TITLE THE EFFECT OF AGE, DIET, AND CARBON TETRACHLORIDE-INDUCED LIVER INJURY ON THE CHOLESTEROL CONTENT OF BLOOD AND CERTAIN OTHER TISSUES IN THE ALBINO RAT

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INTRODUCTION

Cholesterol, one of the most important naturally occurring sterols, is present in all cells of animal organism both in the free state and in the form of fatty acid esters.

Its origin is endogenous as well as exogenous. It can be absorbed from dietary sources, synthesized from simple molecules such as acetate, acetaldehyde, acetone, pyruvate, isovalerate and short fatty acids as shown by Bloch, Rittenberg, Brady, and Gurin (1, 2, 3, 4), and destroyed during metabolic processes. Under normal conditions the quantity or the ratio of the free to the combined forms present in the blood and tissues varies only within narrow limits, for absorption, synthesis, and destruction are so accurately attuned.

However, this is not the case if the individual is under the disturbing influences of unbalanced diet, or impairment of any of the organs. In dietary fatty livers or when large quantities of cholesterol are given to animals the substance accumulates in the liver largely as cholesterol esters. When the parenchyma of liver is extensively damaged the concentration of cholesterol esters in the blood plasma falls and renal lesions appear. In biliary obstruction both free and ester cholesterol in the blood rise, and the dis-

proportion between free and ester forms increases as the obstruction persists.

In patients with diabetes mellitus and nephritis a general rise in all of the lipids of the blood occurs. Hypercholesterolemia has been held responsible for arteriosclerosis which often accompanies diabetes. The general opinion is that arteriosclerosis must be connected with a disturbance of cholesterol metabolism.

Much work has been done in connection with this disease using the chick, rabbit, dog and sometimes the guinea pig and golden hamster as the experimental animal. The rat, although much used in other metabolic studies, has not been considered quite appropriate for this purpose. Therefore only scanty data are available from the literature.

It was the object of the present investigation to study the effect of age, of diets both adequate and inadequate in essential nutrients, and of carbon tetrachloride-induced liver damage on cholesterol levels in blood, liver, heart, and kidney of albino rats.

LITERATURE REVIEW

I. Cholesterol Levels as Influenced by Various Factors

A. Age and Sex

Early in 1928 Shope (5) reported from his observation on calves that changes in serum cholesterol and cholesterol ester content as related to age were of two types: first a marked and rather rapid increase from birth for a relatively short period and then a less marked and more gradual decline with advancing age. He noticed that changes with age were more uniform and regular in male than in female animals. Page et al (6) did not find variations of age in men from 20-90 years a determinable influence on either the amount or the composition of the plasma lipids. Turner and Steiner (7) reported, from weekly determinations of serum cholesterol in several patients over a period of 7-14 months, that the level of cholesterol is remarkably constant. In 1937 Sperry (8) concluded from a long-term study on 25 healthy adult persons that the variation in a given individual over considerable periods of time is much less than the variation among individuals and that in most persons in health the serum cholesterol seems to be maintained at a constitutional level which is characteristic for each individual. In 1950 he and Webb (9) made further studies on the same subjects and

observed that in 8 of the 14 men and 1 of the 8 women there was no appreciable change; in 6 men and 6 women increases of 15-30 percent were found. They concluded that serum cholesterol concentration increases with age in some persons but the increase is not an obligatory concomitant of ageing. Foldes and Murphy (10) noticed from their study of 20 young healthy adults and 20 old patients with no known disorder of lipid metabolism that practically no difference was found in the total cholesterol, cholesterol esters, and phospholipid phosphorus of the two age groups. Gram and Leverton (11) studying the serum cholesterol in women of 5 different age groups, 18-70, found the increase of cholesterol in serum with increasing age to be significant. Keys et al (12) made a cross-sectional examination of 1,492 men from 17-78 years old and 564 women from 17-30 years old. They found that over the age range 17-30 the cholesterol values from men and women were not significantly different as to averages, individual variability, and age trend. Over this range there was an average increase per year amounting to 2.2 mg. of total cholesterol per 100 ml. of serum. For the age range 17-78 years in men there was a pronounced curvilinear relation between age and serum cholesterol concentration with a maximum in the sixth decade. The evidence, they believe, points clearly to rising values for serum cholesterol from youth through middle age to lower values in the oldest persons in the population samples. However, they did not postulate that the cholesterol level actually

declined in old age. Kountz et al (13) reported, from studying 912 patients aged 40-85, that the females showed an average blood-cholesterol level of 237 mg. percent, the males 196 mg. percent. Atherosclerosis appeared earlier and more frequently among the men. Kornerup (14) observed from studies made on 87 men and 38 women from 19-96 years and 96 children from 1-16 years that sex and age differences were small and generally insignificant, but the serum free cholesterol levels of elderly men were significantly higher than those of young men.

With rats, Mounier et al (15) reported similar blood cholesterol levels for both young and old animals.

B. Certain Physiological Conditions

Fasting. Sure, Kik and Church (16) observed that in the albino rat during fasting there was a marked decrease of blood fatty acids and lecithin, but no change in the concentration of blood cholesterol. Entenman et al (17) observed that there was no lipemia, or rise in the levels of total cholesterol, total fatty acids, and phospholipids during acute fasting or chronic undernutrition. Schettler (18) reported from his studies on mice that liver total cholesterol rose on the second day of fasting but dropped by the sixth day. In the other tissues of the animals the cholesterol remained unchanged.

Pregnancy and menstruation. Okey and Stewart (19) noticed that blood cholesterol in women tends to be high just

preceding menstruation, with a fall near the time of onset and a rise afterwards. Lea (20) reported that non-pregnant female mice had considerably less blood cholesterol than males, and pregnant females had about the same as males.

Hypothyroidism and hyperthyroidism. Johnson and Riegel (21) and Foldes and Murphy (22) found that the blood cholesterol of dog and man increased significantly in hypothyroidism and fell to very low values in hyperthyroidism, but the cell lipid values were relatively constant. Fleischmann and Shumacker (23) obtained similar results at an earlier date, concluding that thyroxine having no specific effect on cholesterol metabolism influenced only a shift of cholesterol to and from the blood plasma. Horlick and Havel (24) noticed that cholesterol and propylthiouracil alone or combined failed to produce atherosclerotic vascular lesions in the rat, but cholesterolemia of 2 to 3 times normal values resulted with cholesterol or propylthiouracil feeding and approximately 6 times normal with combined feeding.

Development of tumors. Bennett (25) reported early in 1922 that the outer, more actively growing portions of the tumor contained more cholesterol than the central portion. Jowett (26) noticed that pure malignant tissues are higher in phosphatide and cholesterol than are malignant tissues admixed with normal tissues. Malignant tissues also showed a high proportion of bound cholesterol.

Knudson et al (27) observed that during irradiation

with ultraviolet light the cholesterol content of the skin of rats was increased, practically all of the increase being in the ester form; the tumors showed a high cholesterol content, about 80 percent being in the free form; and the total blood cholesterol was somewhat below the normal. Khaletskaya (28) reported that in mice the blood cholesterol varied with the stage of the development of the tumor, showing a gradual increase from the beginning, reaching the highest (about 30 percent above the normal) at papilloma stage, and dropping to near normal when definite cancer had developed.

C. Dietary Factors

General nutrition. Bloor (29) demonstrated that in dogs single overfeedings with fat or carbohydrate generally resulted in high plasma phospholipid and fat in the post absorptive state, whereas the cholesterol level was not affected. Schmidt et al (30) in a study of the blood cholesterol levels of 10-20 normal persons during 1942-1947, noticed a decrease in total, free, and esterified plasma cholesterol. Lowered fat metabolism due to poor nutrition was held responsible. Recently Schettler (31) reported an increase in total plasma cholesterol in 50 men and 50 women since 1947-1949. He believed the improved nutrition normalized the previously low cholesterol values.

Fat. Treadwell and Eckstein (32) observed that fat contents of diets fed to rats did not influence the blood cholesterol, and the neutral fat, phospholipid and choles-

terol of the liver. Similarly Alfin-Slater et al (33) noticed that the amount of cholesterol synthesized in the liver under the different dietary conditions was the same. However considerable differences were noted by Schettler (34) in mice fed plant oils and those fed animal fats. The former showed an increase in the total blood cholesterol, especially the esterified fraction, accompanied by a lowering in the organs; whereas in the latter there was no definite evidence of hypercholesterolemia. The same author (35) observed an increase in organ cholesterol when cholesterol, and sodium and potassium salts of organic acids were fed to white mice on a basal diet poor in protein and rich in fat. Harris et al (36) reported that a high fat-low carbohydrate diet raised the serum cholesterol level in patients with high blood pressure, and that injection of insulin decreased the serum fat and cholesterol levels.

Vitamins. There is much controversal information regarding vitamin A and cholesterol metabolism. Lasch (37) reported that vitamin A given 3 times a day to human beings, in doses of 40,000-80,000 international units caused an increase in the serum cholesterol (ester fraction) within 5-10 days. Chalier et al (38) found that in guinea pigs deprived of vitamin A the blood cholesterol remained within normal limits. Extra vitamin A increased the blood cholesterol level but heavy doses decreased their blood cholesterol to mere traces. Usuni (39) noticed an increase in serum cholesterol in rabbits receiving a diet deficient in vitamin A. Knapp and Blackberg (40) using rats reported that

deficiencies in vitamin A and in vitamin B complex and in caloric intake produced lesions in the eyes resembling senile arteriosclerosis in human subjects. Choline, methionine, and meso-inositol have been found effective in lowering blood and liver cholesterol (41, 42). Forbes (43) reported that administration of nicotinic acid caused an increased production of cholesterol in the fatty livers. The effects of biotin upon fat synthesis and storage of cholesterol in liver have been reported by Gavin, and McHenry (44) and Okey et al (45). Biotin when given to rats, caused fatty livers which were characterized by a high content of cholesterol. This condition could be prevented by the simultaneous feeding of egg white, lipocaic, or inositol. When rats are fed a biotin-deficient and cholesterol-rich diet they failed to store excess liver cholesterol ester. They believed biotin must be connected with cholesterol metabolism. Myasnikov (46) noticed that in experimental cholesterol atherosclerosis of rabbits, vitamin C reduced blood cholesterol and inhibited the growth of fatty deposits in the aorta. Vitamin E was found by Dam (47) without effect on the deposition of cholesterol in the aorta in rabbits. Max et al (48) noticed that in general tissue and blood cholesterol levels in young rats were not altered by either low or high intakes of vitamin E. However a deficiency caused deposition of cholesterol in aorta.

Favorable factors in treating high blood pressure and

arteriosclerosis. Roffo (49) observed a marked decrease in blood cholesterol in patients injected with an extract from eggplant. Similar properties were found to exist in the artichoke. The author believed the action due to their content of magnesium and potassium salts. However Wilkinson et al (50) could not confirm this from their studies. Recently a rice-fruit diet (51) was reported to have a marked effect in lowering the serum cholesterol, but the specific mechanism remains unknown. Rodbard et al (52) found that restriction of dietary intake even to the point of emaciation gave no protection against atheromatosis or hypercholesterolemia in chicks on a diet supplemented with cholesterol.

D. Production of Arteriosclerosis in Experimental Animals

Species differences have to be considered in producing experimental arteriosclerosis in chicks, guinea pigs, rabbits and dogs.

In chicks the condition may be produced either by cholesterol feeding or diethylstilbesterol administration. Considerably more cholesterol is deposited after cholesterol feeding than after estrogen treatment.

