SERUM LIPID CONCENTRATIONS DURING DMBA-INDUCED MAMMARY CARCINOGENESIS OF RATS FED DIETS OF DIFFERENT TYPES OF PROTEIN WITH 20 % DIETARY RESTRICTION

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JEAN C. RIM, B. S., M. S.

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ABSTRACT

Serum Lipid Concentration during DMBA-Induced Mammary Carcinogenesis of Rats Fed Diets of Different Types Protein with 20% Dietary Restriction

Jean C. Rim
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One hundred and thirty female Sprague-Dawley weanling rats were given free access of AIN-76 diet until 9 weeks of age. At 8 weeks of age, 10 rats were used to determine concentrations of total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and triacylglycerol (TG). Half of the remaining 120 rats received 7,12-dimethylbenz(a)anthracene (DMBA) and the other half received sesame oil alone. Fifty DMBA- and 50 sesame oil-treated rats were randomly assigned to one ad libitum group, casein diet (C-AL) and four 20% dietary restricted groups fed soy protein isolate diet, (SPI-R), defatted cottonseed flour diet (CS-R), wheat gluten diet (W-R), and casein diet (C-R). Serum TC, HDL-C, and TG concentrations were also determined at one week post DMBA and sesame oil treatment and at the time when the first tumor from each rat grew to 1-2 cm in diameter, along with their counterpart in the sesame oil-treated group. The C-AL group exhibited shorter latent period and higher incidence for rats bearing first palpable tumor > 1 cm. The SPI-R rats developed the least number of multiple tumors. All the tumors with the size of 1-2 cm in diameter except one were mammary adenocarcinomas.

No consistent changes in the concentrations of the serum TC, HDL-C, and TG during the development of mammary adenocarcinomas. The concentration of serum TC in the SPI-R rats bearing tumors was significantly higher than those received sesame oil. All groups of rats except the W-R group bearing mammary adenocarcinomas had elevated TG concentrations in comparison to the baseline

values.

In conclusion, serum TC, HDL-C, and TG concentrations are not directly affected by the development of DMBA-induced adenocarcinomas. Feeding a diet containing plant protein to rats or subjected the rats to 20% dietary restriction could influence the concentrations of serum lipids as well as the outcome of tumorigenesis. The changes occurred in the serum lipids were independent to the development of the tumors.

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CHAPTER I

INTRODUCTION

According to the American Cancer Society, approximately one of every eight American women will develop breast cancer in 1993 (1). The incidence of breast cancer has been increasing by about 3% per year since 1980. Breast cancer is now the second leading cause of death among women in The United States (1). The principal risk factors for breast cancer include female gender, family history of breast cancer, early age of menarche, nulliparity, giving first birth after age 30, increasing age, late age at menopause, previous benign breast disease, obesity, lifestyle, and environmental risk factors (2, 3). Gender and age are not readily modifiable for the purpose of risk reduction, but environmental risk factors are. Wynder and Gori (3) estimated that as much as 80% of human cancers to have been caused by environmental factors.

Epidemiological studies have shown a positive relationship between breast cancer incidence and dietary intake of total fat, animal protein and calories from animal products (4). Higher incidence rates for breast cancer are usually found in Western industrially developed nations where diets are rich in fat, animal products and total calories but low in fiber (5-7). The role of dietary protein, independent of total caloric intake, dietary fat or animal products, in breast cancer incidence is difficult to establish from epidemiological studies since it is difficult to delineate the effect of animal protein intake from total caloric or fat intake (4). Clear evidence of the relationship between dietary protein and breast cancer may be achieved by using an animal model.

Cancer is defined as unregulated or disorganized proliferation of cell growth (8). Carcinogenesis is a multi-step process. There are at least two known distinctive stages: initiation and promotion. Initiation is a relatively short but irreversible step during which of the carcinogen or its metabolite binds to DNA. The promotion stage of carcinogenesis is a much longer and reversible process. During this latter stage, the process of carcinogenesis can be modified. Diet is one factor that can act as a modifier. Progression is another irreversible stage of carcinogenesis introduced by Fould (9) during which fast rate of cell growth, invasiveness on target site, capability of metastasis and biochemical changes occur in the malignant cell.

Although abundant experimental evidence is available in the literature regarding the relationship between dietary fats and tumorigenesis, relatively few reports concerning the relationship between dietary protein and tumorigenesis are available. Information on the effect of the quality of dietary protein on tumorigenesis is even more scarce. In addition, the results are inconsistent (10-14). In the Department of Nutrition and Food Sciences at Texas Woman's University, experimentation on the effect of the quality of dietary protein in carcinogenesis has been conducted during the past six years. When the dietary intake of the rats was reduced by approximately 20% during both initiation and promotion, there was a beneficial effect of consuming a good-quality-protein diet on 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumor incidence in rats (15). Such effect was not observed when the experimental diets were fed to the rats only during the initiation phase of DMBA-induced carcinogenesis (16). It is reasonable to speculate that the beneficial effect may be related to the consumption of good quality protein at a restricted level during the promotion phase of carcinogenesis.

In 1974, Rose et al. (17) tested the hypothesis of blood-cholesterol level as a predictor of colon cancer. This hypothesis was formed due to the presence of a strong correlation at a population level between coronary heart disease (CHD) incidence and mean blood-cholesterol concentration or colon cancer mortality rate. The result on the test of the hypothesis revealed an inverse relationship between colon cancer mortality rate and blood-cholesterol concentration. In 1981, Williams et al. (18) assembled cancer data from 5,209 subjects in the Framingham study and found an inverse relation between serum cholesterol concentration and colon cancer incidence in man. Carroll (19) also suggested that fat intake and serum cholesterol concentrations have a possible causal relationships with breast cancer.

Case-control studies have reported higher (20), lower (21), or similar (22) plasma cholesterol levels in breast cancer cases compared to the controls. The trend of lower blood-cholesterol values with more advanced colon cancer was also reported (23). However, the same trend was not found in breast cancer (24). Boyd and McGuire (25) reviewed the association between plasma levels of high density lipoprotein cholesterol (HDL-C) and the risk of breast cancer, and reported low levels of HDL-C in populations with low breast cancer risk. These authors suggested a need for further investigation of the relationships between plasma HDL-C levels and breast cancer risk because if such a relationship truly exists, plasma HDL-C could potentially be used as a marker to identify subjects at increased risk for breast cancer. Potischman et al. (21) also found higher plasma triacylglycerol (TG) concentration in patients with breast cancer than that of the controls. Plasma lipids and lipoproteins have also been reported to change with dietary protein changes (26).

There have not been experiments in which the concentrations of blood lipids were studied during the course of carcinogenesis. The purpose of this study was thus to determine serum lipids concentrations of rats fed diets containing either a vegetable or an animal protein with a 20% dietary restriction during DMBA-induced mammary tumorigenesis.

The null hypotheses were:

1. There would be no differences in the concentrations of serum high density lipoprotein cholesterol (HDL-C), total cholesterol (TC) and triacylglycerol (TG) among groups of rats fed different types of dietary protein subjected to 20% dietary restriction.
2. There would be no relationship between mammary tumor development and serum levels of HDL-C, TC and TG during the course of carcinogenesis.

CHAPTER II

REVIEW OF LITERATURE

Epidemiological studies have shown a positive association between the intake of total fat, total saturated fat, total protein or animal protein, and breast cancer incidence or mortality (5, 7, 27-30). However, these epidemiological studies have failed to delineate the effect of protein from other dietary factors on breast cancer incidence or mortality (4, 31). The per capita intake of calories has also been shown to be indirectly correlated to the incidence of breast cancer in females and colorectal cancer in males (5, 32). Reduced breast cancer risk was observed in women who ingested less amount of fat (28% of total calories) (33). A recent review by Adlercreutz et al. (34) reaffirmed that a Westem diet is the main factor causing the high incidence of breast cancer.

Experimentation with either mice or rats on caloric intake and tumor development was first reported in the 1940s by Tannenbaum who showed an inhibitory effect of caloric restriction in inhibitory to chemically-induced (35-37) or spontaneous (38-41) tumorigenesis. In 1943, Lavik et al. (42) also reported a reduction of 3-methlycholanthrene-induced skin tumors in mice fed a diet high in calories but low in fat . There was a pause in the literature for almost 40 years conceming the effect of caloric intake and tumorigenesis. Not until the 1980s, studies on the effect of caloric restriction was rekindled. Kritchevsky and associates showed a significant reduction in DMBA-induced mammary tumors in rats fed a 40% energy restricted diet during the initiation and promotion phase (43, 44) or any time during the promotion phase (45). When DMBA-treated rats were subjected to intermittent ad libitum feeding and caloric restriction over a four month period, the incidence of tumors correlated with total food intake, and palpable tumor growth spurted from restriction to free feeding and vice versa (46). Caloric contribution from fat was suggested to be the major component of the effect of fat on carcinogenesis although the type of fat also exerted an influence (47). Lowered mammary tumor risk (DMBA-induced) was also observed by Clinton et al. (11, 12) in the rats that had their energy intake reduced. Klurfeld et al. (44) concluded that dietary fat is a tumor promoter only when the rats had free access to food, and that the effect of energy intake is stronger than the effect of dietary fat. The inhibition of tumor growth was also observed to be proportional to the degree of energy restriction (44). Cohen, Rose and Wynder (48) in an up-date review of new evidence from epidemiological, laboratory animal model studies, and preliminary feasibility trials suggested that a more appropriate dietary goal for breast cancer would be a reduction in fat intake to 15% of total calories.

