rat 3	4.43	4.20	2.45	3.85	3.38	5.04
Rat 4	1.44	1.89	0.85	4.54	4.09	8.85
Cage Average	2.94	3.04	1.60	4.19	3.74	6.95
Rat 5	2.09	4.50	4.26	--	3.95	6.12
Rat 6	1.87	8.08	4.07	2.52	2.16	6.33
Cage Average	1.98	6.29	4.17	2.52	3.06	6.23
Rat 7	2.18	4.71	2.01	2.74	9.66	5.95
Rat 8	2.13	5.36	1.87	3.24	2.65	9.26
Cage Average	2.16	5.04	1.94	2.99	6.16	7.61
Rat 9	5.26	6.12	5.26	5.42	3.49	6.69
Rat 10	3.20	8.78	3.45	5.21	5.06	6.05
Cage Average	4.23	7.45	4.36	5.32	4.28	6.37
Split-Plot Average	3.37	5.41	2.90	3.93	4.55	6.33

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 41 Lipid Phosphorus in Blood Plasma (mg/100ml) of Female Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	6.64	7.93	4.38	--[a]	7.03	5.16
Rat 2	7.51	10.79	6.01	10.32	5.43	8.07
Cage Average	7.08	9.36	5.20	10.32	6.23	6.62
Rat 3	4.37	10.65	4.26	7.05	10.73	5.91
Rat 4	5.30	5.87	5.61	9.18	5.01	5.28
Cage Average	4.84	8.26	4.94	8.12	7.87	5.60
Rat 5	7.61	16.67	4.86	--	7.07	8.79
Rat 6	6.64	5.33	7.44	8.14	5.66	5.86
Cage Average	7.13	11.00	6.15	8.14	6.37	7.33
Rat 7	5.69	7.15	7.40	10.75	6.90	5.90
Rat 8	6.58	9.48	7.56	9.47	13.24	6.89
Cage Average	6.14	8.32	7.48	10.11	10.07	6.39
Rat 9	9.31	4.48	3.78	5.76	2.87	8.71
Rat 10	6.60	10.50	4.77	9.64	5.37	7.17
Cage Average	7.96	7.49	4.28	7.45	4.12	7.94
Split-Plot Average	6.63	8.89	5.61	8.83	6.93	6.78

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 42 Lipid Phosphorus in Blood Plasma (mg/100ml) of Male Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	3.23	--[a]	5.22	7.03	2.77	4.70
Rat 2	2.63	5.22	4.11	5.81	--	7.70
Cage Average	2.93	5.22	4.67	6.42	2.77	6.20
Rat 3	4.41	5.42	3.21	5.47	4.12	8.90
Rat 4	4.03	6.80	3.85	6.54	4.26	12.52
Cage Average	4.22	6.11	3.53	6.01	4.19	10.71
Rat 5	4.32	4.75	2.98	--	5.07	4.70
Rat 6	3.59	5.40	5.06	3.87	4.90	7.49
Cage Average	3.96	5.08	4.02	3.87	4.98	6.09
Rat 7	3.49	4.63	3.66	5.22	6.58	6.19
Rat 8	4.86	3.10	--	3.78	6.41	5.08
Cage Average	4.18	3.87	3.66	4.50	6.50	5.64
Rat 9	3.56	5.27	--	6.33	5.30	10.66
Rat 10	4.94	5.18	4.90	6.19	5.73	6.02
Cage Average	4.25	5.23	4.90	6.26	5.52	8.34
Split-Plot Average	3.91	5.10	4.16	5.41	4.79	7.40

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 43 Packed Cell Volume (per cent) of Female Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	42.0	40.0	39.5	41.5	45.5	45.0
Rat 2	42.5	40.7	--[a]	46.0	41.0	--
Cage Average	42.3	40.4	39.5	43.8	43.3	45.0
Rat 3	41.0	44.0	45.0	44.5	41.5	43.0
Rat 4	43.0	45.5	44.0	34.0	39.0	41.5
Cage Average	42.0	44.8	44.5	39.3	40.3	42.3
Rat 5	39.0	40.5	43.5	45.0	44.0	47.0
Rat 6	43.0	42.0	40.0	43.0	43.0	48.0
Cage Average	41.0	41.3	41.8	44.0	43.5	47.5
Rat 7	44.0	45.5	40.5	46.5	37.5	42.0
Rat 8	43.5	41.0	42.5	45.0	41.0	43.5
Cage Average	43.8	43.3	41.5	45.8	39.3	42.8
Rat 9	44.0	43.0	43.0	--	41.5	37.5
Rat 10	37.5	--	42.5	38.5	39.0	--
Cage Average	40.8	43.00	42.8	38.5	40.3	37.5
Split-Plot Average	42.0	42.6	42.0	42.3	41.3	43.0

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 44 Packed Cell Volume (per cent) of Male Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	40.0	45.0	48.0	45.0	39.0	43.0
Rat 2	41.0	43.0	41.5	--[a]	38.0	42.0
Cage Average	40.5	44.0	44.7	45.0	38.5	42.5
Rat 3	--	--	41.0	43.0	46.0	43.0
Rat 4	--	--	43.0	--	44.0	46.0
Cage Average	(44.3)[b]	(46.3)	42.0	43.0	45.0	44.5
Rat 5	44.0	46.0	40.5	--	44.0	44.5
Rat 6	46.0	47.5	40.5	44.0	44.0	44.5
Cage Average	45.0	46.8	40.5	44.0	44.0	44.5
Rat 7	48.0	48.0	--	45.5	41.0	44.5
Rat 8	48.0	--	--	43.0	44.0	46.0
Cage Average	48.0	48.0	(42.3)	44.3	42.5	45.3
Rat 9	46.5	47.0	41.5	46.0	41.5	43.5
Rat 10	41.5	45.5	42.0	48.0	43.5	46.0
Cage Average	44.0	46.3	41.8	47.0	42.5	44.8
Split-Plot Average	44.4	46.3	42.3	44.7	42.5	44.3

a. Dash indicates death of animal or quantity not sufficient for analyses.
b. Average values were determined from other animals in split-plot group for computer analyses.

Table 45 Packed Cell Volume (per cent) of Female Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS: 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	43.0	--[a]	43.0	48.0	43.5	43.0
Rat 2	40.5	44.5	--	40.5	40.0	43.5
Cage Average	41.8	44.5	43.0	44.3	41.8	43.3
Rat 3	44.5	45.0	43.5	45.0	40.5	40.5
Rat 4	40.5	40.0	41.0	--	39.0	43.5
Cage Average	42.5	42.5	42.3	45.0	39.8	42.0
Rat 5	42.0	42.0	40.5	41.0	40.5	44.0
Rat 6	37.0	40.5	44.0	--	41.5	36.0
Cage Average	39.5	41.3	42.3	41.0	41.0	40.0
Rat 7	44.0	45.5	22.5	37.0	40.0	42.0
Rat 8	43.5	45.0	40.0	41.5	42.0	40.0
Cage Average	43.8	45.3	31.3	39.3	41.0	41.0
Rat 9	40.0	33.0	42.5	47.5	31.0	41.0
Rat 10	34.0	37.0	39.0	46.0	40.5	48.0
Cage Average	37.0	35.0	40.8	46.8	35.8	44.5
Split-Plot Average	40.9	41.7	39.9	43.3	39.9	42.2