Feeding cholesterol to guinea pigs results in anemia and an increase in the free cholesterol content of liver, spleen, heart, lungs and blood as shown by Okey (53). A high cholesterol diet will induce arteriosclerosis.

Member et al (54) reported that cholesterol fed with cholic or glycocholic acid to rabbits increased the choles-

terol content of the blood and aorta more than the same amount of cholesterol fed alone. Capritti and Magnani (55) observed that addition of pyridoxine to cholesterol further increased the free and esterified levels in rabbits and also the arteriosclerosis was induced more rapidly.

The production of arteriosclerosis in dogs requires feeding of high cholesterol diet and thiouracil. It is usually necessary to keep the dog in good health by allowing plenty of exercise and maintaining a hearty appetite (56).

Cholesterol feeding to rats produced fatty liver, the cholesterol ester and total lipid concentration increasing after a few days and reaching a maximum after 250 days. No changes occur in the kidney, heart, brain and blood. Hypercholesterolemia in blood is produced only by rather drastic means and then for a short duration only. Byers and Friedman (57) noticed a marked rise in free-cholesterol in plasma after an intravenous injection of a cholesterol suspension. A gradual fall lasting over 48 hours followed each injection. Cholesterol esters rose moderately while the free cholesterol concentration was falling. They reported later (58) that ligation of the bile duct resulted in 200-400 percent increase in plasma cholesterol, and oral administration of large amounts of cholic acid daily for 3 days resulted in extensive hypercholesterolemia. Dent and Hayes (59) noticed that in animals whose adrenals were intact, ascorbic acid caused an increase in serum cholesterol. There are no reports in the literature about atherosclerosis being produced in the rat.

II. Carbon Tetrachloride-induced Liver Injury

Carbon tetrachloride has long been known for its ability to produce fatty liver and hepatic cirrhosis. It is therefore much used to produce hepatoma in cancer studies. The damage it produces and means of protection and prevention have often been investigated.

A. Absorption

Robbins (60), by injection CCl_4 into the stomach, small intestine, or colon of dogs, found no absorption from the stomach, some from the colon, and most from the small intestine. He noticed also that the rate of absorption was increased by giving ethyl alcohol or fat at the same time. Analysis of tissues indicated that bone marrow contained the most CCl_4, with the liver content next highest. Small quantities were found in blood, brain, kidney and lungs. Practically all the CCl_4 was excreted through the lungs; none was found in urine.

B. Biochemical Changes After Carbon Tetrachloride Injury

Kretchmer *et al* (61), by feeding mice 0.1 ml. of 40 percent CCl_4 in olive oil every 4 days, found an appreciable initial decrease in the cytoplasmic succinic oxidase activity of the liver cells. However after 200 days or at the time of tumor induction the value was only slightly below normal. Bodansky *et al* (62) found the liver damage from CCl_4 did not reduce the acetyl-choline esterase activity of the plasma or liver in the rabbit although it did in the rat.

They believed this species difference is due to differences in the choline-ester-hydrolyzing-enzymes in the plasma and liver in each species. Hirvatashi (63) found the asparaginase activity of the liver, lung, and kidney much depressed in phosphorus and CCl_4 poisoning. Richter (64) showed that the liver of rats given 0.1 ml. of CCl_4 intraperitoneally 24 hours previously had impaired ability to methylate nicotinamide and guanidoacetic acid, to form urea from $(NH_4)_2SO_4$ in the presence of ornithine, to hydroxylate phenylalanine, and to conjugate morphine. The oxygen uptake of slices, the succinoxidase, and choline oxidase activities, and the organic phosphate content were higher than normal in the livers of CCl_4-treated rats.

Kurihara (65) showed that the vitamin C contents of the aqueous humor, crystalline lens, liver, spleen, and suprarenal gland of rabbit were greatly reduced when the liver was damaged by CCl_4 and other liver poisons. Rosin and Doljanske (66), by injecting 0.1 ml. of CCl_4 per 100 g. body weight intraperitoneally into young rats, showed that about one hour later the pyroninophilic granules had completely disappeared from hepatic cells, indicating disturbed protein metabolism as one of the first effects of CCl_4 poisoning. Valori (67), by giving rats and guinea pigs CCl_4 in oil solution subcutaneously found very low liver glycogen values in the guinea pig, but only moderately low in the rat. The muscle, and kidney glycogen values were reduced more in the rat than in the guinea pig. The water

content of the liver in both species was not changed appreciably, but the increase in liver fat and decrease in liver protein were much greater in the guinea pig than in the rat.

Guimaraes Villela and Mells (68) obtained a higher phosphatase content of the blood of rats poisoned with CCl_4 or other toxicants.

Dervillee et al (69), by injecting 0.15-0.75 ml. CCl_4 per kg. into rabbits, observed a small increase in total blood cholesterol. Chebotarev (70) found that CCl_4 given to horses resulted in a decrease of the number of erythrocytes, sugar, cholesterol, fibrinogen, calcium, chlorides, and alkaline reserve of the blood, and an increase of bilirubin, non-protein nitrogen, and lactic acid in the blood. Liver glycogen was decreased.

De Senarclens (71) gave subcutaneous injections of CCl_4 to rats kept on a diet rich in cholesterol. The liver at death showed granulomatous lesions composed of vacuolated reticulum cells and containing lecithin. Blood cholesterol and blood lipids increased. Pierce and Gofman (72) investigated the effect of CCl_4 injections on serum cholesterol levels and lipoproteins of the S_f 3-12, 12-20, and 20-24 groups in normal and cholesterol-fed rabbits. They found a marked increase in all classes of lipoproteins and in cholesterol in the non-cholesterol-fed rabbits, with a gradual decrease to control levels after cessation of CCl_4 injections. No macroscopic atherosclerosis developed in this group. In the cholesterol-fed rabbits cholesterol and all classes of

lipoproteins increased not only during CCl_4 injections but also continued to do so after the cessation of CCl_4 injections. Very large quantities of serum lipoproteins and cholesterol developed by the end of 10 weeks, and at this time all animals had developed atherosclerosis. The authors suggested that the increase in the normally occurring S_f 12-20, and S_f 20-24 classes of lipoproteins may occur as a result of impaired function of the degradation and synthetic system (possibly in the liver) involved in the metabolism of these molecules.

C. Effects of Dietary Factors

Diet exerts an enormous influence upon the extent of CCl_4 injury. Barrett, Best, MacLean and Ridout (73) demonstrated that rats maintained on a diet low in lipotropic factors or choline developed very fatty livers during the 20-day period after the administration of CCl_4, whereas animals treated similarly but given a liberal supplement of choline had almost normal livers suggesting that choline or other lipotropic factors are essential for the removal of the excess fat caused by CCl_4 poisoning. Post et al (74), found that growth inhibition and lipodosis of the liver caused by CCl_4 injections in rats was essentially the same whether the amounts of yeast fed were grossly inadequate, or adequate. However when large amounts of brewers yeast were added to the basal diet the harmful effects of CCl_4 were considerably moderated. The amount of food consumed

also influenced the severity of the cirrhosis. Rats fed 8-11 g. of food daily had more severe liver lesions than those fed 14 g. daily. Bollman (75, 76) studying the influence of dietary factors on the resistance of rats to CCl_4 found that a diet containing 6 percent protein, 79 percent carbohydrate, 13 percent fat and 2 percent salt mixture gave the best survival time. In diets of the same caloric intake replacement of the fat by either protein or carbohydrate was found to afford protection to the liver. Drill and Loomis (77, 78) demonstrated that supplements of methionine decreased the degree of liver damage produced by hepatic toxins in protein-depleted animals, but not in animals receiving a normal amount of protein. Later together with Belford, they studied the effect of protein and carbohydrate intake on liver injury by CCl_4 and by bromsulfalein-retention tests found a greater degree of hepatic dysfunction in animals fed the normal protein diet than those receiving a diet low in protein and high in carbohydrate. The incidence of necrosis was also much less in the latter group. Campbell and Kosterlitz (79) also found the least damage in rats on a protein-free and carbohydrate-high diet. However, Hoffbauer (80) did not get good results from either a high protein or a high carbohydrate diet.

D. Protective and Therapeutic Means

Minot (81) observed that dogs fed well-balanced diets showed no outward signs of intoxication even with large doses of CCl_4, but those on low-calcium diets frequently

died with convulsions when given similar doses. The symptoms were relieved by repeated intravenous injections of $CaCl_2$. Ravina (82) also found treatment with Ca ($CaCl_2$) to be successful in CCl_4 intoxication. Forbes, Leach and Williams (83) showed that administration of sulfanilamide to rats retarded development of liver cirrhosis from chronic CCl_4 poisoning, and that para-aminobenzoic acid did not decrease the protective action of this drug in acute CCl_4 poisoning. Miklos Szabo (84) observed that the protective effect of glucose against liver damage by CCl_4 was considerably enhanced when thiamine was administered in liberal amounts. Dillard, Spence, and Forbes found (85) that the administration of large amounts of either glucose or sucrose prior to anesthesia with CCl_4 exerted no inhibitory effect on the development of fatty livers. However, administration in sufficient amounts after anesthesia greatly reduced or completely inhibited the fatty infiltration. The degree of liver necrosis was, on the whole, not effected by the various supplements employed. The authors suggested that carbohydrate administration through inhibition of fatty infiltration of the liver may enable that organ to regenerate when otherwise it would not be possible. De Carvalho Lima and Koch-Weser (86) found that complete protection was afforded by 100 g. of sodium xanthate, but the loss of body weight was accentuated. Aschkenasy and Rolland (87) obtained no significant protective action from the administration of methionine or thiamine or both in chronic and

acute poisoning of adult rats with CCl_4, although earlier workers, Beattie et al (88) clained their treatment with methionine was successful. Drill and Loomis (89) also found methionine supplements not to be beneficial to dogs with liver injury produced by acute or chronic CCl_4 poisoning. Leites and Yakusheva (90) tried the effect of pancreatic lipotropic factor in both guinea pigs and rats. They found no improvement in guinea pigs, but obtained a drop of 20 percent of liver lipids in rats with daily administration of 0.3-0.5 g. of a preparation from pancreatic tissue, and both cholesterol and glycogen decreased slightly. Inositol and insulin did not seem to have a similar effect. De Dominicis (91) found nicotinamide lowered the mortality rate by increasing the resistance to some phases of CCl_4 liver damage in guinea pigs. Hove (92) observed that D-α-tocopherol gave excellent protection against CCl_4 toxicity in rats on a 10 percent casein-vitamin E-free diet. Nevertheless Di Bella (93) did not notice any difference in the toxic effect of CCl_4 between the α-tocopherol-injected guinea pigs and the controls. Papper et al (94, 95) showed that 15μg. vitamin B_{12} per 100 g. body weight of rat given prior to administration of CCl_4 ameliorated the effect of this hepatoxin. The liver weight was less, the quantity of fat deposited in the liver was decreased, and histologic changes in the liver were also modified. Chemical analysis, however, did not show any difference. Also it was not effective when its administration followed that of

toxic compound. The authors were of the opinion that the vitamin is active through mechanisms whereby it prevents accumulation of fat in liver cells and retards the necrotic process.

E. Mechanism of Action by Carbon Tetrachloride

Opie (96) postulated that CCl_4 acts by lowering the osmotic pressure of the cells of liver and of kidney to the level of the medium that surrounds them, but is restored when recovery from the injury occurs.

Hirade and Minomiya (97) believed that the toxic action is due to its combination with sulfhydryl groups of protein molecules, especially enzyme proteins.