Relatively few laboratory studies have examined the relationship between dietary protein and mammary carcinogenesis. The results on the effect of the quantity of dietary protein and carcinogenesis have been inconsistent. Increasing dietary protein intake has been found to have no effect (49, 50), enhancing effect (13, 14, 51) or inhibitory effect (10,12, 52) on spontaneous or chemically induced tumors in rodents. Even fewer reports on the effect of the quality of dietary protein on carcinogenesis are available. Schulsinger et al. (53) reported an inhibition of chemically-induced tumor incidence in rats consuming a low quality protein diet (wheat gluten diet). When such diet was supplemented with lysine, the inhibiting effect was reversed. Hsueh et al. (15) have shown that when rats were fed a reduced amount of a casein diet, the incidence of DMBA-induced mammary tumors was significantly less than in those fed a diet with the same amount of a wheat gluten. This study suggested that when the intake was reduced, the quality of the dietary protein might play an important role in DMBA-induced carcinogenesis.

Recently, attention has focused on the possible role of soybean consumption in reducing cancer risk. Two epidemiological studies identified populations consuming soybean products as their staple diet to have a reduced risk of breast cancer (29, 54). Barnes et al. (55) reported significant inhibitory effect of consuming soy protein on DMBA-induced mammary tumors in rats. These investigators further showed isoflavones to compete with estrogens to bind to estrogen receptors. It was suggested by these authors that the reduced incidence of DMBA-induced tumorigenesis in rats fed the soy protein diet was due to the isoflavones in the soy protein. Hawrylewicz et al. (56) also demonstrated a reduction of N-nitrosomethylurea (NMU)-induced mammary tumor incidence from 80% to 42.3% in rats fed a soy-protein-isolate diet. In contrast, Carroll (19) did not find any differences in DMBA-induced mammary tumorigenesis in rats fed diet containing soy protein isolate or casein. Feeding animals with diet containing plant proteins (e.g. soy, wheat, rice, or cottonseed), has been shown to lower serum concentration of very low density lipoprotein-cholesterol (VLDL-C), low density lipoprotein-cholesterol (LDL-C) or elevate the concentration of high density lipoprotein-cholesterol (HDL-C) in comparison to those fed a casein diet (26, 57, 58, 59).

Very few studies have reported the relationship between breast cancer and blood lipids such as HDL-C, total cholesterol (TC) and triacylglycerol (TG). Potischman et al. (21) reported lower plasma TC concentration and higher TG concentrations in patients diagnosed with breast cancer than the controls. Boyd and

McGuire (60) have noted the blood HDL-C concentration in premenopausal women with mammographic dysplasia to be higher than expected.

CHAPTER III

MATERIALS AND METHODS

Animals and Diets

One hundred and thirty Sprague Dawley female rats, 3 weeks of age, were purchased from Sasco, Houston, Texas. Upon arrival, the rats were individually housed in stainless steel cage with suspended wire-bottoms. The animal room was controlled for temperature (22 ± 2^{o} C), relative humidity (55 ± 5%), and light (12-hour light/12-hour dark). The study protocol was approved by the Animal Care and User Committee of the Texas Woman's University and the approval form is included in Appendix A.

All the rats were given free access to the AIN-76 diet (61, 62) until 9 weeks of age. At the age of 8 weeks, 10 rats were randomly selected and killed to study serum lipid concentrations. One half of the remaining 120 rats were given DMBA (intragastrically, 5 mg/100 g body weight). The concentration of the DMBA solution was 5 mg DMBA in 0.1 mL sesame oil. The remaining 60 rats were each given sesame oil according to their body weights. For example, a rat of 250 grams would receive 0.25 mL DMBA solution or 0.25 mL sesame oil. The food jars were removed 4 hours prior to and 4 hours after the administration of DMBA or sesame oil to avoid any interference on the absorption of DMBA. During the week immediately following DMBA administration, all the rats were kept in disposable cages and housed in the biohazard area of the animal facility. At the age of 9 weeks, 10 DMBA-administered and 10 sesame oil-administered rats were killed for the determination of serum lipid concentrations. The remaining 50 DMBA-administered and 50 sesame oil-administered rats were each randomly assigned to 5 dietary treatment groups and fed the four experimental diets (Table 1). The rats were also returned to the stainless steel wire-bottomed cages and the regular animal rooms. The four experimental diets were modeled after the AIN-76 diet to contain 18% protein, 10% fat, 15% cornstarch, 5% cellulose, 3.5% AIN-76 mineral mixture, 1% AIN-76 vitamin mixture, 0.2% choline bitartrate and amount of sucrose to provide 100% of ingredients. These diets were isocaloric and isoprotein with different type of dietary protein (casein, soy protein isolate, defatted cottonseed flour, and wheat gluten). The diet composition is shown in Table 1. The micronutrients of these diets were not adjusted to compensate for the 20% reduction of the intake. In order to compare the results from the present study and the previous studies, in which these micronutrients were not adjusted, it was decided that no adjustments were made on the quantities of these micronutrients in the diets. In addition, a 20% reduction in the intake of these micronutrients would still meet the requirements of the rats for growth and maintenance (63). The experimental diets were prepared by the investigator and were sent to Pope Testing laboratories in Dallas, Texas for proximate analysis. The results of the proximate analysis are presented in Appendix B. The proximate analyses of the diets indicated that the amount of the macronutrients were as expected. The first group of rats (C-AL) was fed the diet containing casein, ad libitum. The remaining 4 groups of rats were fed diets containing soy protein isolate (SPI-R), cottonseed flour (CS-R), wheat gluten (W-R), and casein (C-R) respectively. The intake of these 4 groups of rats was 80% of the intake of C-AL. For example, if the average intake of the C-AL group was 20 grams, the other four groups of rats would be given 16 grams of their respective diet on the following day. The feeding pattern is illustrated in Table 2. Food intake of the rats

Table 1

Dietary Ingredients and Caloric Density

Ingredient	Casein	Wheat gluten	Soy protein isolate	Defatted cottonseed flour
		g / kg		
Casein[1]	197.4	-	-	-
Wheat gluten[2]	-	251.6	-	-
Soy protein isolate[3]	-	-	206.8	-
Cottonseed flour[4]	-	-	-	328.4
DL-methionine	3.0	-	-	-
Sucrose	452.6	401.4	446.2	324.6
Corn starch	150.0	150.0	150.0	150.0
Corn oil	100.0	100.0	100.0	100.0
Cellulose	50.0	50.0	50.0	50.0
AIN-76 Mineral mix	35.0	35.0	35.0	35.0
AIN-76A Vitamin mix	10.0	10.0	10.0	10.0
Choline bitartrate	2.0	2.0	2.0	2.0
Caloric density (kcal/g diet)	4.0	4.0	4.0	3.9

1. High nitrogen casein (Teklad Test Diets, Madison, WI) Protein, 91.20%; carbohydrate 3.10%; fat, 0.00%.
2. Wheat gluten (ICN Biochemicals, Cleveland, OH) Protein, 71.54%; carbohydrate, 18.50%; fat, 0.75%.
3. Soy protein isolate (ICN Biochemicals, Cleveland, OH) Protein, 87.06%; carbohydrate, 2.79%; fat, 0.45%.
4. Defatted glandless cottonseed flour (Rogers Delinted Cottonseed Co., Waco, TX) Protein, 54.81%; carbohydrate, 25.44%; fat, 2.63%.

Table 2

Feeding Pattern

Dietary group	Dietary protein source	Feeding Pattern
C-AL	Casein	Ad libitum
W-R	Wheat gluten	80% intake of C-AL
SPI-R	Soy protein isolate	80% intake of C-AL
CS-R	Defatted Cottonseed flour	80% intake of C-AL
C-R	Casein	80% intake of C-AL

was expressed as gram per day. Body weights were measured weekly from 4 to 12 weeks of age and biweekly thereafter (until 26 week of age).

A diagram of the experimental design is shown in Diagram 1. Four weeks after DMBA administration, each rat was palpated weekly for mammary tumor development. The location and number of palpable tumors for each rat were recorded and the size of the first tumor was measured by a vernier caliper (Type 6914; Bel Art, Swizerland). When the first palpable tumor of each rat measured 1-2 cm in diameter, the rat was sacrificed and the tumor was excised, measured again, weighed, and preserved in 10% buffered formalin for histopathological examination. Any other palpable or nonpalpable tumors were also counted, excised, measured, weighed, and preserved in 10% buffered formalin in a separate specimen jar.

Determination of Serum Triacylglycerol (TG), Total Cholesterol (TC) and High-Density Lipoprotein-Cholesterol (HDL-C)

Serum lipid concentrations, specifically total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and triacylglycerol (TG) of the rats, were determined at 4 time periods: (a) at the age of 8 weeks - before the administration of DMBA or sesame oil (baseline); (b) at the age of 9 weeks - one week after the administration of DMBA or sesame oil but before assigned to the experimental diets; (c) at the time when the first palpable tumor measured 1-2 cm in diameter (age varied); and (d) at termination (26 weeks of age) regardless of the presence or absence of mammary tumor.