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 46 Packed Cell Volume (per cent) of Male Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	46.5	--	43.5	44.5	46.0
Rat 2	40.0	42.0	43.5	44.5	49.5	45.5
Cage Average	40.0	44.2	43.5	44.0	47.0	45.8
Rat 3	42.0	51.0	46.0	46.0	40.0	45.0
Rat 4	44.0	42.5	43.5	42.5	42.5	43.5
Cage Average	43.0	46.8	44.8	44.3	41.3	44.3
Rat 5	42.0	42.0	46.5	--	43.0	44.5
Rat 6	45.0	51.5	44.0	45.5	43.5	40.5
Cage Average	43.3	46.8	45.3	45.5	43.3	42.5
Rat 7	43.3	48.0	43.0	48.0	43.5	46.5
Rat 8	46.0	45.5	45.0	45.0	46.5	35.5
Cage Average	44.5	46.7	44.0	46.5	45.0	41.0
Rat 9	35.5	42.0	41.0	44.0	39.5	41.0
Rat 10	50.0	38.0	42.5	46.0	41.5	44.0
Cage Average	42.7	40.0	41.8	45.0	40.5	42.5
Split-Plot Average	42.7	44.9	43.9	45.1	43.4	43.2

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 47 Packed Cell Volume (per cent) of Female Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
	DIETS					
Rat 1	43.0	43.5	44.0	--[a]	43.0	43.0
Rat 2	44.0	38.0	43.0	40.0	40.0	40.0
Cage Average	43.5	40.7	43.5	40.0	41.5	41.5
Rat 3	41.0	46.0	45.5	--	40.5	42.0
Rat 4	44.0	45.0	40.5	45.5	40.0	39.5
Cage Average	42.5	45.5	43.0	45.5	40.3	40.7
Rat 5	44.0	48.0	40.5	--	44.0	42.5
Rat 6	38.0	39.0	40.5	43.0	43.5	40.5
Cage Average	41.0	43.5	40.5	43.0	43.8	41.5
Rat 7	41.0	46.5	41.5	44.0	36.5	44.5
Rat 8	39.5	41.0	43.0	44.0	42.5	42.0
Cage Average	40.3	43.7	42.3	44.0	39.5	43.3
Rat 9	43.5	44.5	41.5	44.0	41.0	43.5
Rat 10	43.5	39.5	39.5	44.0	38.0	42.0
Cage Average	43.5	42.0	40.5	44.0	39.5	42.8
Split-Plot Average	42.2	43.1	42.0	43.3	40.9	41.9

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 48 Packed Cell Volume (per cent) of Male Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS: 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	42.0	54.0	35.5	49.0	36.5	54.0
Rat 2	42.5	49.5	40.0	42.0	--[a]	45.0
Cage Average	42.3	51.7	37.7	45.5	36.5	49.5
Rat 3	45.5	44.0	47.0	45.0	42.0	40.5
Rat 4	42.0	46.5	45.0	44.5	40.0	47.0
Cage Average	43.7	45.3	46.0	44.8	41.0	43.7
Rat 5	40.5	44.5	44.0	--	38.0	43.0
Rat 6	48.0	49.0	39.0	47.0	42.0	47.5
Cage Average	44.3	46.7	41.5	47.0	40.0	45.3
Rat 7	42.5	44.0	42.5	46.0	44.0	42.5
Rat 8	41.5	45.0	--	45.5	46.0	46.0
Cage Average	42.0	44.5	42.5	45.8	45.0	44.3
Rat 9	42.5	42.0	--	50.0	42.5	46.5
Rat 10	42.0	46.0	47.0	39.5	45.0	48.0
Cage Average	42.3	44.0	47.0	44.8	43.7	47.3
Split-Plot Average	42.9	46.4	42.9	45.6	41.2	46.0

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 49 Hemoglobin (gm/100ml) of Female Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B12 Deficient	25% Protein Vit.B12 Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	14.0	13.7	13.7	14.3	14.8	15.0
Rat 2	14.0	13.5	--[a]	15.8	14.3	--
Cage Average	14.0	13.6	13.7	15.1	14.6	15.0
Rat 3	13.6	14.3	14.3	15.6	14.4	16.0
Rat 4	14.7	15.4	15.1	11.8	12.8	14.2
Cage Average	14.2	14.9	14.7	13.7	13.6	15.1
Rat 5	13.3	14.2	14.4	14.8	15.2	15.5
Rat 6	14.4	14.4	13.6	14.3	14.4	15.8
Cage Average	13.9	14.3	14.0	14.6	14.8	15.7
Rat 7	14.7	15.0	12.8	15.8	13.2	14.3
Rat 8	14.4	14.3	14.4	15.6	14.8	15.0
Cage Average	14.6	14.7	13.6	15.7	14.0	14.7
Rat 9	14.4	15.0	14.0	--	13.4	14.8
Rat 10	13.0	--	13.3	11.8	14.0	--
Cage Average	13.7	15.0	13.7	11.8	13.7	14.8
Split-Plot Average	14.1	14.5	13.9	14.2	14.1	15.1

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 50 Hemoglobin (gm/100ml) of Male Rats Fed a Basal Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	14.5	14.8	17.6	15.6	12.8	15.3
Rat 2	13.6	14.4	13.0	--[a]	13.6	15.0
Cage Average	14.1	14.6	15.3	15.6	13.2	15.2
Rat 3	--	--	13.3	15.2	15.3	14.4
Rat 4	--	--	14.5	--	13.4	15.4
Cage Average	(14.8)[b]	(15.5)	13.9	15.2	14.3	14.9
Rat 5	14.8	14.8	13.6	--	14.0	15.6
Rat 6	14.8	14.8	13.5	16.5	16.5	16.5
Cage Average	14.8	14.8	13.5	16.5	15.3	16.1
Rat 7	15.0	16.3	--	15.4	13.5	16.0
Rat 8	15.8	--	--	15.0	14.0	15.0
Cage Average	15.4	16.3	(14.4)	15.2	13.8	15.5
Rat 9	15.4	16.0	16.0	15.8	15.6	14.2
Rat 10	14.0	16.5	13.8	15.8	14.7	16.9
Cage Average	14.7	16.3	14.9	15.8	15.2	15.5
Split-Plot Average	14.8	15.5	14.4	15.7	14.4	15.4

a. Dash indicates death of animal or quantity not sufficient for analyses.
b. Average values were determined from other animals in split-plot group for computer analyses.

Table 51 Hemoglobin (gm/100ml) of Female Rats Fed a Cholesterol Supplemented Diet

ANIMALS	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	14.0	--[a]	13.5	14.8	14.5	14.4
Rat 2	14.2	13.3	--	14.0	13.5	13.6
Cage Average	14.1	13.3	13.5	14.4	14.0	14.0
Rat 3	15.3	15.3	14.8	14.8	13.4	14.3
Rat 4	13.8	14.0	14.4	--	14.8	15.3
Cage Average	14.6	14.7	14.6	14.8	14.1	14.8
Rat 5	14.6	15.6	13.4	13.3	12.7	16.0
Rat 6	12.3	14.4	14.6	--	14.0	14.0
Cage Average	13.5	15.0	14.0	13.3	13.4	15.0
Rat 7	14.4	15.4	7.5	13.8	14.4	14.3
Rat 8	14.4	14.8	13.4	14.0	14.8	13.5
Cage Average	14.4	15.1	10.5	13.9	14.6	13.9
Rat 9	13.6	12.5	14.4	16.5	12.8	14.4
Rat 10	11.5	13.0	13.3	14.8	13.5	14.4
Cage Average	12.6	12.8	13.9	15.7	13.2	14.4
Split-Plot Average	13.9	14.2	13.3	14.4	13.9	14.4