EXPERIMENTAL PROCEDURE

I. General

The animals used for the study of the effects of age and tumor growth were obtained from the Rodent Laboratory, Department of Zoology, and those for the dietary factors and the CCl_4-induced liver injury were from the Vitamin Assay Laboratory, Department of Chemistry.

Studies of the cholesterol content of blood, liver, heart and kidney were made in 45 aged rats (5-37 months old), and 11 tumor rats (14-24 months old). Similar analyses were performed in young rats fed 6 different rations: stock, a diet of good nutritive value, a diet of somewhat lower value, two choline deficient diets, and a rachitogenic diet, and in animals with CCl_4-induced liver injury either by periodic exposure to CCl_4 vapors or by intraperitoneal injection of a solution of the compound in mineral oil. Nitrogen, moisture and total fat in the liver and the esterified fatty acids in the plasma were also determined.

II. Composition of the Diets Employed

Stock Diet	
Yellow corn meal (ground)	32.5
Ground wheat	25.0
Milk powder (whole)	22.5
Linseed oil meal	10.0
Alfalfa	6.0
Brewers yeast	3.0
Iodized salt	1.0

Rice Diet*	
Fine rice	66.0
Milk powder (whole)	30.0
Alfalfa	3.0
Iodized salt	1.0

Diet I	
Sucrose	59.5
Yeast	6.0
Casein	10.0
Fat (lard)	5.0
Milk powder	15.0
Alfalfa	2.0
Salt mixture	2.0
Iodized salt	0.5

Diet II	
Sucrose	72.5
Yeast	3.0
Casein	10.0
Fat (lard)	5.0
Milk powder	5.0
Alfalfa	2.0
Salt mixture	2.0
Iodized salt	0.5

Low Choline Diet I	
Casein	12.0
Lard	10.0
Yeast	6.0
Sucrose	66.0
Salt mixture	4.0
Codliver oil	2.0

Low Choline Diet II	
Casein	12.0
Lard	20.0
Yeast	6.0
Sucrose	56.0
Salt mixture	4.0
Codliver oil	2.0

Rachitogenic Diet	
Cornmeal (yellow table meal)	69.0
Wheat gluten	20.0
Yeast	4.0
Casein	3.0
$CaCO_3$	3.0
NaCl	1.0

* The old and the tumor rats were fed this ration.

III. Administration of Carbon Tetrachloride

Carbon tetrachloride was administered in two different ways. By the first method about 1 ml. CCl_4 was introduced into a glass jar of 4 liter capacity, and the rat was then

put into the jar until it showed signs of nervous strain and intention to fight off the vapor. This usually took 2-4 minutes, and the exposure was repeated 3 times a week until the experiment was terminated. By the second method different concentrations of CCl_4 in mineral oil (0.033-0.132 ml. CCl_4 per 100 g. body weight) were injected intraperitoneally twice weekly for two weeks.

IV. Method of Obtaining Blood and Tissues

The old and tumor rats were generally sacrificed within a day or two after they were received from the Rodent Laboratory. Definite periods of feeding had to be allowed for the study of the effect of various diets and of carbon tetrachloride injury. In order to avoid the influence of the absorption of food all the animals were fasted 12-16 hours before they were anesthetized with ethyl ether. Plasma was obtained by centrifuging oxalated blood drawn from heart puncture (lithium oxalate being used as the anticoagulant). The liver, heart, and kidney were removed and weighed immediately. They were then macerated into uniformly fine particles in a Waring blender and made to definite volumes with distilled water. An aliquot of each was extracted. The details of extraction and analysis are given below.

V. Analytical Methods

A. Water Content of the Liver

A portion of liver was weighed and dried at 50-55° C.

under reduced pressure until constant weight was obtained. The loss in weight was calculated as percent moisture in the liver.

B. Nitrogen Content of the Liver

Nitrogen was determined according to the semi-micro Kjeldahl method described by Clark (98), except that H_2O_2 and KOH were used instead of ethyl alcohol and NaOH respectively. One milliliter of the blended liver suspension was digested for 3 hours with 0.75 g. of a mixture of HgO and K_2SO_4 (8 g. HgO and 100 g. anhydrous K_2SO_4) and 1.5 ml. of concentrated H_2SO_4. The digest was then cooled, 2 drops of 30 percent H_2O_2 added, and the mixture again heated until it became clear and colorless. For distillation a 56 percent solution of KOH containing 5 percent $Na_2S_2O_3$ (crystalline) was used. The condensate was collected in a flask containing 5 ml. of 4 percent boric acid. Titration was carried out with 0.02 M. HCl using a mixture of methylene blue, 0.05 percent, and methyl red, 0.1 percent, as indicator.

C. Total Fat in the Liver

Dried liver from the moisture determination was ground and weighed in a thimble for Soxhlet extraction using anhydrous ethyl ether as the solvent. After 12 hours' extraction the solvent was evaporated off and the residue dried at 50-55° C. under reduced pressure until constant weight was reached. The weight of the residue was taken as the total fat in the liver.

D. Cholesterol in Blood and Tissues

Extraction of cholesterol from blood and tissues was accomplished by adding slowly a definite volume of plasma (usually 2 ml.) or an aliquot of the blended tissue suspension (5 ml. in most cases) to approximately 30 ml. of a 1-1 mixture of ethanol and acetone while the flask was being rotated. A fine precipitate of protein resulted, and the flask containing both the precipitate and the extract was then brought to boiling on a water bath to insure complete extraction. After cooling to room temperature this was made to a definite volume, usually 50 ml., and aliquots were used for the determination of cholesterol and the esterified fatty acid. The latter was determined only for blood plasma.

Cholesterol was determined according to Sperry's revised method (99). For the estimation of free cholesterol the alcohol-acetone extract was used directly, whereas hydrolysis with 50 percent aqueous KOH at 42° C. for 30 minutes followed by acidification with 10 percent acetic acid was the first step in the determination of the total cholesterol. Precipitation with digitonin was carried out in slightly acidic solution by the addition of a drop of 10 percent acetic acid and 2 ml. of a 0.5 percent digitonin solution in 50 percent alcohol. After standing 10-12 hours to assure complete precipitation the precipitate was centrifuged, washed once with ether-acetone mixture and twice with ether, and dried at 110-115° C. for 1 hour. The dried

digitonide was then dissolved in glacial acetic acid and the Liebermann-Burchard reaction applied for the colorimetric estimation of cholesterol. A mixture of ice-cooled acetic anhydride-concentrated H_2SO_4 (20-1) was added for the production of the bluish-green color which was allowed to develop in darkness at a constant temperature of 25° C. Thirty minutes later the transmission was read in a photelometer supplied with a red filter. The color of a standard cholesterol solution was developed every time a series of determinations was made. The content of cholesterol was read from a standard curve prepared by the use of standard cholesterol solutions.

E. Esterified Fatty Acids in Blood Plasma

The esterified fatty acid in blood plasma was determined according to the method of Bauer and Hirsch (100), the principle of which is based upon the conversion of fatty acid esters into the corresponding hydroxamic acids by hydroxylamine hydrochloride and NaOH, and their subsequent conversion into colored ferric salts. The reactions may be represented by the following equations:

$$RCOOR_1 + NH_2OH \longrightarrow RCONHOH + R_1OH$$

$$3\ RCONHOH + Fe(ClO_4)_3 \longrightarrow 3\ HClO_4 + Fe(RCONHO)_3$$

An aliquot (usually 5 ml.) of the alcohol-acetone extract of the blood plasma was evaporated to dryness at 60° C.; 15 ml. freshly redistilled ethyl ether was added, followed

by 0.3 ml. of 2.5 percent NaOH in 95 percent alcohol and 0.3 ml. of $NH_2OH \cdot HCl$ (2.5 percent in 95 percent alcohol). The contents were thoroughly mixed after the addition of each reagent. The solution was then evaporated just to dryness at 60° C. Color was developed by the addition of a solution of ferric perchlorate in 95 percent alcohol which was prepared one hour before use by a 1-20 dilution with 95 percent alcohol of a stock solution containing 2 g. ferric perchlorate, 20 ml. 71 percent perchloric acid and 10 ml. distilled water. After 20 minutes the transmission was read in a photelometer with a green filter. The amount of fatty acid esters was read from a standard curve which was prepared using tristearin as the standard.

RESULTS

Table 1 presents the data obtained from analyzing 45 aged animals. The age ranged from 5 to 36.5 months and the distribution of the two sexes was about equal. In the liver the moisture content ranged from 64.5 to 73.8 percent, total fat from 5.59 to 15.7 g. per g. nitrogen, and the total cholesterol from 2.33 to 4.62 mg. per g. of wet tissue, of which the cholesterol esters made up 40.2 percent for the male and 26.6 percent for the female as shown in the detailed data in the appendix. In the blood plasma total cholesterol varied from 44.2 to 102 mg. per 100 ml. Free cholesterol averaged 25.4 percent for the male and 28.4 percent for the female. The esterified fatty acid in the blood had a wide range, 0.15 to 11.5 milliequivalents per liter. The cholesterol content of heart and kidneys ranged from 0.99 to 4.50 and 3.29 to 7.13 mg. per gram of the wet tissue respectively. There seemed to be little change due to age and sex except that the esterified cholesterol content of the liver of the female rats was lower than that of males.

The results obtained from studying 11 tumor rats are assembled in Table 2 in the order of increasing size of the tumor. The liver moisture ranged from 67.2-72.0 percent, total fat 5.73-16.3 g. per g. nitrogen, and cholesterol

TABLE 1

EFFECT OF AGE AND SEX ON CHOLESTEROL CONTENT OF BLOOD, HEART, AND KIDNEY IN AGED RATS

Number of animals	Sex	Age in months	Body weight in grams (average)	Percent moisture in liver	Total fat in liver g./g. nitrogen	Liver cholesterol mg. per gram	Blood cholesterol mg./100 ml. plasma	Blood esterified fatty acids m.eq./liter	Heart cholesterol mg. per gram	Kidney cholesterol mg. per gram
1	M	5	383	69.1	6.49	3.01	62.0	0.15	3.57	7.13
1	M	10	578	66.7	11.0	2.54	60.0	1.33	2.24	4.87
15	M	12.5-16.5	395	67.4	11.1	3.75	61.5	3.66	1.67	4.45
1	M	20	484	68.7	10.1	3.55	56.0	0.52	1.26	4.40
2	M	30	379	70.7	11.4	4.62	67.5	0.91	3.30	4.80
1	M	33	308	73.8	8.4	2.50	80.0	0.52	4.17	4.40
1	M	34	470	69.3	13.2	3.65	77.0	0.51	2.98	4.72
1	F	6	252	70.7	9.60	3.18	72.0	0.68	3.19	5.15
2	F	11	321	68.4	9.4	3.98	55.0	0.98	2.12	5.46
10	F	12.0-16.5	324	69.2	8.59	2.81	63.0	3.73	1.39	4.41
1	F	17	267	69.9	6.78	3.38	92.0	1.25	3.28	5.32
2	F	18	264	69.6	9.49	2.54	60.0	1.18	3.38	6.52
2	F	20	320	69.0	12.3	2.74	66.0	0.47	3.22	4.93
2	F	23-24	227	70.2	10.72	3.48	102.0	5.57	1.61	4.00
2	F	28.5	332	64.5	8.17	2.85	72.5	0.72	2.02	6.08
1	F	36.5	245	72.2	7.23	4.11	61.0	1.69	4.50	4.56