All blood samples were drawn after a 14-hour fasting period. The blood was drawn via cardiac puncture after anesthetized with metofane. Serum was separated by centrifuging at 2,000 X g for 15 minutes (Sorvall RC-5B Du Pont). Serum TC

DIAGRAM 1

Experimental Design

|-------Initiation Phase-------|-------Promotion Phase-------|

C-AL (n=20) --------DMBA or Sesame oil-treated
(Casein diet, ad libitum) (n=10) (n=10)

W-R (n=20) --------DMBA or Sesame oil-treated
(Wheat gluten diet, 80% of C-AL) (n=10) (n=10)

C-R (n=20) --------DMBA or Sesame oil-treated
(Casein diet, 80% of C-AL) (n=10) (n=10)

SP-R (n=20) --------DMBA or Sesame oil-treated
(Soy protein diet, 80% of C-AL) (n=10) (n=10)

CSP-R (n=20)--------DMBA or Sesame oil-treated
(Cottonseed protein diet, 80% of C-AL) (n=10) (n=10)

Diet: AIN-76 diet AIN-76 diet

Age: 4 wks-------8 wks-------9 wks-------termination of the study (26 wks)
(n=130) (n=130) (n=120) (n=100)

4 wks: Experiment was begun

8 wks:
(1) Sacrificed 10 rats to determine serum Chol, HDL-C & TG concentrations
(2) Administerd DMBA and sesame oil to 60 rats each.

9 wks:
(1) Sacrificed 10 DMBA- and 10 sesame oil-treated rats to determine serum Chol, HDL-C, & TG concentrations.
(2) The remaining rats were randomly assigned to 5 dietary treatment groups.

Termination of the study (26 wks):
(1) Sacrificed rats in DMBA group when the first tumor reached 1-2 cm. Tumors were excised and pathologically examined.
(2) Serum Chol, HDL-C, and TG were determined from 10 tumor-bearing rats and 10 sesame oil-treated rats in each dietary treatment group.
(3) Serum Chol, HDL-C, and TG were determined from the remaining rats in the each dietary treatment group at the termination (26 weeks of age).

Chol = Total cholesterol
HDL-C = HDL-cholesterol
TG = Triglyerides

concentration was determined colorimetrically according to the method of Bucolo and David (64). Serum HDL-C concentration was determined by using the supernatant of specimen after precipitated out low density lipoprotein (LDL) cholesterol and very low density lipoprotein (VLDL) cholesterol fractions from serum by means of HDL-C precipitating reagent ($MgCl_2$ 1 mol/L in 1% w/v dextran sulfate) and followed by colorimetrically according to the method of Allain et al (65). Serum TG concentration was determined colorimetrically according to the method of Warnick et al.(66).

Statistical Analysis

Body weights and food intakes were statistically analyzed using a repeated measures analysis of variance (ANOVA). The effect of dietary treatment on latent period (week bewteen DMBA administration and detection of the first palpable tuomr) as well as all the parameters on mammary tumorigenesis were analyzed by Kruskal-Wallis one way analysis of variance (ANOVA). The student-Newman-Keuls test was the post hoc test to determine statistical significances between and among the groups. The level of significance was at 5%.

Concentrations of serum total cholesterol, HDL-cholesterol and triacylglycerol were analyzed by one way analysis of variance (ANOVA). The student-Newman-Keuls procedure was the post hoc test. The level of significance for serum lipid concentration test was also at 5%. Pearson product-moment correlation coefficient was used to determine the correlation between the serum lipoprotein lipids and parameters of mammary tumorigenesis. The level of significance for the Pearson product-moment correlation coefficient test was at 1%.

CHAPTER IV

RESULTS

Bodt Weight

The present study was conducted to determine the serum lipid concentrations of rats during the course of DMBA-induced mammary carcinogenesis. During the 4 weeks prior to the DMBA administration (from 4 weeks to 8 weeks of age) and the one week immediately after the administration of DMBA, all the rats were fed the AIN-76 diet, ad libitum. These 5 weeks are generally recognized as the initiation phase of carcinogenesis in experimentations using rats. Starting from the second week after the administration of DMBA until the development of malignant tumors, it is considered as the promotion phase of carcinogenesis. This promotion phase usually is a much longer period than the initiation phase and it could be as long as 18 to 26 weeks depending upon the type and dosage of the carcinogen administered. It was during the promotion phase of DMBA-induced mammary carcinogenesis that the rats in the present study were fed diets containing different protein-quality and were also subjected to 20% dietary restriction.

The mean body weights of the 5 groups of the rats during the initiation phase (4 to 9 weeks of age) are shown in Table 3. Since these rats had free access to the same AIN-76 diet, all the rats had similar body weight gain as expected. During the promotion phase, the experimental diets were fed to the 5 groups of rats. Each dietary group consisted of 20 rats, one half was administerd with DMBA while the other half received sesame oil. The mean body weights of the rats are presented

TABLE 3

Mean body weights of rats fed AIN-76 diet from four weeks to nine weeks of age[1]

Age (weeks)	Group[2]				
	C-AL	SPI-R	CS-R	W-R	C-R
	gm	gm	gm	gm	gm
4	93.4 ± 7.8	96.4 ± 7.5	92.0 ± 13.3	94.0 ± 6.1	93.0 ± 4.6
9	258.3 ± 6.3	262.2 ± 16.2	258.5 ± 13.5	258.2 ± 13.6	258.2 ± 4.7

[1]Values are means ± S.D. of 20 rats. No statistically significant differences were found among the groups of rats at the same age.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
CS-R=defatted cottonseed flour diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

separately presented according to the treatment of DMBA or sesame oil. The mean body weights (from 9 to 13 weeks) of the 5 groups of rats treated with DMBA are shown in Table 4. One week after consuming their respective experimental diet, the rats that were fed the wheat gluten diet with 20% dietary restriction (W-R-D) began to show a significant slower growth ($p<0.05$) than those fed the casein diet ad libitum (C-AL-D). After the age of 10 weeks, all 4 groups of rats that were subjected to 20% dietary restriction regardless of the quality of dietary protein (SPI-R-D, CS-R-D, W-R-D and C-R-D) weighed less than the C-AL-D group ($p<0.05$). The body weights of the rats that were treated with sesame oil (C-AL-S, SPI-R-S, CS-R-S, W-R-S and C-R-S) followed the same growth pattern as those treated with DMBA (Table 5). Throughout the entire promotion phase, the same growth pattern was seen in the 5 group of rats. The mean body weights (from 14 through the 20th week of age) of the 5 groups of rats treated with DMBA or sesame oil are shown in Table 6 and Table 7, respectively.

Beginning at the age of 18 weeks, some of the rats in the various groups that had developed palpable tumors to greater than 1 cm in diameter. These rats were thus sacrificed according to the original design of the study. The number of rats in each group in Table 6 reflected that some of the rats had already been killed for the removal of their first palpable tumors. For example: None of the rats in any group had their first tumor grown to 1 cm in diameter at the age of 16 weeks, but by the age of 18 weeks, two rats in the C-AL-D group had been killed because their respective first palpable tumor had grown to be 1 cm in diameter. Each time when a DMBA-treated rat was killed, a sesame oil-treated rat with comparable body weight was also killed to serve as a control. As a result the number of rats treated with DMBA

TABLE 4

Mean body weights from 9 to 13 weeks of age of DMBA-treated rats fed experimental diets[1]

Age (weeks)	Group[2]				
	C-AL-D	SPI-R-D	CS-R-D	W-R-D	C-R-D
	gm	gm	gm	gm	gm
9	258.3 ± 6.3^{a}	258.6 ± 16.2^{a}	258.5 ± 13.5^{a}	258.2 ± 13.6^{a}	258.2 ± 4.7^{a}
10	280.0 ± 18.2^{a}	262.2 ± 16.2^{ab}	264.0 ± 14.4^{ab}	257.6 ± 18.7^{b}	262.9 ± 22.2^{ab}
11	300.6 ± 22.5^{a}	270.3 ± 13.3^{b}	270.4 ± 13.3^{b}	269.0 ± 12.4^{b}	268.8 ± 18.4^{b}
12	315.7 ± 27.6^{a}	284.1 ± 12.6^{b}	282.2 ± 14.4^{b}	282.5 ± 9.6^{b}	281.3 ± 18.0^{b}
13	331.7 ± 26.4^{a}	294.0 ± 11.2^{b}	292.0 ± 15.8^{b}	293.3 ± 10.4^{b}	293.2 ± 18.6^{b}

[1]Values are means ± S.D. of 10 rats. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$ using Student Newman Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20 % dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction.

TABLE 5

Mean body weights from 9 to 13 weeks of age of sesame oil-treated rats fed experimental diets[1]

Age	Group[2]				
(weeks)	C-AL-S	SPI-R-S	CS-R-S	W-R-S	C-R-S
	gm	gm	gm	gm	gm
9	260.6 ± 16.7^{a}	260.7 ± 8.7^{a}	260.0 ± 17.5^{a}	260.6 ± 18.0^{a}	260.5 ± 17.7^{a}
10	284.4 ± 21.0^{a}	263.4 ± 9.4^{a}	262.3 ± 14.7^{a}	261.8 ± 21.4^{a}	268.2 ± 18.6^{a}
11	303.6 ± 18.9^{a}	272.6 ± 12.0^{b}	268.0 ± 12.4^{b}	269.2 ± 15.8^{b}	276.5 ± 17.1^{b}
12	322.1 ± 21.5^{a}	280.3 ± 14.0^{b}	277.3 ± 13.6^{b}	279.0 ± 17.4^{b}	285.0 ± 16.5^{b}
13	341.1 ± 27.1^{a}	291.6 ± 15.3^{b}	284.4 ± 16.7^{b}	294.2 ± 14.9^{b}	297.7 ± 15.9^{b}

[1]Values are means ± S.D. of 10 rats. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$, using student Newman Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20 % dietary restriction; CS-R=defatted-cottonsee flour with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction.