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 52 Hemoglobin (gm/100ml) of Male Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	15.6	--	15.2	14.6	16.0
Rat 2	13.3	14.0	14.5	15.6	16.8	15.0
Cage Average	13.3	14.8	14.5	15.4	15.7	15.5
Rat 3	14.0	14.4	17.0	17.0	13.6	16.0
Rat 4	14.8	14.8	14.0	15.8	14.6	15.3
Cage Average	14.4	14.6	15.5	16.4	14.1	15.7
Rat 5	14.8	14.4	15.6	--	14.4	16.0
Rat 6	16.5	17.5	14.4	15.6	14.6	14.8
Cage Average	15.7	15.9	15.0	15.6	14.5	15.4
Rat 7	14.3	16.5	14.0	16.0	14.4	16.0
Rat 8	14.7	15.4	15.4	16.3	12.3	11.3
Cage Average	14.5	16.0	14.7	16.2	13.4	13.7
Rat 9	12.0	14.6	15.0	15.3	13.4	14.4
Rat 10	13.7	15.5	14.4	13.6	14.7	15.5
Cage Average	12.9	15.1	14.7	14.5	14.1	15.0
Split-Plot Average	14.2	15.3	14.9	15.6	14.4	15.1

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 53 Hemoglobin (gm/100ml) of Female Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS: 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	14.0	14.4	14.0	--[a]	14.8	14.0
Rat 2	14.8	13.8	14.3	14.8	14.4	14.4
Cage Average	14.4	14.1	14.2	14.8	14.6	14.2
Rat 3	13.6	15.6	15.0	14.6	13.8	13.6
Rat 4	12.8	15.0	13.6	14.8	13.3	14.0
Cage Average	13.2	15.3	14.3	14.7	13.6	13.8
Rat 5	15.0	15.6	13.0	13.8	13.6	15.0
Rat 6	13.8	13.3	14.8	15.3	15.6	14.6
Cage Average	14.4	14.4	13.9	14.6	14.6	14.8
Rat 7	14.8	15.8	12.6	14.0	12.5	14.8
Rat 8	13.3	15.3	14.4	15.0	14.0	14.8
Cage Average	14.1	15.6	13.5	14.5	13.3	14.8
Rat 9	14.8	15.2	14.0	15.3	14.8	15.0
Rat 10	12.5	14.6	14.8	14.4	14.4	14.4
Cage Average	13.7	14.9	14.4	14.9	14.6	14.8
Split-Plot Average	14.0	14.9	14.1	14.7	14.1	14.5

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 54 Hemoglobin (gm/100ml) of Male Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS: 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	13.6	16.0	10.6	16.0	11.7	16.0
Rat 2	14.5	14.7	14.0	14.2	--[a]	14.4
Cage Average	14.1	15.4	12.3	15.1	11.7	15.2
Rat 3	15.2	15.5	14.8	14.5	13.6	14.8
Rat 4	12.8	16.0	14.8	15.6	13.6	14.8
Cage Average	14.0	15.8	14.8	15.1	13.6	14.8
Rat 5	12.9	14.8	15.0	--	13.8	15.6
Rat 6	15.0	15.5	13.2	16.0	14.8	16.5
Cage Average	14.0	15.2	14.1	16.0	14.3	16.1
Rat 7	13.0	15.2	14.4	16.3	14.8	16.8
Rat 8	14.4	15.0	--	15.0	15.2	15.2
Cage Average	13.7	15.1	14.4	15.7	15.0	16.0
Rat 9	14.4	13.3	--	17.0	12.5	17.0
Rat 10	12.6	13.6	15.6	13.5	13.5	16.9
Cage Average	13.5	13.5	15.6	15.3	13.0	16.9
Split-Plot Average	13.9	15.0	14.2	15.4	13.5	15.8

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 55 Total Leukocytes (number/cmm) of Female Rats Fed a Basal Diet

ANIMALS	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
	DIETS					
Rat 1	3250	3000	5025	5010	7750	5030
Rat 2	5100	5500	--[a]	3650	5650	--
Cage Average	4175	4250	5025	4330	6700	5030
Rat 3	8000	7400	5850	4550	3550	6500
Rat 4	5450	6100	6550	3000	5550	5900
Cage Average	6725	6750	6200	3775	4550	6200
Rat 5	7800	5200	5450	5650	7500	5400
Rat 6	6900	4600	5000	3800	9000	8050
Cage Average	7350	4900	5225	4725	8250	6725
Rat 7	7000	6500	4000	6850	4025	4350
Rat 8	6700	4400	3500	5100	6200	5000
Cage Average	6850	5450	3750	5975	5112	4675
Rat 9	8000	6000	6100	--	4000	4550
Rat 10	7600	--	5600	8450	9250	--
Cage Average	7800	6000	5850	8450	6625	4550
Split-Plot Average	6580	5470	5210	5451	6247	5436

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 56 Total Leukocytes (number/cmm) of Male Rats Fed a Basal Diet

ANIMALS	10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
	DIETS					
Rat 1	6100	6750	8300	8250	6100	4250
Rat 2	5700	6650	12950	--[a]	9200	8600
Cage Average	5900	6700	10625	8250	7650	6425
Rat 3	--	--	8000	10600	7000	7350
Rat 4	--	--	6000	--	6050	9700
Cage Average	(6488)[b]	(8081)	7000	10600	6525	8525
Rat 5	7750	11950	12000	--	8150	9400
Rat 6	5350	9200	6250	12550	9700	6600
Cage Average	6550	10575	9125	12550	8925	8000
Rat 7	7250	8500	--	5850	4700	4500
Rat 8	8200	--	--	8050	6250	8350
Cage Average	7725	85--	(8213)	6950	5475	6425
Rat 9	5200	7600	8000	6150	5250	4000
Rat 10	6350	5500	8200	6350	6750	6000
Cage Average	5775	6550	8100	6250	6000	5000
Split-Plot Average	6487	8081	8612	8920	6915	6875

a. Dash indicates death of animal or quantity not sufficient for analyses.
b. Average values were determined from other animals in split-plot group for computer analyses.

Table 57 Total Leukocytes (number/cmm) of Female Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	6250	--[a]	3500	7500	7500	7300
Rat 2	4500	7950	--	5000	5400	5000
Cage Average	5380	7950	3500	6250	6450	6150
Rat 3	4000	7100	6100	5900	5900	3000
Rat 4	5450	4000	3750	--	4600	5400
Cage Average	4725	5550	4925	5900	5250	4200
Rat 5	8300	6200	9300	5000	4600	5250
Rat 6	7300	10050	8650	--	3500	4300
Cage Average	7650	8125	8975	5000	4050	4775
Rat 7	5650	5000	6250	6700	7250	7300
Rat 8	3750	6300	5150	5500	7250	4100
Cage Average	4700	5650	5700	6100	7250	5700
Rat 9	5000	6400	8900	7950	6150	7100
Rat 10	7000	7950	5750	11050	5200	9400
Cage Average	6000	7175	7325	9500	5675	8250
Split-Plot Average	5691	8890	6085	6550	5735	5815

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 58 Total Leukocytes (number/cmm) of Male Rats Fed a Cholesterol Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	7050	--	8000	5000	10050
Rat 2	10350	10050	7850	8600	5650	9150
Cage Average	10350	8550	7850	8300	5325	9600
Rat 3	6250	8150	7800	6550	5300	7150
Rat 4	11700	8150	6000	8500	8950	7500
Cage Average	8975	8150	6900	7525	7125	7325
Rat 5	7400	5900	8300	--	5700	5450
Rat 6	12100	9750	7850	3950	5550	7450
Cage Average	9750	7825	8075	3950	5625	6450
Rat 7	7500	10350	6500	6100	7250	8750
Rat 8	7250	10750	10600	9000	9600	9200
Cage Average	7375	10550	8550	7550	8425	8975
Rat 9	11500	9200	8400	10150	5400	11050
Rat 10	8500	8600	9400	6350	6450	5200
Cage Average	10000	8900	8900	8250	5925	8125
Split-Plot Average	9290	8795	8055	7115	6485	8095