TABLE 2

EFFECT OF SPONTANEOUS MAMMARY TUMOR ON CHOLESTEROL CONTENT OF BLOOD, LIVER, HEART, AND KIDNEY IN AGED RATS

Animal number	Sex	Age in months	Body weight in grams	Tumor weight in grams	Percent moisture in liver	Total fat in liver g./g. nitrogen	Liver cholesterol mg. per gram	Blood cholesterol mg./100 ml. plasma	Blood esterified fatty acids m.eq./liter	Heart cholesterol mg. per gram	Kidney cholesterol mg. per gram	Tumor cholesterol mg. per gram
335	F	19.5	249	17.3	67.8	8.10	3.98	96.0	2.4	1.05	5.07	2.37
287	F	17.0	284	33.6	67.5	11.3	3.44	82.2	16.5	1.29	6.13	3.34
328	F	16.5	264	35.7	68.4	9.78	3.90	78.1	12.0	1.98	7.38	1.64
334	F	18.5	279	39.4	67.2	16.3	2.11	91.0	2.0	1.18	4.60	2.64
327	F	19.0	232	33.4	70.0	10.2	4.23	138.0	10.5	2.24	6.23	1.87
308	M	15.5	470	70.0	67.8	11.0	4.36	98.3	11.0	1.94	5.06	2.69
302	F	15.5	246	39.5	67.6	10.3	2.76	167.0	18.3	1.35	5.50	2.56
239	F	14.5	242	44.0	72.0	11.3	2.03	67.5	3.5	1.76	4.99	3.07
276	F	24.0	259	59.4	68.9	8.64	2.10	106.0	12.8	1.31	4.86	2.94
238	F	14.0	239	65.9	70.0	5.73	2.17	65.0	4.0	1.69	4.88	2.24
336	F	18.5	328	100.0	69.1	5.83	3.32	58.4	5.0	1.47	4.75	0.85

2.10-4.36 mg. per gram of wet tissue with an average of 23.6 percent for the ester form. The blood cholesterol (total) ranged from 58.4-167 mg. percent, of which 29.3 percent was in the free form. The esterified fatty acid also varied greatly, 2.0-18.3 milliequivalents per liter. The cholesterol content of heart and kidney ranged from 1.05 to 2.24 and 4.60 to 7.38 mg. per gram respectively. The tumor tissue showed a cholesterol content of 0.85-3.34 mg. per gram, being higher than that of heart and lower than that of kidney tissue. It approximated that of the liver.

Table 3 shows the effect of six different diets. In a comparison of the stock, experimental diets I and II there seemed to be little or no difference in the liver cholesterol, blood esterified fatty acids, and heart cholesterol. However in the animals fed the poorer diet the liver showed a slightly lower moisture content, being 65.5 percent as compared to 67.8 and 67.6 percent for the stock and experimental diet I respectively. The total fat was 21.5 g. per g. nitrogen as compared to 12.4 and 15.9 g. per g. nitrogen respectively. The blood cholesterol was 86.4 mg. per 100 ml. as compared to 66.2 and 80.5 mg. Animals on the stock diet seemed to have a higher kidney cholesterol content (5.98 mg. per gram) than the others (4.08 and 4.64 mg. per gram).

The low choline diet II seemed to exert more influence than the low choline diet I. On a 10-day period the liver moisture content was lowered to 62.2 by diet CII whereas

TABLE 3

EFFECT OF DIET ON CHOLESTEROL CONTENT OF BLOOD, HEART, AND KIDNEY IN YOUNG RATS

Number of animals	Initial wt. (ave) g.	Final wt. (ave) g.	Diet*	Duration (wks. or da)	Percent moisture in liver	Total fat in liver g./g. nitrogen	Liver cholesterol mg. per gram	Blood cholesterol mg./100 ml. plasma	Blood esterified fatty acids m.eq./liter	Heart cholesterol mg. per gram	Kidney cholesterol mg. per gram
(a) Comparison of stock, experimental diets I and II											
4	48	152	S	5	67.8	12.4	2.18	66.2	3.6	1.13	5.98
4	48	136	I	5	67.6	15.9	2.38	80.5	5.5	1.89	4.08
3	48	139	II	5	65.5	21.5	2.69	86.4	5.8	2.05	4.64
(b) Low choline effect and experimental diets I and II											
4	34	45	CI	10 d	68.6	18.4	1.72	86.2	5.62	1.42	3.56
4	35	55	CII	10 d	62.2	29.0	2.25	75.8	7.62	1.28	5.39
4	34	125	CI I	10 d 3 w	69.0	26.0	4.08	85.0	9.75	1.90	6.44
4	34	98	CI II	10 d 4 w	65.3	27.2	7.53	65.8	14.5	1.85	4.74
4	36	142	CII I	10 d 3 w	58.4	33.4	6.49	53.0	12.0	2.62	6.15
3	35	123	CII II	10 d 4 w	48.7	66.1	6.24	92.5	10.0	1.46	5.13
(c) Rachitogenic diet											
4	50	119	S	3	69.5	13.0	2.74	76.0	7.20	1.84	6.29
8	48	77	R	3	70.0	13.5	2.05	79.0	6.90	1.86	5.59

* S - stock diet
I - experimental diet I
II - experimental diet II
CI - low choline diet I
CII - low choline diet II
R - rachitogenic diet

little change was observed in animals fed diet CI. The total fat content of the liver showed similar trends, values increased from 12.4-29.0 g. per g. nitrogen being found with CII, and from 12.4-18.0 g. per g. nitrogen with CI. The esterified cholesterol in liver was greatly increased for both groups of animals fed diets CI and CII, averaging 71.6 percent as shown in the detailed data in the appendix.

When the animals which had been fed CI and CII for 10 days were transferred to experimental diets I and II marked differences in moisture, total fat and total cholesterol (mostly in the ester fraction) in the liver and esterified fatty acids in the blood were observed. For animals fed CII previously the moisture content was decreased to 48.7 percent with diet II and to 58.4 percent with diet I. The fat content increased to 33.4 and 66.1 g. per g. nitrogen and liver cholesterol 6.49 and 6.24 mg. per gram for diets I and II. The blood cholesterol was 92.5 mg. percent in animals fed diet II and 53.0 mg. percent in those fed diet I whereas the esterified fatty acid increased to 10.0 and 12.0 milliequivalents per liter respectively. There seemed to be little difference in those animals previously fed CI and changed to diet I and II. Only the total fat in liver increased to 26.0 and 27.2 g. per g. nitrogen and the esterified fatty acids of the blood increased to 9.75 and 14.5 milliequivalents per liter respectively.

A comparison of results obtained from the stock and the rachitogenic diets showed very little difference in the liver constituents and the cholesterol levels of blood and

tissues analyzed.

The data obtained from exposing young rats to carbon tetrachloride vapors are presented in Table 4. Prolonged treatment of 11 weeks showed a decrease in liver moisture both with diets I and II. Total fat was influenced more with diet II than diet I, being 28.4 and 24.7 g. per g. nitrogen respectively whereas the stock diet had very little effect. From the detailed data shown in Table XIII in the appendix there seemed to be quite an individual difference in liver cholesterol. One animal each on diet I and II had very high cholesterol contents, 26.7 and 11.1 mg. per gram respectively (mostly in ester form), and one animal each had only 8.65 and 5.32 mg. per gram respectively. The rest did not differ too much from those on the same diets without CCl_4 treatment. Blood cholesterol was increased more by the poorer diet than by the better one. The value for the former was 94.0 and for the latter, 72.1 mg. per 100 ml. Kidney cholesterol was also higher in those fed diet II.

Intraperitoneal injection of CCl_4 was employed in order to achieve more uniform dosage and the results obtained are shown in Table 5. Here again the total fat in liver increased, especially when the dosage was increased and the animals were fed the poorer diet. The liver cholesterol showed a similar trend. Increasing the dosage beyond the level 0.066 ml. per 100 g. body weight seemed to have little additional effect other than to raise the

TABLE 4

EFFECT OF CCL_4-INDUCED LIVER INJURY ON CHOLESTEROL CONTENT OF BLOOD, LIVER, HEART, AND KIDNEY OF YOUNG RATS

(Administered by exposing to CCl_4 vapors three times a week for 11 weeks)

Number of animals	Initial wt. (ave.) g.	Final wt. (ave.) g.	Diet	Percent moisture in liver	Total fat in liver g./g. nitrogen	Liver cholesterol mg. per gram	Blood cholesterol mg./100 ml. plasma	Blood esterified fatty acids m.eq./liter	Heart cholesterol mg. per gram	Kidney cholesterol mg. per gram
7	45	239	S	70.1	13.6	3.36	61.2	5.91	1.27	5.22
4	55	257	I	60.7	24.7	10.50	72.1	12.40	1.28	3.88
3	56	245	II	61.6	28.4	6.73	94.0	8.70	1.17	7.59

mortality rate which was 60 percent in the case of 0.132 ml. per 100 g. body weight. Blood cholesterol varied a great deal. No definite trend could however be observed. The kidney cholesterol increased quite rapidly at the lowest level 0.033 ml. per 100 g., then dropped to a normal value as the dosage of CCl_4 was further increased. Finally at 0.132 ml. per 100 g. body weight it increased again.

No change was observed in the heart cholesterol and in the esterified fatty acid of the blood plasma.

TABLE 5

EFFECT OF CCL_4-INDUCED LIVER INJURY ON THE CHOLESTEROL CONTENT OF BLOOD, LIVER, HEART, AND KIDNEY OF YOUNG RATS

(Administered by intraperitoneal injection twice weekly for two weeks)

Number of animals	Initial wt. (ave.) grams	Final wt. (ave.) grams	CCl_4 concentration ml./100 g. body weight	Diet	Percent moisture in liver	Total fat in liver g./g. nitrogen	Liver cholesterol mg. per gram	Blood cholesterol mg./100 ml. plasma	Blood esterified fatty acid m.eq./liter	Heart cholesterol mg. per gram	Kidney cholesterol mg. per gram
2	128	152	.033	S	66.9	16.1	4.92	80.4	7.6	2.23	6.38
2	122	150	.033	I	68.9	13.5	3.49	104.0	10.4	2.25	6.38
1	120	139	.033	II	64.2	28.2	6.67	101.0	7.5	2.76	6.55
2	113	128	.066	S	70.0	16.2	2.46	68.5	5.5	1.30	5.87
4	108	118	.066	I	65.8	18.7	3.71	72.1	9.5	1.39	5.55
3	104	108	.066	II	60.9	30.2	4.57	85.0	3.8	1.25	4.50
1	90	117	.099	I	66.5	24.8	5.00	50.0	4.0	1.66	4.62
2	95	110	.099	II	64.6	27.3	4.97	76.5	4.6	1.49	4.71
1	121	118	.132*	I	65.6	24.1	6.55	69.2	2.0	1.74	8.12
1	82	.97	.132	II	69.5	22.5	6.00	164.0	7.2	1.63	6.42

*Mortality was 60 percent on this level

DISCUSSION

The results obtained from studying old animals demonstrated that generally there is little or no influence of age and sex on the blood cholesterol, liver fat, and total cholesterol in the liver, heart, and kidney. However, the ester cholesterol in the liver is lower in the female than in the male, being 26.6 percent and 40.2 percent respectively. This agrees well with the findings of Okey *et al* (101). The cholesterol esters of the liver in the female rat may be more mobilizable than in the male due perhaps to the synthesis of sex hormones required during each estrus cycle.