TABLE 6

Mean body weights from 14 to 20 weeks of age of DMBA-treated rats fed experimental diets[1]

Age (weeks)	Group[2]				
	C-AL-D	SPI-R-D	CS-R-D	W-R-D	C-R-D
	gm	gm	gm	gm	gm
14	345.3 ± 28.6[a] (n=10)	300.2 ± 13.9[b] (n=10)	297.0 ± 13.3[b] (n=10)	301.0 ± 11.6[b] (n=10)	299.4 ± 17.5[b] (n=10)
16	357.5 ± 31.4[a] (n=10)	310.6 ± 13.1[b] (n=10)	302.8 ± 15.3[b] (n=10)	306.3 ± 10.7[b] (n=10)	307.3 ± 18.2[b] (n=10)
18	360.4 ± 37.4[a] (n=8)	317.4 ± 14.5[b] (n=9)	310.3 ± 16.6[b] (n=10)	315.0 ± 12.3[b] (n=10)	315.9 ± 16.0[b] (n=8)
20	375.8 ± 50.1[a] (n=5)	326.5 ± 18.0[b] (n=9)	317.6 ± 16.6[b] (n=8)	323.9 ± 11.4[b] (n=10)	323.6 ± 15.0[b] (n=8)

[1]Values are means ± S.D. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$, using Student Newman Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20 % dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction.

TABLE 7

Mean body weights from 14 to 20 weeks of age of sesame-oil treated rats fed experimental diets[1]

Age (weeks)	Group[2]				
	C-AL-S	SPI-R-S	CS-R-S	W-R-S	C-R-S
	gm	gm	gm	gm	gm
14	351.5 ± 24.8^a (n=10)	299.2 ± 14.0^b (n=10)	291.9 ± 14.2^b (n=10)	300.6 ± 17.9^b (n=10)	307.2 ± 16.0^b (n=10)
16	362.8 ± 23.7^a (n=10)	306.0 ± 13.6^b (n=10)	297.6 ± 16.8^b (n=10)	304.6 ± 15.3^b (n=10)	313.4 ± 15.6^b (n=10)
18	372.8 ± 24.8^a (n=8)	304.4 ± 29.0^b (n=9)	303.5 ± 15.7^b (n=10)	308.5 ± 14.4^b (n=10)	322.2 ± 13.9^b (n=8)
20	393.3 ± 33.0^a (n=5)	319.2 ± 15.6^b (n=9)	308.4 ± 18.3^b (n=8)	317.5 ± 14.7^b (n=10)	326.7 ± 14.0^b (n=8)

[1]Values are means ± S.D. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$, using Student Newman Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20 % dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20 % dietary restriction.

(Table 6) and those treated with sesame oil (Table 7) in the same dietary treatment groups were matched. By the end of 21 weeks of age, all the rats in the CS-R-D and CS-R-S groups were killed whether they were bearing tumors or not. At the initiation of this experiment, this investigator was aware that the available supply of the defatted cottonseed flour in our laboratory was only sufficient to feed 20 rats until they were 20 weeks of age. Due to the difficulty of locating more defatted cottonseed flour for experimentation, it was decided then that the group of rats to be fed the cottonseed flour diet would be included in the study with the understanding that this group of rats might have to be terminated earlier than the remaining 4 groups. This was exactly what happened. While one half of the 10 rats in the C-AL-D group were already killed because their first palpable tumors had reached 1 cm or greater in diameter at 20 weeks of age, only 2 of the 10 rats in the CS-R-D were in the same condition. Since there was no defatted cottonseed flour diet left, all the rats that were fed such diet were killed by 21 weeks of age. The remaining four groups of rats were carried until 26 weeks of age as originally designed. The rats that were subjected to 20% dietary restriction regardless of the type of dietary protein weighed consistently less ($p<0.05$) than those fed the casein diet (Table 8 and Table 9).

<u>Food Intake</u>

The mean daily feed intakes of the experimental diets during the promotion phase of DMBA-induced mammary tumorigenesis are shown in Tables 10, 11, and 12. The mean intake of the C-AL group was calculated from the intakes of all the rats consuming the casein diet ad libitum (DMBA-treated and sesame oil-treated) and 80% of that mean intake was given to the other four groups of rats on the

TABLE 8

Mean body weights from 22 to 26 weeks of age of DMBA-treated rats fed experimental diets[1]

Age	Group[2]				
(weeks)	C-AL-D	SPI-R-D	CS-R-D[3]	W-R-D	C-R-D
	gm	gm	gm	gm	gm
22	382.7 ± 54.5^{a} (n=5)	333.5 ± 14.8^{b} (n=9)	-	331.1 ± 14.6^{b} (n=10)	329.5 ± 22.0^{b} (n=8)
24	387.6 ± 69.3^{a} (n=4)	339.7 ± 15.8^{b} (n=9)	-	339.3 ± 15.0^{b} (n=9)	339.3 ± 18.0^{b} (n=6)
26	435.1 ± 86.2^{a} (n=2)	348.3 ± 17.9^{b} (n=8)	-	351.4 ± 13.0^{b} (n=7)	324.8± 15.8^{b} (n=3)

[1]Values are means ± S.D. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$, using Student Newman Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20 % dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction.

[3]Rats in this group were sacrificed at 21 weeks of age.

TABLE 9

Mean body weights from 22 to 26 weeks of age of sesame oil-treated rats fed experimental diets[1]

Age (weeks)	Group[2]				
	C-AL-S	SPI-R-S	CS-R-S[3]	W-R-S	C-R-S
	gm	gm	gm	gm	gm
22	416.6 ± 43.9^a (n=5)	326.4 ± 17.8^b (n=9)	-	323.2 ± 15.0^b (n=10)	333.9 ± 19.9^b (n=8)
24	420.0 ± 43.6^a (n=4)	332.0 ± 19.0^b (n=9)	-	332.8 ± 16.2^b (n=9)	344.7 ± 17.2^b (n=6)
26	439.2 ± 38.0^a (n=2)	332.1 ± 16.6^b (n=8)	-	342.1 ± 18.8^b (n=7)	33.7 ± 18.6^b (n=3)

[1]Values are means ± S.D. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$, using Student Newman Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20 % dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction.

[3]Rats in this group were sacrificed at 21 weeks of age.

TABLE 10

Mean feed intake from 9 to 12 weeks of age of rats fed experimental diets[1]

Age (weeks)	Group[2]				
	C-AL (D+S)	SPI-R (D+S)	CS-R (D+S)	W-R (D+S)	C-R (D+S)
	gm/day	gm/day	gm/day	gm/day	gm/day
9 - 10	20.2 ± 1.6^{a}	14.4 ± 1.1^{b}	14.6 ± 1.5^{b}	13.7 ± 2.9^{b}	15.2 ± 1.1^{b}
10 - 11	19.7 ± 1.8^{a}	15.4 ± 0.5^{b}	15.5 ± 0.5^{b}	15.3 ± 0.8^{b}	15.4 ± 0.5^{b}
11 - 12	20.5 ± 2.6^{a}	15.6 ± 0.8^{b}	15.8 ± 0.2^{b}	16.0 ± 0.3^{b}	16.0 ± 0.2^{b}

[1]Values are means ± S.D. of 20 rats. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$, using Student Newman Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20% dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction. D+S=including both DMBA and sesame oil-treated rats.

TABLE 11

Mean feed intake from 12 to 20 weeks of age of rats fed experimental diets[1]

Age (weeks)	Group[2]				
	C-AL (D+S)	SPI-R (D+S)	CS-R (D+S)	W-R (D+S)	C-R (D+S)
	gm/day	gm/day	gm/day	gm/day	gm/day
12 - 14	20.4 ± 1.1^{a} (n=20)	15.8 ± 0.8^{b} (n=20)	16.1 ± 0.8^{b} (n=20)	16.0 ± 1.0^{b} (n=20)	16.1 ± 0.8^{b} (n=20)
14 - 16	18.7 ± 1.9^{a} (n=20)	14.5 ± 0.5^{b} (n=20)	14.8 ± 0.5^{b} (n=20)	14.7 ± 0.4^{b} (n=20)	14.7 ± 0.4^{b} (n=20)
16 - 18	18.1 ± 1.9^{a} (n=16)	14.4 ± 0.5^{b} (n=18)	14.6 ± 0.5^{b} (n=20)	14.5 ± 0.5^{b} (n=20)	14.6 ± 0.3^{b} (n=16)
18 - 20	17.9 ± 1.9^{a} (n=10)	13.9 ± 0.7^{b} (n=18)	14.1 ± 0.2^{b} (n=16)	14.1 ± 0.3^{b} (n=20)	14.2 ± 0.2^{b} (n=16)

[1]Values are means ± S.D. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$, using Student Newman Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20% dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction. D+S=including both DMBA and sesame oil-treated rats.

TABLE 12

Mean feed intake from 20 to 26 weeks of age of rats fed experimental diets[1]

Age (weeks)	Group[2]				
	C-AL (D+S)	SPI-R (D+S)	CS-R[3] (D+S)	W-R (D+S)	C-R (D+S)
	gm/day	gm/day	gm/day	gm/day	
20 - 22	18.4 ± 2.1^a (n=10)	14.0 ± 0.6^b (n=18)	-	14.3 ± 0.4^b (n=20)	14.3 ± 0.6^b (n=16)
22 - 24	18.3 ± 2.2^a (n=8)	14.2 ± 0.4^b (n=18)	-	14.3 ± 0.6^b (n=18)	14.6 ± 0.3^b (n=12)
24 - 26	18.7 ± 1.3^a (n=4)	13.8 ± 0.4^b (n=16)	-	14.2 ± 0.1^b (n=14)	14.0 ± 0.5^b (n=6)

[1]Values are means ± S.D. Values not sharing a common letter superscript in the same row are significantly different at $p<0.05$, using Student Newman Keuls post hoc procedure

[2]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20% dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction. D+S=including both DMBA and sesameoil-treaed rats.