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 59 Total Leukocytes (number/cmm) of Female Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	11550	4900	2400	--[a]	7950	5300
Rat 2	6850	6150	9350	8500	5950	6100
Cage Average	9200	5525	5875	8500	6950	5700
Rat 3	6500	6850	4800	4000	4000	4350
Rat 4	10900	8450	5450	5550	4250	6450
Cage Average	8700	7650	5125	4775	4125	5400
Rat 5	5250	4450	5000	--	7200	6150
Rat 6	6800	4000	5950	7900	6450	2000
Cage Average	6025	4225	5475	7900	6825	4075
Rat 7	6100	6000	5900	2800	3600	7150
Rat 8	5650	5900	5000	5800	3500	6050
Cage Average	5875	6450	5450	4300	3550	6600
Rat 9	4500	6050	5150	6150	4500	8100
Rat 10	5750	6350	8500	4100	4700	5350
Cage Average	5125	6200	6825	5125	4600	6725
Split-Plot Average	6985	6010	5750	6120	5210	5700

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 60 Total Leukocytes (number/cmm) of Male Rats Fed a Cholesterol and Fat Supplemented Diet

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	5300	6700	2050	7100	2150	5700
Rat 2	10150	6500	7600	7650	--[a]	10000
Cage Average	7725	6600	4825	7375	2150	7850
Rat 3	7500	10650	8100	7250	5020	7200
Rat 4	7700	9050	10700	7100	11750	7300
Cage Average	7600	9850	9400	7175	8385	7250
Rat 5	13500	11950	8650	--	5030	8350
Rat 6	8200	13400	10200	9650	10150	8850
Cage Average	10850	12675	9425	9650	7590	8600
Rat 7	10800	9250	7350	6800	10900	6000
Rat 8	8200	5100	--	11300	6500	6500
Cage Average	9500	7175	7350	9050	8700	6250
Rat 9	9150	8050	--	9400	8200	8700
Rat 10	6600	8250	8300	5950	5650	6000
Cage Average	7875	8150	8300	7675	6950	7350
Split-Plot Average	8710	8890	7860	8185	6750	7460

a. Dash indicates death of animal or quantity not sufficient for analyses.

Table 61 Blood Lymphocytes (number/cmm) of Experimental Rats

WHOLE PLOT DIETS[a]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	4508.5(8)	5519.3(7)	5692.5(8)	6841.6(7)	4909.7	4551.3
	Female	4869.2	3768.8(9)	4058.6(9)	3755.7(9)	4066.8	3696.5
	Average[b]	4708.8	4534.6	4827.2	5105.8	4488.3	4171.4
B+C	Male	6633.0(9)	6596.3	5549.9(9)	4638.9(9)	4208.8	5512.6
	Female	4461.7	6631.9(9)	4606.3(9)	4264.1(8)	3670.4(9)	4204.2
	Average	5490.2	6613.2	5078.1	4462.5	3953.8	4858.4
B+C+F	Male	5617.9	5796.3	6193.7	6449.7(9)	4137.8(9)	4953.4
	Female	4868.5	4237.0	4105.5(8)	3990.2(9)	3850.2	3876.0
	Average	5243.2	5016.6	5265.6	5219.9	3986.4	4414.7

a. B= Basal Diet
C= 1% Cholesterol
F= 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals per group unless indicated by () after numerical value.

Table 62 Blood Neutrophils (number/cmm) of Experimental Rats

		BASAL DIETS					
WHOLE PLOT DIETS[a]	Sex	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	1855.3(8)	2440.5(7)	2781.7(8)	1989.2(7)	1887.8	2220.6
	Female	1579.2	1630.0(9)	1021.2(9)	1586.2(9)	2005.3	1641.7
	Average	1701.9	1984.5	1849.7	1762.5	1946.5	1963.3
B+C	Male	2406.1(9)	2031.6	2279.6(9)	2284.4(9)	2101.1	2485.2
	Female	1138.2	2026.9(9)	1332.6(9)	2122.2(8)	1978.5(9)	1465.4
	Average	1738.8	2029.4	1806.1	2208.1	2043.0	1975.3
B+C+F	Male	2935.3	2942.6	1540.6	1571.5(9)	2497.5(9)	2327.5
	Female	2032.6	1670.8	1518.0(8)	2019.6(9)	1281.7	1732.8
	Average	2483.9	2306.7	1530.6	1795.5	1857.6	2030.2

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals per group unless indicated by () after numerical value.

Table 63 Blood Monocytes (number/cmm) of Experimental Rats

WHOLE PLOT DIET[a]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	129.7(8)	80.8(7)	94.7(8)	71.4(7)	82.9	82.5
	Female	98.7	32.8(9)	93.8(9)	103.6(9)	99.9	70.7(8)
	Average[b]	112.5	53.8	94.2	89.5	91.4	77.3
B+C	Male	167.2(9)	105.5	144.9(9)	120.9(9)	116.7	89.0
	Female	68.3	124.5(9)	97.4(9)	111.4(8)	74.6(9)	87.2
	Average	115.1	114.5	121.2	116.4	96.8	88.1
B+C+F	Male	87.1	88.9	102.2	114.6(9)	94.5(9)	126.8
	Female	62.9	78.1	74.8(8)	122.4(9)	62.5	62.7
	Average	75.0	83.5	90.0	118.5	77.6	94.8

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. Weighted average - ten animals/group unless indicated by () after numerical value.

Table 64 Blood Eosinophils (number/cmm) of Experimental Rats

WHOLE PLOT DIETS[a]	Sex	BASAL DIETS 10% Protein	25%q Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	25.9(8)	32.3(7)	43.1(8)	17.8(7)	34.6	20.6
	Female	32.9	32.8(9)	20.8(9)	5.5(9)	68.7	27.2(8)
	Average[b]	29.8	32.1	31.3	10.9	51.7	23.5
B+C	Male	55.7(9)	35.2	72.5(9)	71.2(9)	64.8	8.1
	Female	28.5	97.8(9)	54.8(9)	39.3(8)	5.7(9)	52.3
	Average	41.4	64.9	63.6	56.1	36.8	30.2
B+C+F	Male	60.9	62.2	39.3	32.7(9)	13.5(9)	29.8
	Female	20.9	18.0	46.0(8)	48.9(9)	10.4	17.1
	Average	40.9	40.1	42.2	40.8	11.9	23.5

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals/group unless indicated by () after numerical value.

Table 65 Systolic Blood Pressure(mmHg) of Female Rat Fed a Basal Diet (nine months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	121.0	110.0	140.0	150.0	115.0	122.0
Rat 2	119.0	120.0	113.0	137.0	121.0	118.0
Cage Average	120.0	115.0	126.5	143.5	118.0	120.0
Rat 3	110.0	129.0	121.0	138.0	109.0	122.0
Rat 4	119.0	133.0	110.0	123.0	107.0	120.0
Cage Average	114.5	131.0	115.5	130.5	108.0	121.0
Rat 5	115.0	121.0	118.0	143.0	97.0	129.0
Rat 6	118.0	127.0	121.0	125.0	70.0	129.0
Cage Average	116.5	124.0	119.5	134.0	83.5	129.0
Rat 7	114.0	137.0	119.0	129.0	120.0	151.0
Rat 8	122.0	141.0	114.0	135.0	108.0	134.0
Cage Average	118.0	139.0	116.5	132.0	114.0	142.5
Rat 9	137.0	139.0	109.0	---[a]	123.0	119.0
Rat 10	120.0	149.0	83.0	132.0	119.0	110.0
Cage Average	128.5	145.0	96.0	132.0	121.0	114.5
Split-Plot Average	119.5	130.8	114.8	134.4	108.9	125.4

a. Dash indicates death of animal or unable to determine blood pressure.