Mounier *et al* (15) found similar values for blood cholesterol in both young and old rats. In the present study the variation was large, 44.2-102 mg. percent, although no definite increase or decrease due to age was observed. Two female animals, 23-24 months, had blood cholesterol values as high as 100 and 104 mg. percent. Probably in the rat there is also a constitutional level characteristic for each animal as suggested by Sperry (8) for human beings. Some rats might be influenced by age much more than others, thus contributing to the wide range observed. The oldest rats of this group, 28-36.5 months of age, showed values of 57.0-90.0 mg. percent. Keys *et al* (12) reported similar findings from an investigation made on human

subjects living in Minnesota.

The results of the study of 11 rats with spontaneous mammary tumors showed that the blood cholesterol was generally high although the variations were considerable. The blood cholesterol of rats No. 327 and No. 302 was 138 and 167 mg. percent respectively. They were the highest values observed in this investigation. Rats No. 238 and No. 336 had comparatively large tumors. Their blood cholesterol was 65.0 and 58.4 mg. percent respectively. Two explanations might be offered. These two rats probably had not been eating well due to their poor physical conditions caused by the advanced stage of the tumor growth, or they might belong to the class that has low blood cholesterol by constitution. The esterified fatty acids in the blood generally increased with the blood cholesterol.

The differences in results with stock ration, diet I and diet II were observed only in the fat content of the liver and the blood cholesterol. The liver fat was appreciably higher with the poorer diet. From the composition of the two experimental diets it is clear that they differ only in the amount of yeast and milk powder. Yeast comprised 3 percent of the former and 6 percent of the latter whereas the content of milk powder was 5 percent and 15 percent respectively. A diet comparatively low in vitamin B complex supplied chiefly by the yeast, and low in protein might be expected to lead to an increase in the fat content of the liver. Diet II was chosen for study because in

human dietaries deficiencies of a multiple nature rather than single deficiencies are encountered and they commonly involve the B complex vitamins and protein. Probably the combination of slight deficiency in the B complex and protein also results in a disturbance in the mechanism of maintaining the usual blood cholesterol level.

The influence of the low choline diets manifested itself in a higher fat content, a lower percentage of moisture and an increased ester cholesterol content of the liver. The blood cholesterol was normal. A comparison of the two choline diets showed that low choline diet II produced more severe symptoms, higher fat and ester cholesterol contents and lower percentage of moisture in the liver. The difference in the composition of the two diets was chiefly in the percentage of fat, being twice as high with the CII (20 percent) as with the CI diet (10 percent). This confirms that choline deficiency is produced more easily with a high fat diet, other constituents being the same.

In the study of influence of diets I and II on rats previously maintained on the choline diets several differences were noted. Only small differences were observed in effect of diets I and II on animals previously fed CI. However with those previously fed CII the feeding of diet II resulted in a lower moisture and higher fat content in the liver and also in higher blood cholesterol values. This would indicate that diet CII had produced more severe changes than diet CI. It also follows that the deviation from the

normal was much greater in the case of diet II than with diet I as indicated by the fact that the liver fat was approximately twice as high.

The experiment with rachitic animals showed that there was no apparent effect on the cholesterol metabolism because all of the tissues studied were of essentially normal cholesterol content. It is quite likely that in view of the characteristically low consumption of food no difficulties involving cholesterol and fat metabolism would be encountered.

The 11-week period of exposure to CCl_4 vapors of young rats resulted in a slightly less degree of fatty liver on the diet I. This is probably due to the greater intake of food in this group of animals. Those on stock diet showed even less degree of fatty liver. Post et al (74) found that rats fed larger amounts of food developed less severe cirrhosis by CCl_4 poisoning. Probably the rats fed the stock diet and diet I ate more nutritious food and therefore were in better condition to resist and to recover from the exposure to CCl_4. On the whole the condition of the rats was not made worse by prolonged exposure. It is quite likely that as in the case of many other toxic substances rats might develop a capacity for making a successful adjustment to the CCl_4.

Intraperitoneal injections of CCl_4 at different levels showed that the degree of fatty liver produced increased as the concentration of CCl_4 was raised from 0.033 to

0.066 ml. per 100 g. body weight. At each of these two levels the fat content of the liver of animals fed diet I was lower than those fed the poorer diet. Beyond the two levels mentioned above no further change was observed except that the blood cholesterol slightly decreased, probably due to the impairment of the appetite and consequent low food intake. With concentration as high as 0.132 ml. per 100 g. body weight, the mortality rate was very high. Thus it indicates a concentration of 0.066 ml. per 100 g. body weight would be more suitable for the experimental study of liver damage by CCl_4 in rats.

From the results of this study it may be concluded that in the rat the cholesterol content in the blood, liver, heart, and kidneys does not appear to be much influenced by age and sex, except that the ester fraction in the liver is lower in the female than that in the male. Rats with spontaneous mammary tumors in general showed a tendency toward higher blood cholesterol values. Diet was observed to markedly affect liver fat, and liver cholesterol, and moderately affect blood cholesterol in the rat. Diet II, somewhat low in B complex and protein, markedly increased the liver fat and raised the blood cholesterol. Choline deficient diets greatly increased the fat and cholesterol content (ester fraction) of the liver. A rachitogenic diet had no influence on the distribution of cholesterol in the tissues studied. Carbon tetrachloride produced fatty liver and increased the liver cholesterol content (ester fraction).

Deviations from the normal were greater in animals fed diet II, probably due largely to the smaller intake of less nutritious food.

SUMMARY

1. The analysis of the cholesterol content of blood, liver, heart, and kidney tissues of 45 rats from 5-36.5 months of age showed no appreciable difference with regard to age and sex, except that the average ester fraction of liver cholesterol in female rats was considerably lower than that in the male.
2. Similar studies with 11 rats with spontaneous mammary tumors showed that the blood cholesterol levels were generally high although considerable variations occurred.
3. A comparison of the effect of the stock diet, experimental diets I and II showed that diet II which contained less B complex and protein produced a moderate degree of fatty liver and slight increase in blood cholesterol.
4. The feeding of choline deficient diets I and II resulted in fatty livers, decrease in moisture content and increase in liver cholesterol, mainly the ester fraction. No appreciable change was noted in the blood cholesterol, although a decrease has been reported in the literature. Diet II containing the larger amount of fat produced the more severe symptoms.
5. When rats previously maintained on choline deficient diet I were fed experimental diets I and II relatively small differences were observed in the fat content of

the liver. With choline deficient diet II differences occurred in which the deviation from the normal was markedly greater with the diet of lower nutritional value.

6. Rats fed a rachitogenic diet showed a normal cholesterol distribution in the tissues studied.
7. Liver injury induced by periodic exposure to CCl_4 vapors resulted in fatty liver and an increase in liver cholesterol (ester cholesterol mainly). The degree of tissue change was influenced by diet being greatest in the case of diet II which was of lower nutritional value.
8. Intraperitoneal injection of CCl_4 in mineral oil produced similar results. A dosage of 0.066 ml. per 100 g. body weight was found most suitable for experimental study.

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APPENDIX

EFFECT OF AGE ON LIVER CONSTITUENTS OF MALE RATS

Rat no.	Age in mos.	Body wt. gms.	Liver wt. gms.	% liver in body wt.	Percent moisture	N mg./g. wet wt.	Total lipids			Cholesterol		
							% dry basis	gm./gm. wet wt.	gm./gm. N	Total mg./g	Free mg./g	Ester %
138	5	383	14.5	3.78	69.1	33.0	13.8	.214	6.49	3.01		
191	10	578	19.8	3.42	66.7	29.0	20.4	.320	11.0	2.54		
255	12.5	330	8.59	2.60	65.9	32.5	31.6	.487	15.0	4.42	3.10	29.8
219	13.5	356	9.46	2.63	68.4	31.5	18.7	.274	8.70	4.56	2.49	45.4
218	14	422	10.9	2.58	70.0	29.7	22.0	.314	10.6	4.43	2.52	43.2
233	14	345	10.9	3.16	68.9	29.4	26.0	.378	12.8	3.27	1.90	41.9
220	14	456	10.6	2.33	66.0	33.5	17.2	.261	7.80	3.94	2.44	38.0
252	14.5	381	8.23	2.16	67.2	34.9	27.9	.415	11.9	4.30	2.73	36.4
145	15	432	11.3	2.62	70.4	29.8	16.5	.234	7.85	3.54		
222	15	334	10.5	3.15	68.7	33.8	14.0	.204	6.05	3.18	1.81	43.2
232	15	344	11.7	3.40	67.9	28.9	30.8	.454	15.7	3.89	1.96	49.6
235	16	409	12.5	3.16	68.3	31.7	22.2	.325	10.2	3.59	2.13	40.6
254	16.5	486	8.91	1.84	67.4	34.3	23.0	.342	9.97	3.67	2.93	20.2
228	16.5	340	9.76	2.87	67.6	32.6	28.5	.422	12.9	3.65	2.09	42.7
229	16.5	335	10.4	3.11	68.4	32.4	26.1	.368	11.4	3.09	1.70	45.0
230	16.5	356	11.2	3.15	67.9	29.1	25.3	.373	12.8	3.32	1.57	52.7
231	16.5	329	10.6	3.22	68.0	31.6	26.5	.390	12.3	3.43	2.25	34.4
154	20	484	10.3	2.14	68.7	31.4	21.9	.318	10.1	3.55		
162	30	354	9.9	2.80	70.0	29.7	21.4	.306	10.3	4.10		
163	30	404	10.7	2.65	71.5	28.4	25.6	.358	12.6	5.14		
159	33	308	10.5	3.41	73.8	31.5	19.4	.263	8.35	2.50		
160	34	470	9.4	2.00	69.3	31.0	28.3	.408	13.2	3.65		

EFFECT OF AGE ON CHOLESTEROL CONTENT OF BLOOD, HEART, AND KIDNEY OF MALE RATS

Rat no.	Age in mos.	Body wt. gms.	Blood				Heart			Kidney		
			Cholesterol			Esterified fatty acid m.e./liter	wt. gms.	% in body wt.	Total cholesterol mg/g	wt. gms.	% in body wt.	Total cholesterol mg/g
			Free mg. %	Total mg. %	Ester mg. %							
138	5	383		62.0		0.15	1.00	.261	3.57	2.20	.575	7.13
191	10	578		60.0		1.33	1.49	.258	2.24	3.48	.603	4.87
255	12.5	330	17.5	65.0	73.1	7.25	0.81	.248	1.38	1.78	.540	6.18
219	13.5	356	10.0	50.0	80.0	4.00	0.99	.268	1.18	2.05	.576	4.46
218	14	422	15.9	70.0	77.3	3.25	1.22	.296	3.39	2.22	.526	3.29
233	14	345	10.3	51.0	79.8	2.00	0.97	.281	1.44	1.99	.577	3.97
220	14	456	15.6	70.0	77.8	4.25	1.08	.237	1.37	2.33	.511	4.02
252	14.5	381	17.5	69.0	74.7	8.25	0.95	.249	1.24	1.86	.488	5.55
145	15	432		96.0		0.86	1.16	.268	3.65	2.22	.514	5.60
222	15	334	29.7	62.8	52.7	4.75	1.06	.318	1.04	1.93	.578	4.45
232	15	344	7.5	44.2	83.1	1.00	0.98	.285	1.56	1.95	.577	3.72
235	16	409	7.7	62.0	87.6	1.25	1.15	.281	1.38	2.70	.661	4.07
254	16.5	486	26.8	67.5	60.3	11.5	1.12	.251	1.38	1.95	.402	4.88
228	16.5	340	11.7	50.5	77.2	1.50	0.96	.282	1.46	2.00	.588	4.30
229	16.5	335	13.7	50.5	72.8	1.75	0.91	.272	1.50	1.76	.526	4.66
230	16.5	356	13.9	53.0	74.2	1.75	0.98	.275	1.19	2.20	.618	4.18
231	16.5	329	15.7	61.5	74.4	3.25	0.96	.292	1.22	2.04	.620	3.67
154	20	484		56.0		0.52	1.26	.260	4.17	2.42	.500	4.40
162	30	354		57.0		0.98	1.17	.331	3.37	2.25	.635	5.04
163	30	404		78.0		0.84	1.26	.312	3.23	2.75	.680	4.56
159	33	308		80.0		0.52	1.26	.409	4.17	2.42	.786	4.40
160	34	470		77.0		0.51	1.42	.302	2.98	2.57	.547	4.72