[3]All the rats in this group were killed at 21 weeks of age.

following day. The intake of C-AL group was significantly higher ($p<0.05$) than those of the SPI-R, CS-R, W-R and C-R as expected since those latter 4 groups were subjected to a 20% dietary restriction. Consequently, no statistically significant differences were observed among the groups of rats subjected to 20% dietary restriction.

Tumor Development

Table 13 presents several parameters related to tumor development. The mean latent period (time between DMBA administration and the detection of the first palpable tumor) for the five groups of rats ranged from 9.7 to 13.5 weeks which were not significantly different. The average latent period was the longest for the W-R group and shortest for the C-AL group. The average time for the tumor to grow to 1-2 cm in diameter was not different among the five groups of rats. The CS-R group exhibited the shortest time period which was 0.7 weeks. At the time when all the CS-R rats had to be killed (21 weeks of age), about half of the 10 rats in the C-AL or the CS-R group had tumors measured 1-2 cm in diameter. Only one of the 10 rats in either SPI-R or C-R had a tumor greater than 1 cm in diameter while none in the W-R group had tumors of the same size. At termination, four additional rats in each of the C-AL, SPI-R, and W-R groups had tumors measured ≥ 1 cm while 6 more rats in the C-R groups developed tumors of ≥ 1 cm. This brought to a total of 9, 5, 4, and 7 rats in C-AL, SPI-R, W-R, and C-R respectively having their first palpable tumors measured ≥ 1 cm when the study was terminated.

At necropsy (26 weeks of age), all palpable and nonpalpable tumors were excised, counted, measured with a caliper, and weighed. The data are presented in

TABLE 13

Number of first palpable tumors measured 1-2 cm in diameter in rats fed experimental diets

	Group[1]				
	C-AL	SPI-R	CS-R[2]	W-R	C-R
Total number of rats given DMBA	10	10	10	10	10
Latent periods[3] (weeks)[4]	9.7±2.2 (n=9)	13.2±3.9 (n=5)	10.8±2.1 (n=4)	13.5±1.3 (n=4)	11.8±2.8 (n=7)
Mean weeks for tumors to reach ≥ 1cm in diameter[4]	3.1±2.3 (n=9)	2.6±1.5 (n=5)	0.7±0.6 (n=4)	3.0±0.6 (n=4)	2.0±1.4 (n=7)
Number of rats bearing first palpable tumor of ≥1 cm in diameter before 21 weeks of age	5/10	1/10	4/10	0/10	1/10
Number of rats bearing first palpable tumor ≥1 cm in diameter after 21 weeks of age	4/5	4/9	-	4/10	6/9

[1]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20% dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction.

[2]All rats were sacrificed at 21 weeks of age due to the shortage of the cottonseed flour diet.

[3]Weeks between DMBA administration and detection of the first palpable tumor.

[4]Values are mean ± S.D. Values are not significantly different among the five treatment groups.

Table 14. Since all the CS-R rats were sacrificed about five weeks earlier than the other 4 groups of rats, no data from CS-R are in Table 14. Neither the number of palpable tumor per tumor-bearing rat nor the weight of the first palpable tumor when it was greater than 1 cm in diameter was significantly different among the four groups of rats.

Data on multiple tumor development are presented in Table 15. More than 80% of the rats that were fed a diet containing wheat gluten of 20% dietary restriction developed multiple tumors. On the other hand, 50% of the tumor-bearing rats consuming defatted cottonseed flour diet and 29% of the soy protein isolate diet rats developed multiple tumors. The number of multiple tumors per tumor-bearing rat was similar among the five groups of rats although rats that were fed the soy protein diet (SPI-R) seemed to have more multiple palpable tumors per tumor-bearing rat.

Histopathological Examination of Tumors

Histopathological examination of all the first palpable tumors that most of them had reached at least 1 cm in diameter were conducted by Texas Veterinary Medical Diagnostic Laboratory in Bryan, Texas. Table 16 presents the results of the pathological analysis. All the tumors examined except one were mammary adenocarcinoma. One of the tumors from the W-R group was a mammary fibroadenoma. A copy of the original report of the histopathology analysis is included in Appendix C.

Serum Total Cholesterol (TC) Concentrations

The concentrations of serum total cholesterol (TC), high density lipoprotein-

TABLE 14

Tumor counts and weight of the first tumor from rats fed diets of different protein and subjected to 20% dietary restriction

	Group[1]				
	C-AL	SPI-R	CS-R[2]	W-R	C-R
Number of rats bearing palpable tumors and non-palpable tumors at necropsy	10	7	-	6	8
Total number of palpable tumors	21	13	-	11	17
Total number of nonpalpable tumors	11	4	-	6	4
Number of palpable tumors per tumor-bearing rat[3]	2.0 ±1.2 (n=10)	1.8 ± 1.6 (n=7)	-	1.8 ± 0.8 (n=6)	2.1 ± 1.4 (n=8)
Weight (gm) of first palpable tumor (≥ 1 cm in diameter) per tumor-bearing rat[3]	1.8 ±0.9 (n=9)	1.5 ± 0.6 (n=5)	-	1.7 ± 0.8 (n=4)	1.9 ± 1.0 (n=7)

[1]C-AL=casein diet, ad libitum; SPI-R=soy protein isolate diet with 20% dietary restriction; CS-R=defatted-cottonseed flour diet with 20% dietary restriction; W-R=wheat gluten diet with 20% dietary restriction; C-R=casein diet with 20% dietary restriction.

[2]All rats were sacrificed at 21 weeks of age due to the shortage of the cottonseed flour diet.

[3]Values are mean ± S.D. Values are not significantly different at $p<0.05$ among the 4 dietary groups.

TABLE 15

Multiple tumor development in rats fed experimental diets

	Group[1]				
	C-AL	SPI-R	CS-R	W-R	C-R
Number of rats bearing multiple palpable and nonpalpable. tumors	7/10 (70%)	2/7 (29%)	2/4 (50%)	5/6 (83%)	5/8(62%)
Number of multiple palpable tumors per tumor-bearing rat	2.8	4.0	2.0	2.3	2.8
Number of multiple (palpable + nonpalpable) tumor per tumor-bearing rat	3.7	5.0	2.0	2.2	3.6

[1]C-AL=casein diet, ad libitum;

SPI-R=soy protein isolate diet with 20% dietary restriction;

CS-R=defatted-cottonseed flour diet with 20% dietary restriction;

W-R=wheat gluten diet with 20% dietary restriction;

C-R=casein diet with 20%dietary restriction.

TABLE 16

Summary of histopathologic examination of first palpable mammary tumors from rats fed experimental diets[1]

	Group[2]				
	C-AL	SPI-R	CS-R	W-R	C-R
Total number of first palpable tumors examined (number of rats)	10 (10)	7 (7)	4 (4)	6 (6)	8 (8)
Number of mammary adenocarcinomas (%)	10 (100%)	7 (100%)	4 (100%)	5 (83.3%)	8 (100%)
Number of mammary fibroadenoma	0 (0%)	0 (0%)	0 (0%)	1 (16.7%)	0 (0%)

[1]Tumors were examined by Texas Veterinary Medical Diagnostic Laboratory in Bryan, Texas.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

cholesterol (HDL-C) and triacylglycerol (TG) were determined before DMBA administration (baseline-8 weeks of age), one week after DMBA administration (9 weeks of age), at the time when the first palpable tumor measured 1-2 cm in diameter, and at the termination of the study (26 weeks of age). Fasting blood samples were drawn via cardiac puncture and sera were separated by centriifuging at 4,000 x g for 20 minutes. Table 17 presents the concentrations of serum TC at baseline and one week post DMBA-administration. The mean serum TC concentration of the rats treated with DMBA (2.17 mmol/L) was significantly lower ($p<0.05$) than that of the rats either treated with sesame oil (2.44 mmol/L) or at the baseline (2.60 mmol/L). No significant difference was found in the TC concentrations of rats at the baseline and those treated with sesame oil.

Table 18 presents the serum total cholesterol (TC) concentrations of rats bearing first palpable tumor of 1-2 cm in diameter and those did not bear any tumors (treated with sesame oil). Rats in the C-AL group showed the highest TC concentration whether they were treated with DMBA or sesame oil and these TC concentrations were significantly higher ($p<0.05$) than those fed diets containing soy, cottonseeds or wheat gluten. Rats in the SPI-R group showed the lowest TC concentration in both DMBA- and sesame oil treated groups. When the rats were treated with the sesame oil their mean TC concentrations was significantly different ($p<0.05$) from those treated with DMBA and fed the same soy protein diet. No significant difference ($p <0.05$) was found in the concentrations of serum TC of rats in the C-AL group and the C-R group regardless of the treatment. Serum TC concentration of rats in the C-AL group bearing tumors of 1-2 cm in diameter was significantly higher than that of the rats at the baseline (prior to DMBA administration),

TABLE 17

Serum total cholesterol concentrations of rats at baseline and one week post DMBA or sesame oil-treatment[1,2]

Baseline (8 weeks of age)	1 week post the administration of (9 weeks of age)	
	DMBA	Sesame oil
mmol/L	mmol/L	mmol/L
2.60 ± 0.48[a] (n=10)	2.17 ± 0.40[b] (n=9)	2.44 ± 0.53[a] (n=10)

[1]Values are means ± S.D. Values not sharing a common letter superscript are significantly different at $p<0.05$ using Student Newman-Keuls post hoc procedure.