Table 66 Systolic Blood Pressure (mmHg) of Male Rats Fed a Basal Diet (nine months on test)

ANIMALS	DIETS: 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	95.0	130.0	--[a]	140.0	116.0	153.0
Rat 2	127.0	139.0	120.0	130.0	80.0	149.0
Cage Average	111.0	134.5	120.0	135.0	98.0	151.0
Rat 3	--	--	117.0	119.0	121.0	147.0
Rat 4	--	--	--	--	142.0	135.0
Cage Average	(116.4)[b]	(134.5)	117.0	119.0	131.5	141.0
Rat 5	115.0	138.0	135.0	141.0	118.0	140.0
Rat 6	109.0	138.0	--	--	120.0	147.0
Cage Average	112.0	138.0	135.0	141.0	119.0	143.5
Rat 7	111.0	135.0	--	140.0	120.0	140.0
Rat 8	119.0	--	--	141.0	139.0	123.0
Cage Average	114.0	135.0	(120.3)	140.5	129.5	131.5
Rat 9	117.0	129.0	111.0	147.0	--	121.0
Rat 10	140.0	132.0	107.0	143.0	127.0	150.0
Cage Average	128.5	130.5	109.0	145.0	127.0	135.5
Split-Plot Average	116.4	134.5	120.3	136.1	119.0	140.5

a. Dash indicates death of animal or unable to determine blood pressure.
b. Average values were determined from other animals in split-plot group for computer averages.

Table 67 Systolic Blood Pressure (mmHg) of Female Rats Fed a Cholesterol Supplemented Diet (nine months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	110.0	--[a]	128.0	129.0	88.0	124.0
Rat 2	110.0	127.0	103.0	131.0	141.0	137.0
Cage Average	110.0	127.0	115.5	130.0	114.5	130.5
Rat 3	107.0	132.0	111.0	109.0	119.0	134.0
Rat 4	111.0	137.0	101.0	129.0	128.0	128.0
Cage Average	109.0	134.5	106.0	119.0	123.5	131.0
Rat 5	126.0	120.0	123.0	121.0	141.0	124.0
Rat 6	90.0	118.0	131.0	130.0	117.0	120.0
Cage Average	108.0	119.0	127.0	125.5	129.0	122.0
Rat 7	112.0	119.0	124.0	110.0	116.0	133.0
Rat 8	98.0	122.0	109.0	120.0	104.0	119.0
Cage Average	105.0	120.5	116.5	115.0	110.0	126.0
Rat 9	130.0	141.0	141.0	159.0	141.0	122.0
Rat 10	120.0	112.0	109.0	124.0	142.0	127.0
Cage Average	125.0	126.5	125.0	141.5	141.5	124.5
Split-Plot Average	111.4	125.5	120.0	126.2	123.7	126.8

a. Dash indicates death of animal or unable to determine blood pressure.

Table 68 Systolic Blood Pressure (mmHg) of Male Rats Fed a Cholesterol Supplemented Diet (nine months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	137.0	138.0	--	117.0	132.0
Rat 2	--	130.0	110.0	--	150.0	125.0
Cage Average	(105.0)[b]	133.5	124.0	(128.5)	133.5	128.5
Rat 3	131.0	120.0	112.0	127.0	138.0	140.0
Rat 4	120.0	121.0	113.0	135.0	125.0	140.0
Cage Average	125.5	120.5	112.5	131.0	131.0	140.0
Rat 5	123.3	139.0	117.0	116.0	134.0	134.0
Rat 6	119.0	128.0	150.0	133.0	131.0	140.0
Cage Average	121.0	133.5	133.5	123.5	132.5	137.0
Rat 7	115.0	135.0	117.0	132.0	122.0	130.0
Rat 8	129.0	146.0	127.0	132.0	122.0	130.0
Cage Average	122.0	140.5	122.0	132.0	122.0	130.0
Rat 9	131.0	129.0	112.0	129.0	150.0	137.0
Rat 10	112.0	168.0	121.0	126.0	130.0	136.0
Cage Average	121.5	148.5	116.5	127.5	140.0	136.5
Split-Plot Average	119.0	135.3	121.7	128.5	131.9	134.4

a. Dash indicates death of animal or unable to determine blood pressure.

b. Average values were determined from other animals in split-plot group for computer analyses.

Table 69 Systolic Blood Pressure (mmHg) of Female Rats Fed a Cholesterol and Fat Supplemented Diet (nine months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	137.0	137.0	141.0	120.0	117.0	126.0
Rat 2	130.0	122.0	138.0	--[a]	--	130.0
Cage Average	133.5	129.5	139.5	120.0	117.0	128.0
Rat 3	141.0	119.0	133.0	112.0	130.0	129.0
Rat 4	118.0	118.0	135.0	--	129.0	--
Cage Average	129.5	118.5	134.0	112.0	129.5	129.0
Rat 5	120.0	122.0	130.0	129.0	127.0	133.0
Rat 6	119.0	122.0	140.0	131.0	131.0	133.0
Cage Average	119.5	122.0	135.0	130.0	129.0	133.0
Rat 7	130.0	123.0	111.0	140.0	125.0	--
Rat 8	118.0	124.0	123.0	134.0	--	129.0
Cage Average	124.0	123.5	117.0	137.0	125.0	129.0
Rat 9	112.0	158.0	123.0	130.0	--	109.0
Rat 10	115.0	126.0	108.0	137.0	--	121.0
Cage Average	113.5	142.0	115.5	133.5	(116.0)[b]	115.0
Split-Plot Average	124.0	127.1	130.2	126.5	123.3	126.8

a. Dash indicates death of animal or unable to determine blood pressure.

b. Average values were determined from other animals in split-plot group for computer analyses.

Table 70 Systolic Blood Pressure (mmHg) of Male Rats Fed a Cholesterol and Fat Supplemented Diet (nine months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	119.0	133.0	149.0	133.0	110.0	150.0
Rat 2	133.0	153.0	115.0	137.0	112.0	158.0
Cage Average	126.0	143.0	132.0	135.0	111.0	154.0
Rat 3	127.0	136.0	129.0	--[a]	129.0	130.0
Rat 4	154.0	139.0	138.0	119.0	130.0	147.0
Cage Average	145.5	137.5	134.5	119.0	129.5	138.5
Rat 5	125.0	143.0	139.0	139.0	131.0	129.0
Rat 6	131.0	141.0	161.0	123.0	122.0	151.0
Cage Average	128.0	142.0	150.0	131.0	126.5	140.0
Rat 7	115.0	147.0	119.0	117.0	130.0	128.0
Rat 8	125.0	145.0	--	121.0	129.0	141.0
Cage Average	120.0	146.0	119.0	119.0	129.5	134.5
Rat 9	131.0	141.0	--	155.0	112.0	160.0
Rat 10	135.0	143.0	111.0	122.0	100.0	148.0
Cage Average	133.0	142.0	111.0	138.5	106.0	154.0
Split-Plot Average	130.5	142.1	129.3	128.5	120.5	144.2

a. Dash indicates death of animal or unable to determine blood pressure.