EFFECT OF AGE ON LIVER CONSTITUENTS [illegible]

Rat no.	Age in mos.	Body wt. gms.	Liver wt. gms.	% liver in body wt.	Percent moisture	N mg./g. wet wt.	Total lipids			Cholesterol		
							% dry basis	gm./gm. wet wt.	gm./gm. N	Total mg./g	Free mg./g	Ester %
199	6	252	11.2	4.41	70.7	30.3	20.6	.292	9.64	3.18		
197	11	354	11.8	3.34	70.5	29.5	20.3	.288	9.76	3.40		
198	11	289	9.20	3.19	66.4	29.5	17.7	.267	9.06	4.46		
221	12.5	237	7.50	3.15	68.5	31.0	21.2	.301	9.72	2.79	2.03	27.2
234	14	182	6.33	3.48	69.8	28.6	25.2	.362	12.7	3.19	2.03	26.3
224	15	206	6.30	3.05	68.7	35.3	13.5	.197	5.59	3.12	2.02	25.2
216	15.5	212	7.55	3.56	68.9	33.8	21.2	.308	9.12	2.49	1.96	21.2
217	15.5	189	6.50	3.42	66.9	30.8	12.2	.183	5.95	2.71	2.02	24.8
236	16	197	5.64	2.87	69.7	32.4	28.6	.410	12.7	3.16	1.89	40.2
237	16	201	7.30	3.61	70.0	31.7	26.5	.378	11.9	2.73	2.54	7.0
225	16.5	198	6.50	3.27	70.0	32.7	14.6	.209	6.39	2.89	1.94	32.8
226	16.5	188	7.14	3.80	69.2	33.1	14.4	.208	6.29	2.68	2.00	25.4
227	16.5	196	7.43	3.79	70.0	32.3	12.5	.179	5.54	2.33	2.04	12.4
144	17	267	9.58	3.73	69.9	30.0	14.2	.203	6.78	3.38		
142	18	218	6.67	3.06	68.5	32.0	20.7	.302	9.44	2.81		
196	18	311	16.7	5.37	70.7	25.2	16.9	.240	9.53	2.54		
195	20	321	12.98	4.04	64.2	28.2	29.6	.461	16.3	2.03		
153	20	318	7.08	2.23	73.8	31.6	19.5	.265	8.39	3.45		
253	23.7	244	7.12	2.92	70.5	33.7	27.4	.389	11.5	2.98	2.64	11.4
223	24	211	5.95	2.82	70.0	32.8	22.8	.326	9.95	3.27	2.23	31.8
178	28.5	313	10.9	6.20	68.1	33.5	20.8	.306	9.14	3.48		
179	28.5	342	13.7	4.02	60.9	33.1	14.5	.238	7.19	2.22		
161	36.5	245	10.7	4.37	72.2	32.4	16.9	.234	7.23	4.11		

EFFECT OF AGE ON CHOLESTEROL CONTENT OF BLOOD, HEART, AND KIDNEY OF FEMALE RATS

Rat no.	Age in mos.	Body wt. gms.	Blood				Heart			Kidney		
			Cholesterol			Esterified fatty acid m.e./liter	wt. gms.	% in body wt.	Total cholesterol mg/g	wt. gms.	% in body wt.	Total cholesterol mg/g
			Free mg. %	Total mg. %	Ester mg. %							
199	6	252		72.0		0.68	0.73	.290	3.19	1.49	.591	5.15
197	11	354		50.0		0.98	0.96	.271	2.27	1.97	.557	5.69
198	11	289		60.0		0.98	1.02	.353	1.97	1.87	.647	5.22
221	12.5	237	12.0	61.0	80.3	3.75	0.86	.363	1.32	1.33	.561	4.56
234	14	182	21.7	64.5	66.3	2.25	0.63	.346	2.11	1.10	.605	3.93
224	15	206	17.3	61.5	71.8	5.00	0.68	.330	0.99	1.27	.617	3.78
216	15.5	212	15.9	46.7	66.0	2.75	0.63	.297	1.12	1.13	.536	5.07
217	15.5	189	22.6	60.0	62.4	1.75	0.65	.344	1.20	1.45	.718	4.60
236	16	197	27.7	82.0	66.2	4.25	0.67	.340	1.50	1.15	.584	5.56
237	16	201	10.0	50.5	80.2	3.25	0.63	.313	1.62	1.21	.602	4.06
225	16.5	198	15.8	60.0	73.7	6.25	0.64	.323	0.99	1.15	.581	3.82
226	16.5	188	15.3	70.0	78.1	6.25	0.81	.431	1.65	1.39	.740	4.17
227	16.5	196	17.3	74.0	76.6	1.75	0.83	.423	1.41	1.52	.776	4.51
144	17	267		92.0		1.25	0.83	.311	3.28	1.58	.592	5.32
142	18	218		60.0		1.10	0.83	.380	4.47	1.41	.647	8.64
196	18	311		60.0		1.25	0.92	.296	2.18	2.06	.670	4.40
195	20	321		74.0		0,55	0.87	.271	2.46	1.73	.539	5.55
153	20	318		58.0		0.39	0.85	.267	3.95	1.67	.525	4.31
253	23.7	244	26.0	104.	77.0	6.00	0.69	.283	1.40	1.32	.542	4.09
223	24	211	39.0	100.	61.0	5.25	0.73	.346	1.83	1.28	.655	3.91
178	28.5	313		90.		0.59	0.97	.309	1.36	1.81	.579	6.02
179	28.5	342		55.		0.95	1.12	.327	2.69	1.93	.565	6.14
161	36.5	245		61.		1.69	1.09	.445	4.50	2.00	.842	4.56

EFFECT OF TUMOR ON LIVER CONSTITUENTS OF TUMOR RATS

Rat no.	Sex	Age in mos.	Body wt. gms.	Liver wt. gms.	% liver in body wt.	Percent moisture	N mg./g. wet wt.	Total lipids			Mg. per gram cholesterol wet basis		Percent esterified cholesterol
								% dry basis	gm./gm. wet wt.	gm./gm. N	Free	Total	
238	F	14.0	239	7.64	3.19	70.0	29.7	11.9	.170	5.73	1.94	2.17	10.8
239	F	14.5	242	9.79	4.08	72.0	28.0	22.7	.315	11.3	1.33	2.03	34.5
276	F	24.0	259	6.98	2.69	68.9	33.9	20.2	.293	8.64	1.47	2.10	30.0
287	F	17.0	284	9.40	3.31	67.5	31.2	23.7	.351	11.3	3.18	3.44	7.5
302	F	15.5	246	9.99	4.05	67.6	29.3	20.3	.301	10.3	2.20	2.76	20.2
308	M	15.5	470	9.57	2.03	67.8	33.4	25.0	.369	11.0	2.78	4.36	36.2
327	F	19.0	232	7.79	4.22	70.0	31.5	22.4	.320	10.2	3.29	4.23	22.2
328	F	16.5	264	11.5	4.36	68.4	30.4	20.3	.297	9.78	2.58	3.90	33.8
334	F	18.5	279	11.0	3.94	67.2	25.2	27.7	.412	16.3	1.72	2.11	18.4
335	F	19.5	249	8.94	3.59	67.8	34.1	18.7	.276	8.10	2.41	3.98	39.4
336	F	18.5	328	7.16	2.18	69.1	47.7	19.2	.278	5.83	3.08	3.32	7.2

TABLE VI

CHOLESTEROL CONTENT IN BLOOD, HEART, KIDNEY AND TUMOR OF TUMOR RATS

Rat no.	Sex	Age mos.	Body wt. gms.	Blood				Heart			Kidney			Tumor		
				Cholesterol			Esterified fatty acid m.e./liter	Wt. gms.	% body wt.	Cholesterol mg. %	Wt. gms.	% body wt.	Cholesterol mg. %	Wt. gms.	% body wt.	Cholesterol mg. %
				Free mg.%	Total mg. %	Ester %										
238	F	14.	239	20.7	65.0	69.2	4.0	.59	.25	1.69	1.27	.53	4.88	65.9	27.5	2.24
239	F	14.5	242	27.0	67.5	60.0	3.5	.61	.25	1.76	1.27	.53	4.99	44.0	18.2	3.07
276	F	24.	259	28.3	106.	73.3	12.8	.71	.27	1.31	1.44	.56	4.86	59.4	22.9	2.94
287	F	17.	284	19.5	82.2	76.3	16.5	.83	.29	1.29	1.60	.56	6.13	33.6	11.8	3.34
302	F	15.5	246	45.5	167.	72.8	18.3	.77	.31	1.35	1.27	.52	5.50	39.5	16.1	2.56
308	M	15.5	470	23.5	98.3	76.1	11.0	1.01	.22	1.94	2.08	.44	5.06	70.3	15.0	2.69
327	F	19.	232	34.0	138.	75.4	10.5	.70	.30	2.24	1.39	.60	6.23	33.4	14.4	1.87
328	F	16.5	264	34.0	155.	78.1	12.0	.74	.28	1.98	1.38	.52	7.38	35.7	13.5	1.64
334	F	18.5	279	24.4	91.	73.2	2.0	.73	.26	1.18	1.45	.52	4.60	39.4	14.1	2.64
335	F	19.5	249	38.5	96.0	59.8	2.4	.80	.32	1.05	1.38	.56	5.07	17.3	6.96	2.37
336	F	18.5	328	21.0	58.4	64.0	5.0	.90	.27	1.47	1.58	.48	4.75	100.	30.5	.85

TABLE VII

COMPARISON OF EFFECTS OF THE THREE DIETS ON LIVER CONSTITUENTS OF YOUNG RATS

Rat no.	Sex	Diet	Body wt. gms.	Liver wt. gms.	% liver wt.	Percent moisture	N mg./g. wet wt.	Total lipids % dry basis	Total lipids gm./gm. wet wt.	Total lipids gm./gm.N	Mg. per gram cholesterol Free	Mg. per gram cholesterol Total	Percent esterified cholesterol
245*	F	I	148 167	12.2	3.87	68.6	26.9	21.3	.311	11.6	2.14	2.62	18.3
246*	M	I	212 208	19.1	4.55	69.3	26.9	19.3	.279	10.4	1.98	2.19	9.5
316	M	I	125	4.81	3.84	68.8	26.5	26.5	.386	14.5	1.86	3.54	47.4
317	M	I	115	4.89	4.26	68.7	32.1	37.0	.538	16.7	3.02	5.85	48.3
318	F	I	148	6.29	4.25	66.7	28.1	30.5	.457	16.3	2.09	2.92	28.4
319	F	I	155	6.29	4.06	66.2	30.7	33.3	.503	16.4	2.53	6.08	48.3
247*	F	II	156 127	12.6	4.45	66.5	23.6	27.5	.414	17.6	1.84	3.32	44.5
248*	M	II	143 196	18.6	5.49	65.1	22.3	36.4	.559	25.1	1.91	2.80	31.8
320	M	II	146	6.42	4.39	65.5	30.3	37.6	.574	19.0	2.20	4.46	50.1
321	F	II	137	4.91	3.58	65.6	31.0	45.8	.697	22.4	3.72	4.06	8.3
322	F	II	133	5.01	3.77	65.4	29.8	45.2	.692	23.2	2.16	4.25	49.1
329	F	Stock	137	6.41	4.68	67.2	34.5	22.3	.332	9.62	2.16	2.88	25.0
330	F	Stock	161	7.30	4.53	67.0	32.6	18.2	.331	10.1	2.40	3.06	11.5
331	M	Stock	149	9.44	6.33	69.2	25.6	24.3	.351	13.7	1.92	2.52	23.8
332	M	Stock	159	9.32	5.86	68.0	26.7	29.3	.431	16.1	2.25	3.00	24.9