[2]All rats were fed the AIN-76 diet.

TABLE 18

Serum total cholesterol concentrations of rats bearing first tumor measured 1-2 cm in diameter induced by DMBA[1]

Group[2]	Treatment		
	DMBA (Diameter of first tuomr ≥ 1cm)	Sesame oil (No tumor)	P
	mmol/L	mmol/L	
C-AL	3.54 ± 1.08^{a} (n=9)	3.31 ± 0.40^{a} (n=9)	N. S.
SPI-R	2.37 ± 0.82^{b} (n=5)	1.09 ± 0.59^{b} (n=5)	<0.05
CS-R	2.42 ± 0.31^{b} (n=4)	2.53 ± 0.57^{c} (n=4)	N. S.
W-R	2.28 ± 0.29^{b} (n=4)	2.56 ± 0.28^{c} (n=4)	N. S.
C-R	2.79 ± 0.30^{a} (n=7)	$3.01 \pm 0.80^{a,c}$ (n=7)	N. S.

[1]Values are means ± S.D. Values not sharing a common letter superscript within a column are significantly different at p<0.05 using Student Newman-Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

one week after DMBA administration or subjected to 20% dietary restriction (Table 19).

A number of rats in each dietary group either did not develop any tumor or had their first palpable tumor measured less than 1 cm in diameter. The TC concentrations of these rats are presented in Table 20. Consuming diets containing different type of protein did not affect the TC concentration if the rats bearing either no tumor or tumors that were smaller than 1 cm in diameter.

Serum High Density Lipoprotein-Cholesterol (HDL-Cholesterol) Concentrations

Serum HDL-C concentrations of rats at baseline and one week post DMBA administration are shown in Table 21. A significant reduction of the HDL-C concentrations ($p<0.05$) was seen in the rats at 9 weeks of age regardless of treatment.

Table 22 presents serum HDL-C concentrations of rats bearing their first palpable tumors of 1-2 cm in diameter and of rats treated with sesame oil. Neither the type of dietary protein nor the imposition of 20% dietary restriction had an effect on the serum HDL-C concentrations if the rats were treated with DMBA. In the sesame oil-treated group, rats that were fed the casein diet ad libitum (C-AL) showed a significantly higher serum HDL-C concentration ($p<0.05$) than those subjected to 20% dietary restriction (SPI-R, CS-R, W-R and C-R). With the same dietary treatment, no difference was found on the serum HDL concentrations between the rats treated with DMBA and those treated with sesame oil.

Statistical comparisons were also made on HDL-C concentrations at different time periods (Table 23). One week after DMBA administration, serum HDL-C

TABLE 19

Serum total cholesterol (TC) concentrations of rats fed diets containing different protein-quality after DMBA administration[1]

Time period	Diet[2] (group)	Restriction	TC concentration
			mmol/L
Baseline	AIN-76A	none	2.60 ± 0.48[a] (n=10)
1 week post DMBA administration	AIN-76A	none	2.17 ± 0.40[a] (n=9)
First palpable tumor > 1 cm in diameter	Casein (C-AL)	none	3.54 ± 1.08[b] (n=9)
	Soy protein isolate (SPI-R)	20%	2.37 ± 0.82[a] (n=5)
	Defatted cottonseed flour (CS-R)	20%	2.42 ± 0.31[a] (n=4)
	Wheat gluten (W-R)	20%	2.28 ± 0.29[a] (n=4)
	Casein (C-R)	20%	2.79 ± 0.30[a] (n=7)

[1]Values are means ± S.D. Values not sharing a common letter superscript within a column including baseline with each column are significantly different at $p<0.05$ using Student Newman-Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

TABLE 20

Serum total cholesterol (TC) concentrations of rats bearing first palpable tumor less than 1 cm in diameter or no tumors[1]

Group[2]	TC concentrations of Rats		
	DMBA-Treated		Sesame oil-treated
	First palpable tumor < 1cm in diameter	No tumors	No tumors
	mmol/L	mmol/L	mmol/L
C-AL	3.12 ± 0.00 (n=1)	-	3.13 ± 0.00 (n=1)
SPI-R	2.22 ± 0.21 (n=2)	2.00 ± 0.27 (n=3)	2.54 ± 0.63 (n=5)
CS-R	-	2.54 ± 0.68 (n=6)	2.75 ± 0.60 (n=6)
W-R	2.70 ± 0.97 (n=2)	2.62 ± 0.37 (n=4)	2.25 ± 0.23 (n=6)
C-R	3.03 ± 0.00 (n=1)	2.62 ± 0.06 (n=2)	2.36 ± 0.61 (n=3)

[1]Values are means ± S.D. None of the values within a column are significantly different at $p<0.05$.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

TABLE 21

Serum high density lipoprotein-cholesterol (HDL-C) concentrations of rats at baseline and one week post DMBA or sesame oil-treatment[1,2]

Baseline (8 weeks of age)	1 week post the administration of (9 weeks of age)	
	DMBA	Sesame oil
mmol/L	mmol/L	mmol/L
1.45 ± 0.43[a] (n=10)	0.89 ± 0.24[b] (n=9)	1.07 ± 0.25[b] (n=10)

[1] Values are means ± S.D. Values not sharing a common letter superscript are significantly different at $p<0.05$ using Student Newman-Keuls post hoc procedure.

[2] All rats were fed the AIN-76 diet.

TABLE 22

Serum high density lipoprotein cholesterol (HDL-C) concentration of rats treated with DMBA or sesame oil[1]

Group[2]	Treatment		
	DMBA (Diameter of first tumor ≥ 1 cm)	Sesame oil (No tumor)	P
	mmol/L	mmol/L	
C-AL	1.85 ± 0.88[a] (n=9)	2.02 ± 0.19[a] (n=9)	N. S.
SPI-R	1.09 ± 0.59[a] (n=5)	1.15 ± 0.36[b] (n=5)	N. S.
CS-R	1.27 ± 0.34[a] (n=4)	1.30 ± 0.54[b] (n=4)	N. S.
W-R	0.93 ± 0.18[a] (n=4)	1.09 ± 0.37[b] (n=4)	N. S.
C-R	1.14 ± 0.27[a] (n=7)	1.43 ± 0.44[b] (n=7)	N. S.

[1]Values are means ± S.D. Values not sharing a common letter superscript within a column are significantly different at $p<0.05$ using Student Newman-Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

concentration of DMBA-treated rats was significantly lowered ($p<0.05$) in comparison to the C-AL group bearing first palpable tumor of > 1 cm in diameter.

Table 24 included HDL-C concentrations from rats bearing tumors smaller than 1 cm in diameter or no tumors at the end of the study. None of the values in Table 24 are significantly different from each other indicating neither the treatment of DMBA nor the bearing of smaller or no tumors had an effect on the concentration of serum HDL-C.

Serum Triaclyglycerol (TG) Concentrations

The serum triacylglycerol (TG) concentrations of rats at baseline and one week post DMBA or sesame oil administration are presented in Table 25. The TG concentration of the rats did not change after DMBA treatment. However rats treated with sesame oil had elevated their TG concentrations significantly ($p<0.05$).

At the time when their first palpable tumors were greater than 1 cm in diameter, serum TG concentration was affected by neither the quality of dietary protein nor dietary restriction (Table 26). On the other hand, rats that were fed the soy protein isolate diet or cottonseed flour diet (SPI-R or CS-R) and were treated with sesame oil demonstrated a significantly lowered concentration of serum TG compared to those fed the casein ad libiitum diet (C-AL).

Data on TG concentration at different time periods are presented in Table 27 to examine whether there was a change in this lipid during the course of tumor development. At the time the first DMBA-induced palpable mammary tumor grew to > 1cm in diameter, rats fed the casein diet ad libitum (CA-L) showed significantly elevated ($p<0.05$) serum TG concentrations than those at the baseline or

TABLE 23

Serum high density lipoprotein-cholesterol (HDL-C) concentrations of rats fed diets containing different protein-quality after DMBA administration[1]

Time period	Diet (group)	Restriction	HDL-C concentration
			mmol/L
Baseline	AIN-76A	none	1.45 ± 0.43[a] (n=10)
1 week post DMBA administration	AIN-76A	none	0.89 ± 0.24[e] (n=9)
First palpable tumor > 1 cm in diameter	Casein (C-AL)	none	1.85 ± 0.88[a,b] (n=9)
	Soy protein isolate (SPI-R)	20%	1.09 ± 0.59[a] (n=5)
	Defatted cottonseed flour (CS-R)	20%	1.27 ± 0.34[a] (n=4)
	Wheat gluten (W-R)	20%	0.93 ± 0.18[d] (n=4)
	Casein (C-R)	20%	1.14 ± 0.27[c] (n=7)

[1]Values are means ± S.D. Values not sharing a common letter superscript within a column including baseline with each column are significantly different at $p<0.05$ using Student Newman-Keuls post hoc procedure.