Table 71 Systolic Blood Pressure (mmHg) of Female Rats Fed a Basal Diet (eleven months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	138.0	128.0	121.0	149.0	122.0	128.0
Rat 2	127.0	132.0	135.0	129.0	124.0	136.0
Cage Average	131.5	130.0	128.0	139.0	123.0	132.0
Rat 3	107.0	117.0	136.0	146.0	134.0	134.0
Rat 4	138.0	141.0	129.0	119.0	86.0	141.0
Cage Average	122.5	129.0	132.5	132.5	110.0	137.5
Rat 5	138.0	122.0	107.0	152.0	137.0	139.0
Rat 6	120.0	113.0	135.0	126.0	125.0	129.0
Cage Average	129.0	117.5	121.0	139.0	131.0	134.0
Rat 7	135.0	122.0	118.0	141.0	133.0	135.0
Rat 8	130.0	129.0	117.0	117.0	127.0	128.0
Cage Average	132.5	125.5	117.5	129.0	130.0	131.5
Rat 9	150.0	140.0	124.0	--[a]	114.0	139.0
Rat 10	119.0	--	111.0	99.0	120.0	--
Cage Average	134.5	140.0	117.5	99.0	117.0	139.0
Split-Plot Average	130.0	128.4	123.3	127.7	122.2	134.8

a. Dash indicates death of animal or unable to determine blood pressure.

Table 72 Systolic Blood Pressure (mmHg) of Male Rats Fed a Basal Diet (eleven months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	121.0	153.0	117.0	129.0	150.0	134.0
Rat 2	120.0	145.0	141.0	139.0	115.0	151.0
Cage Average	120.5	149.0	129.0	134.0	132.5	142.5
Rat 3	--[a]	--	140.0	139.0	120.0	137.0
Rat 4	--	--	139.0	147.0	131.0	120.0
Cage Average	(126.3)[b]	(139.8)	139.5	143.0	124.5	128.5
Rat 5	117.0	145.0	127.0	144.0	138.0	145.0
Rat 6	149.0	117.0	132.0	141.0	127.0	151.0
Cage Average	133.0	131.0	129.5	142.5	131.5	148.0
Rat 7	140.0	159.0	--	121.0	140.0	152.0
Rat 8	113.0	--	--	117.0	144.0	149.0
Cage Average	126.5	159.0	(130.8)	119.0	142.0	150.5
Rat 9	121.0	106.0	124.0	148.0	126.0	145.0
Rat 10	129.0	135.0	127.0	140.0	142.0	151.0
Cage Average	125.0	120.5	125.5	144.0	134.0	148.0
Split-Plot Average	126.3	139.9	130.9	136.5	132.9	143.5

a. Dash indicates death of animal or unable to determine blood pressure.

b. Average values were determined from other animals in split-plot group for computer analyses.

Table 73 Systolic Blood Pressure (mmHg) of Female Rats Fed a Cholesterol Supplemented Diet (eleven months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	107.0	--[a]	137.0	142.0	121.0	119.0
Rat 2	122.0	118.0	128.0	143.0	137.0	132.0
Cage Average	114.5	118.0	132.5	142.5	129.0	125.5
Rat 3	123.0	150.0	111.0	114.0	100.0	127.0
Rat 4	127.0	133.0	127.0	124.0	123.0	134.0
Cage Average	125.0	141.5	119.0	119.0	111.5	130.5
Rat 5	127.0	133.0	129.0	119.0	140.0	111.0
Rat 6	117.0	118.0	124.0	123.0	119.0	137.0
Cage Average	122.0	125.5	126.5	121.0	129.5	124.0
Rat 7	113.0	140.0	135.0	108.0	149.0	131.0
Rat 8	116.0	142.0	118.0	125.0	125.0	128.0
Cage Average	114.5	141.0	126.5	116.5	137.0	129.5
Rat 9	120.0	129.0	133.0	179.0	127.0	129.0
Rat 10	126.0	136.0	132.0	125.0	126.0	130.0
Cage Average	123.0	132.5	132.5	152.0	126.5	129.5
Split-Plot Average	119.8	131.7	127.4	130.2	126.7	127.8

a. Dash indicates death of animal or unable to determine blood pressure.

Table 74 Systolic Blood Pressure (mmHg) of Male Rats Fed a Cholesterol Supplemented Diet (eleven months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	143.0	136.0	137.0	121.0	126.0
Rat 2	101.0	135.0	120.0	143.0	151.0	151.0
Cage Average	101.0	139.0	128.0	140.0	136.0	138.5
Rat 3	120.0	125.0	153.0	121.0	138.0	159.0
Rat 4	124.0	117.0	119.0	131.0	141.0	129.0
Cage Average	122.0	121.0	136.0	126.0	139.5	145.0
Rat 5	120.0	129.0	131.0	--	128.0	140.0
Rat 6	122.0	129.0	134.0	141.0	122.0	130.0
Cage Average	121.0	129.0	132.5	141.0	125.0	135.0
Rat 7	104.0	139.0	138.0	149.0	111.0	149.0
Rat 8	114.0	137.0	138.0	149.0	141.0	135.0
Cage Average	109.0	138.0	138.0	149.0	126.0	142.0
Rat 9	122.0	150.0	127.0	114.0	111.0	126.0
Rat 10	107.0	175.0	121.0	142.0	129.0	146.0
Cage Average	114.5	162.5	124.0	128.0	120.0	136.0
Split-Plot Average	113.5	137.9	131.7	136.8	129.3	139.3

a. Dash indicates death of animal or unable to determine blood pressure.

Table 75 Systolic Blood Pressure (mmHg) of Female Rats Fed a Cholesterol and Fat Supplemented Diet (eleven months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	109.0	122.0	119.0	118.0	130.0	112.0
Rat 2	119.0	133.0	137.0	119.0	109.0	114.0
Cage Average	114.0	127.5	128.0	118.5	119.5	113.0
Rat 3	147.0	134.0	120.0	110.0	111.0	103.0
Rat 4	114.0	111.0	145.0	110.0	129.0	102.0
Cage Average	130.5	122.5	132.5	110.0	119.0	102.5
Rat 5	118.0	119.0	101.0	121.0	120.0	137.0
Rat 6	117.0	107.0	131.0	124.0	124.0	120.0
Cage Average	117.5	113.0	116.0	122.5	122.0	128.5
Rat 7	117.0	129.0	119.0	123.0	108.0	115.0
Rat 8	105.0	131.0	127.0	124.0	129.0	120.0
Cage Average	111.0	130.0	123.0	123.5	118.5	117.5
Rat 9	141.0	123.0	101.0	127.0	116.0	101.0
Rat 10	128.0	131.0	128.0	132.0	110.0	107.0
Cage Average	134.5	127.0	109.5	129.5	113.0	104.0
Split-Plot Average	121.4	123.6	121.8	120.8	118.4	113.1

Table 76 Systolic Blood Pressure (mmHg) of Male Rats Fed a Cholesterol and Fat Supplemented Diet (eleven months on test)

ANIMALS	DIETS					
	10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	117.0	130.0	126.0	144.0	115.0	149.0
Rat 2	132.0	139.0	117.0	146.0	118.0	133.0
Cage Average	124.5	134.5	121.5	145.0	116.5	141.0
Rat 3	109.0	143.0	135.0	151.0	129.0	109.0
Rat 4	125.0	141.0	130.0	140.0	100.0	132.0
Cage Average	117.0	142.0	132.5	145.5	114.5	120.5
Rat 5	119.0	139.0	139.0	--[a]	117.0	141.0
Rat 6	119.0	142.0	159.0	148.0	120.0	139.0
Cage Average	119.0	140.5	149.0	148.0	118.5	140.0
Rat 7	139.0	130.0	155.0	129.0	131.0	129.0
Rat 8	112.0	143.0	--	142.0	122.0	140.0
Cage Average	125.5	136.5	155.0	135.5	126.5	134.5
Rat 9	111.0	146.0	--	140.0	120.0	141.0
Rat 10	140.0	146.0	109.0	143.0	109.0	147.0
Cage Average	125.5	146.0	109.0	141.5	114.5	144.0
Split-Plot Average	122.3	139.9	133.4	143.1	118.1	136.0

a. Dash indicates death of animal or unable to determine blood pressure.