* Determined on composite of two animals
I - Experimental diet I
II - Experimental diet II

TABLE VIII

COMPARISON OF THE EFFECT OF THE THREE DIETS ON BLOOD, HEART, AND KIDNEY CHOLESTEROL OF YOUNG RATS

Rat no.	Sex	Body wt. gms.	Diet	Blood				Heart			Kidney		
				Cholesterol			Esterified fatty acid m.e./liter	wt. gms.	% body wt.	Total cholesterol mg. /g	wt. gms.	% body wt.	Total cholesterol mg. /g
				Free mg. %	Total mg. %	Ester mg. %							
245*	F	148 167	I	11.5	65.9	82.5	29.0	0.86	.27	1.13	1.99	.63	5.70
246*	M	212 208	I	7.5	46.7	83.9	23.0	1.38	.20	1.48	2.72	.65	5.21
316	M	125	I	13.0	67.5	80.7	6.0	0.44	.35	2.55	0.90	.72	4.45
317	M	115	I	18.3	81.8	76.6	2.1	0.40	.35	1.45	0.92	.80	3.44
318	F	148	I	14.5	81.7	82.2	5.4	0.48	.32	1.87	1.06	.72	4.40
319	F	155	I	14.5	90.8	84.3	8.5	0.57	.37	1.72	1.16	.75	4.44
247*	F	156 127	II	17.5	74.3	76.4	13.5	0.89	.31	1.57	0.88	.67	5.00
248*	M	143 196	II	11.5	72.5	84.1	11.3	1.19	.35	1.79	2.63	.78	5.71
320	M	146	II	17.0	75.0	77.3	9.5	0.56	.38	1.91	1.03	.71	5.02
321	F	137	II	18.5	85.8	78.4	5.0	0.53	.39	1.77	1.13	.83	5.02
322	F	133	II	19.5	98.3	80.2	3.0	0.42	.32	2.46	1.03	.78	3.88
329	F	137	Stock	15.5	52.5	70.5	5.5	0.44	.32	1.55	0.99	.72	6.48
330	F	161	Stock	20.0	77.9	74.3	7.0	0.44	.27	1.55	1.01	.63	5.45
331	M	149	Stock	21.0	62.5	66.4	1.0	0.50	.34	1.28	1.16	.78	6.46
332	M	159	Stock	30.0	71.7	58.1	1.0	0.55	.35	1.42	1.35	.85	5.54

* Determined on pooled sample of two animals
I - Experimental diet I
II- Experimental diet II

TABLE IX

EFFECT OF CHOLINE DEFICIENT DIET AND EXPERIMENTAL DIETS I AND II ON LIVER CONSTITUENTS OF YOUNG RATS

Rat no.	Sex	Diet	Body wt. gms.	Liver wt. gms.	% liver wt.	Percent moisture	N mg./g. wet wt.	Total lipids % dry basis	Total lipids gm./gm. wet wt.	Total lipids gm./gm. N	Mg. per gram cholesterol wet basis Free	Mg. per gram cholesterol wet basis Total	Percent esterified cholesterol
269*	F	CI	51 43	6.05	6.44	66.9	24.6	33.8	.505	20.5	0.39	1.47	73.5
270*	M	CI	45 41	4.31	5.02	70.2	27.2	31.1	.443	16.3	0.52	1.98	73.8
271*	M	CII	56 49	2.81	2.59	64.1	21.6	43.0	.670	31.0	0.60	2.26	73.5
272*	F	CII	58 57	10.23	8.89	60.3	28.0	46.4	.754	26.9	0.83	2.24	63.0
288*	M	CI, I	143 132	9.66	3.53	70.3	33.8	22.3	.318	9.41	2.91	3.33	12.5
289*	F	CI, I	113 113	7.60	3.36	67.7	22.7	40.0	.591	26.0	3.26	4.84	32.6
290*	M	CII, I	151 155	15.0	4.91	59.5	26.6	53.9	.906	34.0	2.67	7.15	62.1
291*	F	CII, I	135 128	12.5	4.75	57.3	29.9	56.6	.987	32.9	2.69	8.01	66.4
292*	M	CI, II	115 79	12.6	6.50	64.2	22.2	48.8	.760	34.2	3.10	8.72	65.5
293*	F	CI, II	92 107	7.02	3.53	66.6	30.7	41.6	.625	20.3	1.98	4.27	43.6
294	M	CII, II	126	6.89	2.65	52.0	21.5	68.3	1.31	61.0	1.98	6.67	70.3
295*	F	CII, II	138 104	13.7	5.67	45.3	24.0	80.4	1.71	71.3	2.93	5.81	49.6

* Composite of two rats

269*-272* rats were put on choline deficient diet for 10 days and then sacrificed

The rest * were put on choline deficient diet for [illegible] and then transferred to the specific ration

CI - Choline deficient diet I

CII - Choline deficient diet II

I - Experimental diet I

II - Experimental diet II

EFFECT OF CHOLINE DEFICIENT DIET ON BLOOD, HEART, AND KIDNEY CHOLESTEROL OF YOUNG RATS

Rat no.	Sex	Body wt. gms.	Diet	Blood				Heart			Kidney		
				Cholesterol			Esterified fatty acid m.e./liter	wt. gms.	% body wt.	Total cholesterol mg. /g	wt. gms.	% body wt.	Total cholesterol mg. /g
				Free mg. %	Total mg. %	Ester mg. %							
269*	F	51 43	CI	23.3	67.5	65.5	5.00	0.48	.56	1.60	1.13	1.23	3.40
270*	M	45 41	CI	37.5	106.0	64.6	6.25	0.34	.32	1.25	0.94	1.09	3.72
271*	M	56 49	CII	24.2	75.8	68.0	8.75	0.50	.48	1.28	1.04	1.00	5.45
272*	F	58 57	CII	24.2	75.8	68.0	6.50	0.48	.43	1.29	0.98	0.84	5.33
288*	M	143 132	CI, I	18.5	85.0	78.2	10.5	1.10	.40	1.87	2.10	0.76	6.44
289*	F	113 113	CI, I	12.0	85.0	85.7	9.0	0.94	.42	1.94	1.94	0.86	6.45
290*	M	151 155	CII, I	17.5	59.0	70.4	12.0	1.08	.35	1.81	2.30	0.75	6.09
291*	F	135 138	CII, I	13.5	47.0	71.2	12.0	0.64	.24	3.31	1.96	0.75	6.21
292*	M	115 79	CI, II	42.5	65.8	35.4	17.0	0.70	.36	2.03	1.76	0.91	5.02
293*	F	92 107	CI, II	31.5	65.8	52.0	12.5	0.67	.34	1.70	1.94	0.98	4.47
294	M	126	CII, II	23.5	89.2	73.6	11.0	0.45	.36	1.56	0.93	0.74	4.30
295*	F	138 104	CII, II	31.5	95.8	67.1	9.0	0.87	.36	1.77	1.98	0.82	5.97

* Determined on pooled sample of two animals

CI - Choline deficient diet I

CII - Choline deficient diet II

I - Experimental diet I

II - Experimental diet II

TABLE XI

EFFECT OF RACHITOGENIC DIET ON LIVER CONSTITUENTS OF YOUNG RATS

Rat no.	Sex	Diet	Body wt. gms.	Liver wt. gms.	% liver wt.	Percent moisture	N mg./g. wet wt.	Total lipids % dry basis	Total lipids g./g. wet wt.	Total lipids g./g. N	Mg. per gram cholesterol wet basis Free	Mg. per gram cholesterol wet basis Total	Percent esterified cholesterol
323	M	S	128	5.98	4.67	70.5	34.8	21.3	.302	8.70	2.48	3.81	34.9
324	M	S	123	4.84	3.93	68.9	31.8	33.5	.487	15.3	2.78	3.84	27.6
325	F	S	110	4.36	3.96	70.0	33.1	27.7	.396	12.0	2.85	3.52	21.8
326	F	S	114	5.01	4.40	68.7	33.4	36.2	.526	15.8	2.86	4.07	29.6
309	F	R	71	3.05	4.30	72.1	35.5	32.9	.457	12.9	1.16	1.59	27.0
310	F	R	87	3.31	3.81	68.3	33.4	29.2	.428	12.8	1.93	1.93	0.0
311	M	R	89	3.75	4.22	69.4	33.2	26.6	.383	11.6	1.40	1.77	20.9
285*	F	R	89 94	6.67	3.65	70.6	33.2	24.7	.349	10.5	2.94	4.22	30.3
286*	M	R	146 113	8.54	3.29	69.6	34.0	18.6	.268	7.90	3.06	3.20	4.4
297*	M	R	80 85	7.05	4.27	69.0	30.5	36.3	.526	17.5	1.72	2.90	40.6
298*	F	R	74 77	6.70	4.44	70.0	30.2	25.8	.369	14.3	2.12	2.65	20.0
299*	F	R	74 73	5.76	3.92	70.1	29.2	27.8	.539	18.4	1.65	2.85	42.0
300*	F	R	88 62	6.93	4.12	78.4	40.3	27.1	.346	8.60	3.01	3.84	27.8

* Determined on composite of two rats
S - Stock diet
R - Rachitogenic diet

TABLE XII

EFFECT OF RACHITOGENIC DIET ON BLOOD, HEART, AND KIDNEY CHOLESTEROL IN YOUNG RATS

Rat no.	Sex	Body wt. gms.	Diet	Blood				Heart			Kidney		
				Cholesterol			Fatty acid esterified me./ liter	Wt. gms.	% body wt.	Cholesterol total mg. /g	Wt. gms.	% body wt.	Total cholesterol mg. /g
				Free mg.%	Total mg. %	Ester mg. %							
323	M	128	S	14.5	70.8	79.5	3.0	.60	.47	1.73	1.03	.81	6.31
324	M	123	S	16.0	58.3	72.5	2.5	.52	.42	1.54	1.14	.93	6.40
325	F	110	S	27.0	87.5	69.2	12.0	.46	.42	2.10	0.93	.85	6.10
326	F	114	S	17.5	87.5	80.0	11.5	.48	.42	1.96	0.89	.78	6.37
309	F	71	R	18.9	75.5	75.0	13.0	.40	.57	1.45	0.66	.93	4.55
310	F	87	R	28.0	98.5	71.6	4.5	.40	.46	1.62	0.74	.85	5.18
311	M	89	R	21.0	77.5	72.9	6.0	.42	.47	1.58	0.79	.89	5.10
285*	F	89 94	R	25.0	74.2	66.3	11.5	1.46	.80	3.24	1.48	.81	6.63
286*	M	146 113	R	32.0	85.0	62.3	10.0	.99	.38	2.22	2.58	1.00	5.62
297*	M	80 85	R	17.5	56.7	69.2	6.8	.61	.37	1.85	1.42	.86	6.03
298*	F	74 77	R	22.0	77.5	71.6	3.2	.62	.41	1.94	1.19	.79	6.85
299*	F	74 73	R	34.0	101.7	66.5	3.0	.57	.39	1.74	1.38	.94	5.07
300*	F	88 62	R	21.0	62.5	66.4	2.6	.64	.43	2.40	1.36	.91	5.52