TABLE 24

Serum high density lipoprotein-cholesterol (HDL-C) concentrations of rats bearing first palpable tumor less than 1 cm in diameter or no tumors[1]

Group[2]	HDL-C concentrations of Rats		
	DMBA-Treated		Sesame oil-treated
	First palpable tumor < 1cm in diameter	No tumors	No tumors
	mmol/L	mmol/L	mmol/L
C-AL	0.88 ± 0.00 (n=1)	-	0.94 ± 0.00 (n=1)
SPI-R	0.83 ± 0.19 (n=2)	0.68 ± 0.12 (n=3)	0.89 ± 0.22 (n=5)
CS-R	-	0.88 ± 0.29 (n=6)	1.06 ± 0.16 (n=6)
W-R	1.07 ± 0.16 (n=2)	0.87 ± 0.15 (n=4)	0.68 ± 0.08 (n=6)
C-R	0.86 ± 0.00 (n=1)	0.68 ± 0.00 (n=2)	0.86 ± 0.24 (n=3)

[1]Values are means ± S.D. None of the values within a column are significantly different at $p<0.05$.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

TABLE 25

Serum triacylglycerol concentrations of rats at baseline and one week post DMBA or sesame oil-treatment[1,2]

Baseline (8 weeks of age)	1 week post the administration of (9 weeks of age)	
	DMBA	Sesame oil
mg/dL	mg/dL	mg/dL
70.0 ± 15.2[a] (n=10)	80.6 ± 20.4[a] (n=9)	112.7 ± 28.3[b] (n=10)

[1]Values are means ± S.D. Values not sharing a common letter superscript are significantly different at $p<0.05$ using Student Newman-Keuls post hoc procedure.

[2]All rats were fed AIN-76 diet.

TABLE 26

Serum triacylglycerol concentrations of rats bearing first tumor measured 1-2 cm in diameter during the promotion phase on DMBA-induced tumorigenesis[1]

Group[2]	Treatment		
	DMBA (Diameter of first tumor ≥ 1 cm)	Sesame oil (No tumor)	P
	mg/dl	mg/dL	
C-AL	144.4 ±69.9[a] (n=9)	167.1 ± 82.0[a] (n=9)	N. S.
SPI-R	95.5 ±37.8[a] (n=5)	77.4 ± 30.0[b] (n=5)	N. S.
CS-R	91.7 ±9.7[a] (n=4)	86.3 ± 26.7[b] (n=4)	N. S.
W-R	73.6 ±33.1[a] (n=4)	83.9 ± 17.9[a,b] (n=4)	N. S.
C-R	102.3 ±41.8[a] (n=7)	123.7 ± 35.8[a,b] (n=7)	N. S.

[1]Values are means ± S.D. Values not sharing a common letter superscript within a column are significantly different p<0.05, using Student Newman-Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

one week after the administration of DMBA. Rats in the SPI-R, CS-R, and C-R group also showed elevated serum TG concentration at the time the tumor grew to the size of > 1 cm in diameter in comparison to the baseline concentration ($p<0.05$). On the contrary, rats that were fed the wheat gluten diet (W-R) did not significantly changed their serum TG concentration after their tumors became greater than 1 cm in diameter.

Serum TG concentration of those rats whose first palpable tumors did not grew to 1 cm or greater in diameter or did not develop any tumor at the time the study was terminated (26 weeks of age) are presented in Table 28. Only one DMBA-treated rat was in the C-AL group and this rats had extremely high concentration of serum TG which became significantly higher ($p<0.05$) than those in the other three groups. No significant difference were found in the TG concentrations in the rats bearing no tumors.

Correlation Coefficient Analysis

Correlation coefficient was determined between serum lipid concentrations and tumor parameters of rats fed experimental diets after DMBA administration (Table 29). Significantly negative correlations ($p<0.01$) were found between the latent period (time between the appearance of the first tumor and the administration of DMBA) and the serum HDL-C concentration of the rats when the first palpable tumor was > 1cm (**r** = -0.4416); and between the size of the tumor and the latent period (**r** = -0.4531). There was also a significant negative correlation ($p<0.01$) between tumor size and the time when the rats were sacrificed.

Serum Amino Acids Concentrations

The concentrations of nine essential amino acids in the serum of the rats

TABLE 27

Serum triacylglycerol concentrations of rats fed diets containing different protein-quality after DMBA administration[1]

Time period	Diet (group)	Restriction	TG concentration
			mg/dL
Baseline	AIN-76A	none	70.0 ± 15.2^{a} (n=10)
1 week post DMBA administration	AIN-76A	none	80.6 ± 20.4^{b} (n=9)
First palpable tumor > 1 cm in diameter	Casein (C-AL)	none	144.4 ± 69.9^{c} n=9)
	Soy protein isolate (SPI-R)	20%	$95.5 \pm 37.8^{b,c}$ (n=5)
	Defatted cottonseed flour (CS-R)	20%	$91.7 \pm 9.7^{b,c}$ (n=4)
	Wheat gluten (W-R)	20%	$73.6 \pm 33.1^{a,b,c}$ (n=4)
	Casein (C-R)	20%	$102.3 \pm 41.8^{b,c}$ (n=7)

[1]Values are means ± S.D. Values not sharing a common letter superscript within a column including baseline with each column are significantly different at $p<0.05$ using Student Newman-Keuls post hoc procedure.

TABLE 28

Serum triacylglycerol (TG) concentrations of rats bearing first palpable tumor less than 1 cm in diameter or no tumors[1]

Group[2]	TG concentrations of Rats		
	DMBA-Treated		Sesame oil-treated
	First palpable tumor < 1cm in diameter	No tumors	No tumors
	mg/dL	mg/dL	mg/dL
C-AL	432.0 ±0.0[a] (n=1)	-	131.0± 0.0[a] (n=1)
SPI-R	71.9± 1.3[b] (n=2)	73.0 ± 51.4[a] (n=3)	68.3 ± 22.5[a] (n=5)
CS-R	-	106.7± 37.2[a] (n=6)	98.2 ± 19.3[a] (n=6)
W-R	75.3± 41.0[b] (n=2)	78.3 ± 23.9[a] (n=4)	78.3 ± 30.9[a] (n=6)
C-R	80.9± 10.0[b] (n=1)	80.9 ± 10.0[a] (n=2)	83.6 ±22.8[a] (n=3)

[1]Values are means ± S.D. Values not sharing a common letter superscript within a column are significantly different at $p<0.05$ using Student Newman-Keuls post hoc procedure.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

TABLE 29

Correlations between serum lipid concentrations and tumor parameters of rats fed experimental diets after DMBA treatment

	Total cholesterol	HDL-cholesterol	Triacyl-glycerol	Palpable tumor size (diameter)
	(r)	(r)	(r)	(r)
Latent period (weeks)	-0.3190	-0.4416*	-0.0770	-0.4531*
Number of palpable tumor	0.1179	0.1391	-0.1120	-0.1676
Total palpable tumor weight	0.1120	0.2565	-0.1275	-0.7783
Number of non-palpable tumor	0.0943	0.0230	-0.0864	-0.1160
Total non-palpable tumor weight	0.1042	0.0358	-0.0904	-0.1204
Time at sacrifice (weeks post DMBA administration)	0.0837	-0.1605	0.0862	-0.4946*

[1] r = correlation coefficient.

*Significant at $p<0.01$

received DMBA or sesame oil are presented in Tables 30 and 31, respectively. The concentration of tryptophan is not presented because tryptophan was destroyed during the process of acid hydrolysis. For the purpose of convenience, values in the C-AL group are arbitrarily assigned as 100%. Data from the other four dietary groups are then calculated as percentage of the values of the C-AL group. The concentrations of the nine essential amino acids in the serum of DMBA-treated rats in the C-AL group (Table 30) except threonine were similar to those of sesame oil-treated rats in the C-AL group (Table 31). The concentration of serum threonine of the C-AL-S rats (sesame oil-treated) was about 28% less than that of the C-AL-D (DMBA-treated) rats. In the DMBA-treated rats (Table 30), most of the essential amino acid were lower in concentration in the rats subjected to 20% dietary restriction than the ad libitum fed group (C-AL-D). In general, the patterns of the concentration of the serum essential amino acids are similar in rats treated with DMBA and those treated with sesame oil. The concentrations of the essential amino acids in the serum of the rats reflected the diets that they consumed. For example, the concentration of methionine in the rats consuming soy protein isolate diet was either similar or slightly less than those consumed the casein diet. Rats that were fed the defatted cottonseed flour diet showed less amount of branched amino acids and higher amount of arginine in their sera which is reflective of the amino acid contents of the cottonseed protein (67). On the other hand, rats that were fed the wheat gluten diet had less amount of lysine in their sera. The purpose of determining the concentration of the essential amino acids was no other than to see if the diets that were fed to the rats were indeed reflected in the amino acid pools of the rats. Data in

TABLE 30

Fasting serum essential free amino acid concentrations of rats fed experimental diets containing casein or plant protein with 20 % dietary restriction after DMBA administration[1]

	Group[2]				
Amino acid	C-AL-D	SPI-R-D	CS-R-D[3]	W-R-D	C-R-D
			μmol/L		
Lysine	900(100%)	780(86%)	690(77%)	750(83%)	760(84%)
Histidine	60(100%)	50(83%)	50(83%)	50(83%)	70(117%)
Arginine	220(100%)	240(109%)	250(114%)	160(73%)	210(95%)
Threonine	880(100%)	650(74%)	570(65%)	600(68%)	800(91%)
Valine	300(100%)	260(87%)	230(77%)	230(77%)	250(83%)
Methionine	70(100%)	70(100%)	70(100%)	60(86%)	70(100%)
Isoleucine	16(100%)	14(87%)	13(81%)	13(81%)	14(87%)
Leucine	260(100%)	230(88%)	200(77%)	210(81%)	220(85%)
Phenyalanine	100(100%)	90(90%)	90(90%)	80(80%)	90(90%)

[1]Values represent one pooled sample from 10 rats for each dietary group.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
C-R=casein diet with 20%dietary restriction.