Table 77 Systolic Blood Pressure (mmHg) of Female Rats Fed a Basal Diet (twelve months on test)

ANIMALS	DIETS: 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	149.0	130.0	123.0	127.0	127.0	127.0
Rat 2	138.0	118.0	126.0	130.0	124.0	132.0
Cage Average	143.5	124.0	124.5	128.5	125.5	129.5
Rat 3	110.0	123.0	134.0	133.0	142.0	138.0
Rat 4	138.0	129.0	127.0	129.0	105.0	131.0
Cage Average	124.0	126.0	130.5	131.0	123.5	134.5
Rat 5	144.0	127.0	107.0	135.0	129.0	135.0
Rat 6	128.0	129.0	128.0	121.0	119.0	131.0
Cage Average	136.0	128.0	117.5	128.0	125.0	133.0
Rat 7	139.0	117.0	121.0	139.0	107.0	120.0
Rat 8	127.0	119.0	118.0	111.0	128.0	135.0
Cage Average	131.0	118.0	119.5	125.0	117.5	127.5
Rat 9	130.0	129.0	121.0	--[a]	119.0	138.0
Rat 10	120.0	--	109.0	115.0	123.0	--
Cage Average	125.0	129.0	115.0	115.0	121.0	138.0
Split-Plot Average	131.9	125.0	121.4	125.5	122.5	132.5

a. Dash indicates death of animal or unable to determine blood pressure.

Table 78 Systolic Blood Pressure (mmHg) of Male Rats Fed a Basal Diet (twelve months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	120.0	129.0	113.0	140.0	139.0	149.0
Rat 2	121.0	134.0	122.0	--[a]	130.0	131.0
Cage Average	120.5	131.5	117.5	140.0	134.0	140.0
Rat 3	--	--	117.0	129.0	117.0	123.0
Rat 4	--	--	129.0	--	109.0	110.0
Cage Average	(122.3)[b]	(137.3)	123.0	129.0	113.0	116.5
Rat 5	119.0	135.0	138.0	147.0	119.0	127.0
Rat 6	123.0	142.0	129.0	137.0	120.0	159.0
Cage Average	121.0	138.5	133.5	142.0	119.5	143.0
Rat 7	129.0	141.0	--	127.0	140.0	134.0
Rat 8	132.0	--	--	119.0	131.0	133.0
Cage Average	130.5	141.0	(123.8)	123.0	135.5	133.5
Rat 9	109.0	135.0	119.0	139.0	111.0	123.0
Rat 10	125.0	141.0	123.0	127.0	119.0	150.0
Cage Average	117.0	138.0	121.0	133.0	115.0	136.5
Split-Plot Average	122.3	137.3	123.8	133.4	123.4	133.9

a. Dash indicates death of animal or unable to determine blood pressure.

b. Average values were determined from other animals in split-plot group for computer analyses.

Table 79 Systolic Blood Pressure (mmHg) of Female Rats Fed a Cholesterol Supplemented Diet (twelve months on test)

ANIMALS	DIETS: 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	119.0	--[a]	121.0	123.0	110.0	127.0
Rat 2	115.0	115.0	129.0	130.0	103.0	121.0
Cage Average	117.0	115.0	125.0	126.5	106.5	124.0
Rat 3	127.0	124.0	94.0	113.0	121.0	110.0
Rat 4	129.0	131.0	113.0	125.0	123.0	120.0
Cage Average	128.0	127.5	103.5	119.0	122.0	115.0
Rat 5	137.0	133.0	107.0	104.0	118.0	120.0
Rat 6	119.0	119.0	133.0	121.0	113.0	121.0
Cage Average	128.0	126.0	120.0	112.5	115.5	120.5
Rat 7	112.0	109.0	139.0	101.0	138.0	121.0
Rat 8	120.0	115.0	124.0	117.0	117.0	119.0
Cage Average	116.0	112.0	131.5	109.0	127.5	120.0
Rat 9	130.0	122.0	121.0	163.0	120.0	139.0
Rat 10	113.0	111.0	122.0	128.0	128.0	140.0
Cage Average	121.5	116.5	121.5	145.5	124.0	139.5
Split-Plot Average	122.1	119.4	120.3	122.5	119.1	123.8

a. Dash indicates death of animal or unable to determine blood pressure.

Table 80 Systolic Blood Pressure (mmHg) of Male Rats Fed a Cholesterol Supplemented Diet (twelve months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	--[a]	113.0	--	127.0	133.0	127.0
Rat 2	112.0	129.0	126.0	127.0	168.0	132.0
Cage Average	112.0	121.0	126.0	127.0	150.5	129.5
Rat 3	107.0	128.0	141.0	115.0	125.0	138.0
Rat 4	123.0	110.0	125.0	123.0	117.0	139.0
Cage Average	115.0	118.5	133.0	119.0	121.0	138.5
Rat 5	118.0	131.0	122.0	--	123.0	129.0
Rat 6	117.0	135.0	122.0	133.0	118.0	132.0
Cage Average	117.5	133.0	122.0	133.0	120.5	130.5
Rat 7	120.0	133.0	130.0	133.0	135.0	141.0
Rat 8	125.0	142.0	128.0	145.0	127.0	149.0
Cage Average	122.5	137.5	129.0	139.0	131.0	145.0
Rat 9	119.0	149.0	123.0	129.0	107.0	113.0
Rat 10	131.0	127.0	127.0	133.0	137.0	130.0
Cage Average	125.0	138.0	125.0	131.0	122.0	126.5
Split-Plot Average	118.4	129.6	127.0	129.8	129.0	134.0

a. Dash indicates death of animal or unable to determine blood pressure.

Table 81 Systolic Blood Pressure (mmHg) of Female Rats Fed a Cholesterol and Fat Supplemented Diet (twelve months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B12 Deficient	25% Protein Vit.B12 Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	129.0	130.0	117.0	130.0	117.0	120.0
Rat 2	129.0	116.0	124.0	115.0	128.0	110.0
Cage Average	129.0	123.0	120.5	122.5	121.5	115.0
Rat 3	150.0	131.0	131.0	111.0	109.0	121.0
Rat 4	122.0	113.0	140.0	109.0	124.0	114.0
Cage Average	136.0	122.0	135.5	110.0	116.5	117.5
Rat 5	127.0	109.0	120.0	124.0	125.0	129.0
Rat 6	111.0	120.0	116.0	131.0	133.0	125.0
Cage Average	119.0	114.5	118.0	127.5	129.0	127.0
Rat 7	139.0	131.0	109.0	138.0	129.0	127.0
Rat 8	133.0	120.0	131.0	123.0	130.0	114.0
Cage Average	136.0	125.5	120.0	130.5	129.5	120.5
Rat 9	155.0	135.0	103.0	120.0	113.0	122.0
Rat 10	132.0	129.0	125.0	136.0	118.0	113.0
Cage Average	143.5	132.0	114.0	128.0	115.5	126.5
Split-Plot Average	132.7	123.4	121.6	123.7	122.4	121.3

Table 82 Systolic Blood Pressure (mmHg) of Male Rats Fed a Cholesterol and Fat Supplemented Diet (twelve months on test)