* Determined on pooled sample of two rats
S - Stock diet
R - Rachitogenic diet

TABLE XIII

EFFECT OF STOCK, EXPERIMENTAL DIETS I AND II ON LIVER CONSTITUENTS OF RATS (3½ MONTHS OLD) EXPOSED TO CCl_4 VAPORS 3 TIMES WEEKLY FOR 11 WEEKS

Rat no.	Sex	Diet	Body wt. gms.	Liver wt. gms.	% body wt.	Percent moisture	N mg./g. wet wt.	Total lipids			Mg. per gram cholesterol		Percent esterified cholesterol
								% dry basis	g./g. wet wt.	g./g. N	Free	Total	
265	M	S	321	9.85	3.07	68.8	33.5	27.4	.399	11.9	2.64	3.45	23.5
266	M	S	256	10.3	4.03	70.6	31.6	26.0	.372	11.8	2.07	2.94	29.5
267	F	S	253	8.05	3.18	69.7	34.5	18.7	.268	7.77	2.42	2.92	17.0
268	F	S	209	6.56	3.14	70.7	31.8	49.3	.698	21.9	1.40	2.51	44.2
249	M	S	221	8.78	3.97	69.4	30.9	26.8	.386	12.5	3.14	3.67	14.4
250	M	S	240	7.97	3.32	68.3	31.6	29.1	.412	13.0	1.69	3.20	47.2
251	F	S	170	7.60	4.47	73.4	27.8	33.2	.453	16.3	1.96	2.84	30.9
277	F	I	212	6.42	3.13	56.4	31.6	46.2	.820	26.0	1.57	3.38	53.5
278	M	I	302	14.8	4.91	62.0	27.5	44.7	.722	26.2	4.28	8.65	50.5
279	M	I	281	11.5	4.10	61.6	29.6	44.0	.714	24.1	4.51	26.7	83.1
280	F	I	234	7.33	3.13	64.0	31.3	45.0	.704	22.5	2.33	3.11	25.0
273	F	II	259	13.0	5.02	58.4	28.0	51.9	.889	31.7	2.93	11.1	73.6
274	M	II	210	6.58	3.13	63.8	30.9	53.2	.834	27.0	2.30	5.32	56.8
275	M	II	266	9.68	3.60	62.5	31.0	51.2	.822	26.5	2.69	3.76	31.0

S - Stock diet
I - Experimental diet I
II - Experimental diet II

TABLE XIV

EFFECT OF CCL_4-INDUCED LIVER INJURY ON BLOOD, HEART, AND KIDNEY CHOLESTEROL OF YOUNG RATS (ADMINISTERED BY EXPOSURE FOR 11 WEEKS)

Rat no.	Sex	Body wt. gms.	Diet	Blood				Heart			Kidney		
				Cholesterol			Esterified fatty acid m.e./liter	Wt. gms.	% body wt.	Cholesterol total mg. /g	Wt. gms.	% body wt.	Cholesterol total mg. /g
				Free mg.%	Total mg. %	Ester %							
265	M	321	S	20.0	70.8	71.7	4.00	.94	.29	1.42	2.03	.64	4.84
266	M	256	S	31.5	50.8	38.0	4.0	.81	.32	1.08	1.60	.63	5.10
267	F	253	S	29.5	98.3	70.0	4.3	.75	.30	1.26	1.56	.62	4.89
268	F	209	S	26.0	70.8	63.3	3.5	.70	.34	1.41	1.09	.52	4.91
249	M	221	S	9.0	43.4	79.3	2.3	.65	.39	1.16	1.58	.72	5.27
250	M	240	S	13.5	43.4	68.9	7.5	.75	.31	1.20	1.56	.65	5.55
251	F	170	S	14.4	50.8	71.6	15.8	.57	.34	1.20	1.13	.67	5.90
277	F	212	I	3.0	54.0	94.4	4.0	.62	.29	1.10	1.36	.64	4.16
278	M	302	I	26.6	75.0	64.5	17.5	.89	.29	1.39	2.09	.69	3.34
278	M	281	I	33.3	78.4	57.5	11.5	.83	.30	1.34	1.88	.67	4.43
280	F	234	I	30.1	81.0	62.8	6.5	.69	.30	1.27	1.34	.57	3.60
273	F	259	II	24.2	117.0	79.3	6.5	.76	.29	1.07	0.90	.36	11.20
274	M	210	II	15.8	75.8	79.1	10.8	.61	.29	1.28	1.31	.62	6.35
275	M	266	II	15.8	89.2	82.3	8.8	.86	.32	1.16	1.82	.69	5.22

S - Stock diet
I - Experimental diet I
II - Experimental diet II

TABLE XV

EFFECT OF STOCK, EXPERIMENTAL DIETS I AND II ON LIVER CONSTITUENTS OF RATS INJECTIONED INTRAPERITONEALLY WITH DIFFERENT CONCENTRATIONS OF CCl_4 TWICE WEEKLY FOR 2 WEEKS

Rat no.	Sex	Diet	CCl_4 conc. ml./100 g. body wt.	Body wt. gms.	Liver wt. gms.	% liver wt.	Percent moisture	N mg./g. wet wt.	Total lipids % dry basis	Total lipids g./g. wet wt.	Total lipids g./g. N	Mg. per gram cholesterol Free	Mg. per gram cholesterol Total	Percent esterified cholesterol
240	F	S	0.033	123	6.21	5.05	69.3	31.9	26.2	.378	11.8	3.50	4.11	14.0
241	M	S	0.033	182	8.73	4.79	64.6	29.1	38.9	.601	20.3	1.82	5.75	68.4
242	F	I	0.033	146	7.86	5.38	68.1	26.2	26.0	.382	14.6	2.05	3.27	37.3
243	M	I	0.033	166	7.34	4.42	69.6	29.1	25.2	.362	12.4	2.74	3.71	26.0
244	M	II	0.033	144	8.11	5.63	64.2	27.0	49.5	.761	28.2	2.62	6.67	40.7
256	M	S	0.066	126	5.10	4.04	70.0	30.3	34.4	.491	16.2	2.27	2.49	8.90
257	M	S	0.066	130	5.15	3.96	70.0	31.6	36.8	.512	16.2	2.16	2.42	10.1
258	F	I	0.066	136	4.55	3.35	65.1	32.7	46.0	.708	21.6	2.16	4.05	46.6
259	F	I	0.066	123	4.74	3.85	67.3	31.6	36.7	.546	17.3	2.95	3.41	14.0
260	M	I	0.066	102	4.61	4.52	65.7	35.5	33.8	.515	14.5	2.19	2.98	26.5
261	M	I	0.066	111	5.06	4.56	65.4	30.6	43.0	.658	21.5	2.44	4.42	44.8
262	F	II	0.066	112	5.87	5.23	55.4	27.9	63.2	1.14	40.8	2.13	5.32	40.0
263	M	II	0.066	113	7.18	6.35	60.0	30.6	52.6	.879	28.7	2.04	4.94	58.7
264	M	II	0.066	99	4.44	4.48	67.2	31.7	45.3	.674	21.2	2.60	3.46	24.8
303	F	I	0.099	117	5.15	4.40	66.5	29.2	48.2	.725	24.8	2.42	5.00	51.6
304	F	II	0.099	115	8.08	6.02	62.3	25.4	49.4	.794	31.2	2.02	4.73	52.3
305	M	II	0.099	105	5.46	5.19	66.8	28.5	44.4	.665	23.3	2.71	5.21	47.9
306	F	I	0.132	118	6.68	5.67	65.6	28.7	46.1	.693	24.1	2.54	6.55	61.2
307	M	II	0.132	97	6.48	6.69	69.5	26.5	41.4	.596	22.5	2.74	6.00	54.3

S - Stock diet
I - Experimental diet I
II - Experimental diet II

TABLE XVI

EFFECT OF CCL_4-INDUCED LIVER INJURY ON CHOLESTEROL IN BLOOD, HEART, KIDNEY OF RATS INJECTED INTRAPERITONEALLY WITH DIFFERENT CONCENTRATIONS OF CCL_4 TWICE WEEKLY FOR 2 WEEKS

Rat no.	Sex	Body wt. gms.	Diet	CCl_4 conc. ml./ 100 g. body wt.	Blood				Heart			Kidney		
					Cholesterol			Esterified fatty acid m.eq./ liter	Wt. gms.	% body wt.	Cholesterol total mg. /g	Wt. gms.	% body wt.	Cholesterol total mg. /g
					Free mg. %	Total mg. %	Ester %							
240	F	123	S	.033	23.0	87.5	73.6	10.3	.54	.438	2.34	.97	.789	6.80
241	M	182	S	.033	12.5	73.4	82.8	5.0	.63	.346	2.13	1.14	.627	5.97
242	F	146	I	.033	25.5	111.0	77.0	13.3	.59	.404	2.54	1.12	.768	6.40
243	M	166	I	.033	37.5	98.5	61.9	7.5	.65	.391	2.05	1.31	.790	6.36
244	M	144	II	.033	29.0	100.7	70.1	7.5	.54	.375	2.76	1.07	.743	6.55
256	M	126	S	.066	43.0	62.7	31.4	5.0	.42	.333	1.43	1.00	.795	5.83
257	M	130	S	.066	15.8	74.2	78.3	6.0	.43	.331	1.17	.98	.754	5.95
258	F	136	I	.066	43.3	74.2	41.7	7.0	.46	.338	1.89	.94	.691	6.92
259	F	123	I	.066	24.1	95.8	74.7	7.0	.35	.285	1.48	.98	.797	5.96
260	M	102	I	.066	17.5	43.3	59.5	15.5	.31	.304	.97	.87	.853	5.28
261	M	111	I	.066	19.1	75.0	74.2	7.3	.47	.423	1.21	.87	.784	4.02
262	F	112	II	.066	20.0	64.1	68.8	5.8	.33	.295	1.51	.88	.785	3.60
263	M	113	II	.066	21.6	95.8	77.4	3.0	.41	.363	1.13	.97	.858	4.47
264	M	99	II	.066	29.1	95.0	69.3	2.5	.31	.313	1.10	.86	.870	5.43
303	F	117	I	.099	17.2	50.0	65.6	4.0	.42	.359	1.66	1.01	.864	4.62
304	F	115	II	.099	22.5	104.0	78.4	2.0	.45	.391	1.70	.96	.835	4.52
305	M	105	II	.099	14.0	49.0	61.4	7.2	.39	.371	1.28	.95	.904	4.90
306	F	118	I	.132	9.5	69.2	86.3	2.0	.46	.393	1.74	..76	.644	8.12
307	F	97	II	.132	43.0	164.0	73.8	7.2	.38	.392	1.63	.83	.856	6.42

S - Stock diet
I - Experimental diet I
-- - Experimental diet II