ANIMALS	DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
Rat 1	149.0	123.0	151.0	138.0	108.0	139.0
Rat 2	120.0	129.0	149.0	141.0	113.0	149.0
Cage Average	134.5	126.0	150.0	139.5	110.5	154.0
Rat 3	127.0	143.0	134.0	150.0	160.0	140.0
Rat 4	120.0	130.0	135.0	146.0	121.0	143.0
Cage Average	123.5	136.5	134.5	148.0	140.5	141.5
Rat 5	107.0	131.0	128.0	--[a]	121.0	135.0
Rat 6	123.0	134.0	145.0	131.0	133.0	138.0
Cage Average	115.0	132.5	136.5	131.0	127.0	136.5
Rat 7	122.0	127.0	132.0	130.0	130.0	138.0
Rat 8	119.0	129.0	--	120.0	137.0	132.0
Cage Average	120.5	128.0	132.0	125.0	133.5	135.0
Rat 9	119.0	143.0	--	147.0	128.0	157.0
Rat 10	141.0	128.0	113.0	131.0	131.0	143.0
Cage Average	130.0	135.5	113.0	139.0	129.5	150.0
Split-Plot Average	124.7	131.7	133.2	136.9	128.2	143.3

a. Dash indicates death of animal or unable to determine blood pressure.

Table 83 Body Weight (gms) of Rats on Test (1 wk)

WHOLE PLOT DIETS [a]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	66.3	78.1(9)	64.3	81.1	56.1	67.1
	Female	72.2	79.6	64.0	75.5	63.2	69.8
	Average [b]	69.3	78.9	64.2	78.3	59.7	68.5
B+C	Male	60.3	79.6	64.8	81.5	55.1	60.7
	Female	68.2	79.0	69.9	79.7	56.6	66.5
	Average	64.3	79.3	67.4	80.6	55.9	63.6
B+C+F	Male	62.3	76.7	58.3	74.7	58.3	61.8
	Female	59.6	70.4	58.6	74.5	60.8	64.5
	Average	61.0	73.6	58.5	74.6	59.6	63.2

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals/group unless indicated by () after weight.

Table 84 Body Weight (gms) of Rats on Test (3 wks)

WHOLE PLOT DIETS[a]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	101.9	127.0(9)	92.2	133.8	93.3	133.3
	Female	106.9	125.2	90.7	119.6	90.1	108.2
	Average	104.4	126.1	91.5	126.7	91.7	120.6
B+C	Male	89.9	129.6	97.3	127.7	88.9	112.4
	Female	97.9	121.1	101.4	123.6	91.7	116.1
	Average	93.9	125.4	99.4	125.7	90.3	114.3
B-C-F	Male	101.9	140.3	97.7	130.0	99.1	128.0
	Female	99.5	110.1	91.5	129.8	87.9	115.0
	Average	100.7	125.2	94.6	129.9	93.5	121.5

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil

b. Weighted averages - ten animals/group unless indicated by ( ) after weight.

Table 85 Body Weight (gms) of Rats on Test (13 wks)

WHOLE PLOT DIETS [a]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	227.6	311.1(9)	222.0	299.6	226.0	313.4
	Female	191.8	221.0	181.8	211.0	192.5	203.4
	Average [b]	209.7	263.7	201.9	255.3	209.3	258.4
B+C	Male	215.0	282.8	221.6	273.2	221.9	261.2
	Female	178.1	216.7	188.3	206.9	175.9	195.6
	Average	196.5	249.8	205.1	240.0	198.9	228.4
B+C+F	Male	217.2	319.0	219.3	292.8	214.2	298.3
	Female	191.0	212.7	184.6	220.1	182.7	211.3
	Average	204.1	265.9	202.0	256.5	198.5	254.8

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals/group unless indicated by () after weight.

Table 86 Body Weight (gms) of Rats on Test (24 wks)

WHOLE PLOT DIETS [a]	Sex	10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
		BASAL DIETS					
B	Male	239.9	331.0(8)	237.5	315.4	228.9	320.3
	Female	202.2	234.5	195.7	229.8	203.3	216.0
	Average [b]	221.1	277.4	216.6	272.6	216.1	268.2
B+C	Male	225.2	291.9	227.0	281.2	213.1	271.2
	Female	185.9	227.3	197.6	224.7	173.8	208.7
	Average	205.6	259.6	212.3	252.9	193.5	240.0
B+C+F	Male	228.2	335.1	227.7	300.3	214.3	309.8
	Female	199.5	231.5	193.3	239.4	189.3	222.6
	Average	213.9	283.3	210.5	269.8	201.8	266.2

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals/group unless indicated by () after weight.

Table 87 Body Weight (gms) of Rats on Test (33 wks)

WHOLE PLOT DIETS [a]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	244.9	351.5(8)	258.8(8)	331.2	224.0	336.0
	Female	208.6	240.5	202.7	230.2	202.6	220.2
	Average [b]	226.7	289.8	227.6	280.7	213.3	278.1
B+C	Male	232.1(9)	301.8	239.8	293.1	199.0	282.1
	Female	186.5	234.2(9)	199.1	229.2	155.0	209.4
	Average	208.1	269.8	219.5	261.2	177.0	245.8
B+C+F	Male	239.6	340.0	241.0	309.8	206.3	309.0
	Female	202.0	233.5	197.2	237.7	182.2	224.4
	Average	220.8	286.8	219.1	273.6	194.3	266.7

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals/group unless indicated by () after weight.

Table 88 Body Weight (gms) of Rats on Test (44 wks)

WHOLE PLOT DIET [a]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit. B_{12} Deficient	25% Protein Vit. B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	257.6(8)	359.2(7)	274.1(8)	345.3	243.8	350.4
	Female	212.8	240.2(9)	214.5	239.8	208.9	225.9
	Average [b]	232.7	292.3	241.0	292.6	226.4	288.2
B+C	Male	252.7(9)	317.2	255.0(9)	312.6(9)	206.1	291.0
	Female	204.4	243.3(9)	213.4	232.4	163.5	214.1
	Average	227.3	282.2	233.1	270.4	184.8	252.5
B+C+F	Male	249.9	347.0	248.8	315.5(9)	190.3	313.4
	Female	197.4	228.5	205.3(8)	235.3	170.3	213.6
	Average	223.7	287.8	229.4	273.3	180.3	263.5

a. B = Basal Diet
C = 1% Cholesterol
F = 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals/group unless indicated by () after weight.

Table 89 Body Weight (gms) of Rats on Test (52 wks)

WHOLE PLOT DIETS [a]	Sex	BASAL DIETS 10% Protein	25% Protein	10% Protein Vit.B_{12} Deficient	25% Protein Vit.B_{12} Deficient	10% Protein Choline Deficient	25% Protein Choline Deficient
B	Male	279.8(8)	398.3(7)	299.1(8)	347.0(8)	265.8	365.1
	Female	222.9	248.8(9)	222.4	252.1(9)	213.3	234.0(9)
	Average[b]	248.2	314.2	256.5	296.7	239.6	303.0
B+C	Male	257.3(9)	334.5	278.4(9)	335.0(9)	254.0	311.6
	Female	206.9	243.9(9)	213.0	229.7	187.3	222.4
	Average	230.8	291.6	244.0	279.6	220.7	267.0
B+C+F	Male	277.7	375.9	273.2	333.4(9)	216.6	336.1
	Female	215.4	232.9	219.9(8)	242.4	195.4	222.6
	Average	246.2	304.4	249.5	285.5	206.0	279.4

a. B= Basal Diet
C= 1% Cholesterol
F= 12% Hydrogenated Coconut Oil.

b. Weighted averages - ten animals/group unless indicated by () after weight.