TABLE 31

Fasting serum essential free amino acid concentrations of rats fed experimental diets containing casein or plant protein with 20 % dietary restriction after sesame oil administration[1]

	Group[2]				
Amino acid	C-AL-S	SPI-R-S	CS-R-S	W-R-S	C-R-S
			μmol/L		
Lysine	850(100%)	760(89%)	650(76%)	770(90%)	830(98%)
Histidine	50(100%)	50(100%)	50(100%)	50(100%)	50(100%)
Arginine	230(100%)	190(83%)	270(117%)	190(83%)	220(96%)
Threonine	640(100%)	660(103%)	540(84%)	600(94%)	710(111%)
Valine	280(100%)	230(82%)	230(82%)	240(86%)	260(93%)
Methionine	80(100%)	70(87%)	80(100%)	70(87%)	70(87%)
Isoleucine	16(100%)	13(81%)	12(75%)	13(81%)	15(94%)
Leucine	250(100%)	210(84%)	210(84%)	210(84%)	240(96%)
Phenyalanine	100(100%)	90(90%)	90(90%)	90(80%)	100(100%)

[1]Values represent one pooled sample from 10 rats for each dietary group.

[2]C-AL=casein diet, ad libitum;
SPI-R=soy protein isolate diet with 20% dietary restriction;
CS-R=defatted-cottonseed flour diet with 20% dietary restriction;
W-R=wheat gluten diet with 20% dietary restriction;
C-R=casein diet with 20% dietary restriction.

Tables 30 and 31 showed that our purpose was achieved. The report from the Genetic Screening and Counseling Service (GSCS) in Denton, Texas is included in Appendix D.

CHAPTER V

DISCUSSION

The present study was designed to determine the serum lipid concentration of female rats that were fed different types of dietary protein and subjected to 20% dietary restriction after the administration of DMBA. The body weights of the animals that were subjected to 20% dietary restriction regardless of the type of protein in their diets were significantly reduced ($p<0.05$) in comparison to those fed a casein diet, ad libitum during the 18-week study period. The degree of the reduction in the body weight was approximately from 15 to 25%. During the first half of the study period, the degree of the reduction in the body weight was about 15% (Table 4 to Table 7). As the study progressed, the degree of the reduction in the body weights of those rats subjected to 20% dietary restriction increased to about 25% of those fed the casein diet ad libitum (Tables 8 and 9). Under normal condition when the intake of the rats was ad libitum, the growth of the rats was usually retarded if the diet was a poor protein diet (15, 16, 53). Caloric restriction has been well documented to cause growth retardation (34, 35, 42, 43, 44, 69). In the present study, the quality of the dietary protein did not have any effect on the growth of the rats when 20% dietary restriction was imposed upon them.

Since the protocol of the study called for a 20% dietary restriction to be imposed on four of the five dietary groups, it was expected that the intake of the four restricted groups consumed less feed than those fed ad libitum. The difference in the intake was statistically significant ($p<0.05$). There was a slight difference between the actual and the intended percentage of restriction among the four dietary restricted groups. This difference of about 1-2% was due to spillage and it was not statistically significant .

SPI-R group had the greatest spillage. The same phenomenon was also observed by Handy (16).

Tumor Development and Growth

The results of the present study showed that DMBA-induced mammary tumor development was delayed in those rats that were subjected to 20% dietary restriction. At the age of 21 weeks (Table 13), five of 10 rats consuming casein diet ad libitum (C-AL) developed their first palpable tumors to the size of 1-2 cm in diameter. Fewer rats in the four groups of restricted rats developed their first mammary tumors to the size of 1-2 cm in diameter. Forty percent of the rats (4/10) consuming cottonseed flour diet (CS-R) had tumors grown to 1-2 cm in diameter while 10%, 0% and 10% of the rats consuming soy protein isolate diet (SPI-R), wheat gluten diet (W-R), and casein diet (C-R), respectively, had tumor size of 1-2 cm in diameter (Table 13). Although these percentage are not statistically significantly different, it is obvious that a substantial delay in the development and growth of the mammary tumor was present in the rats subjected to 20% dietary restriction. It is also interesting to note that tumors from the rats consuming the cottonseed flour diet took less than one week to grow to 1-2 cm in diameter (Table 13). Cottonseed protein contains high concentration of arginine (67) which has the characteristic of stimulating growth (68). It is possible that the growth of the tumor was stimulated by the arginine in the cottonseed flour.

By the age of 26 weeks (at termination), additional 4 rats in the C-AL group had tumors greater than 1 cm in diameter which gave a total of 90% of the rats in this group having the size of the tumors greater than 1 cm in diameter. Additional rats in the SPI-R, W-R, and C-R groups also developed tumors of the same size. At termination, 50% of the rats in the SPI-R and W-R groups had their first palpable mammary tumors grown to 1-2 cm in diameter compared to the 70% of the C-R group. There are no data in the literature on tumor size in relation to feeding different dietary protein during tumorigenesis. It was unfortunate that in the present study, the CS-R group was not carried to termination due to the lack of the cottonseed flour diet. Longer latent period was also observed in the groups of rats subjected to 20% dietary restriction (Table 13). However, the latent period was not equally extended among these groups indicating not only 20% dietary restriction but also the quality of dietary protein had an effect on the latent period.

The average number of palpable tumors developed in each tumor-bearing rat (Table 14) was not different among the five groups of rats. This was not surprising since the criteria used to sacrifice the rats was at the time when the first tumor reached 1-2 cm in diameter. Therefore, it was not a long enough time period for more tumor to develop. Fifty percent of the tumor-bearing rats in the CS-R group or 29% in the SPI-R group developed multiple tumors while more than 80% of the tumor-bearing rats in the W-R group developed multiple palpable tumors (Table 15). Unlike the number of rats developed tumors of > 1 cm in diameter (in which the W-R group was the least), as many as 80% of the W-R rats developed multiple tumors. This observation was in agreement with the observations by Handy (16) and Barner (70). The reason for this phenomenon is unknown. Soy, cottonseed and wheat are all plant proteins. Although the former two are better in quality than the latter one, it is not known whether the amino acid composition or some other factors of these two better quality plant proteins are the reason for developing less multiple tumors. Since the intention of the present study was to examine the serum lipid concentrations at the time the rats were bearing DMBA-induced tumors, the number of the rats in each dietary group in the present study was not sufficient to study tumorigenesis. Therefore, no further discussion related discussion related to the dietary protein and tumorigenesis will be provided. Suffice to say that dietary protein in combination with 20% dietary restriction probably will have an influence on both the development and the growth of DMBA-induced mammary tumors. Possibly, a better quality plant protein will be a favorable one as far as tumorigenesis is concerned. Further studies are definitely warranted.

Serum Lipids Concentrations

Serum total cholesterol concentration (TC) and high density lipoprotein-cholesterol (HDL-C) concentration were significantly ($p<0.05$) lowered at one week after the administration of DMBA in comparison to the baseline concentrations (Table 17 and Table 21). Serum triacylglycerol (TG) concentration was not changed after DMBA administration (Table 25). The reason for the change or no change in these serum lipid concentrations is not clear. Since the dietary intake was not measured during the week after the administration of DMBA, it is not certain that these changes in TC and HDL-C concentrations are due to dietary intake. Serum TC concentration of the rats (Table 17) was not changed one week after the administration of sesame oil. However, serum HDL-C concentrations of these rats were lowered ($p<0.05$, Table 21) while the concentration of TG was significantly ($p<0.05$) elevated (Table 25). The administration of sesame oil is unlikely to have any effect on these parameters unless there was stress due to the intubation procedure. The intubation procedure took only one minute per rat and those rats were very calm during the procedure. Therefore, the produce of intubation did not seem to be a factor in changing these serum lipid concentrations.

At the time when the first palpable tumor of the rats grew to 1-2 cm in diameter, the serum concentration of TC of the rats that were fed the casein diet, ad libitum (C-AL) was significantly higher than those fed plant proteins and subjected to 20% dietary restriction (SPI-R, CS-R and W-R) but similar to those fed the same casein diet with 20% dietary restriction (C-R) (Table 18). These results demonstrated that the lowered concentration of serum TC in the rats bearing tumors was not due to dietary restriction rather by consuming diets containing plant proteins. In the sesame oil-treated rats, same trend of changes was observed indicating that it was the dietary treatment, not the development of the tumors that were influencing the concentrations of serum TC. Furthermore, rats that were fed the soy protein diet and received sesame oil had an even lower concentration of TC than those received DMBA and bearing tumors (Table 18). This further indicates the effectiveness of soy protein in lowering serum cholesterol concentration which has been known for sometime (55-58). Both the AIN-76 diet and casein experimental diet contained casein as the sole protein source. When the serum TC concentrations of the rats consumed AIN-76 diet or C-AL were compared, a significantly elevated ($p<0.05$) TC concentration was seen as tumor development advanced (Table 19). The TC concentration in the restricted groups with advanced tumor development did not show such elevation indicating that it is the ad libitum intake of the casein diet that has an influence on the serum TC concentration. In some case-control studies, serum TC concentration of patients with breast cancer was shown to be significantly higher ($p<0.05$) than those in the control group (20, 71). In the present study, rats that were fed the soy diet showed a significant difference ($p<0.05$) in the serum TC concentration between the rats bearing adenocarcinomas and those received sesame oil (control). This difference in the TC concentration probably cannot attribute to the presence of the tumors but rather to the cholesterol lowering effect of soy protein since the TC concentration of tumor-bearing rats was not elevated.

Feeding different dietary proteins to the rats since the administration of DMBA until the development of the first palpable mammary tumor to 1-2 cm did not have a significant effect on the serum HDL-C concentrations (Table 22). When the rats did not develop any tumor (i.e. received sesame oil instead of DMBA), a 20% dietary restriction significantly lowered ($p<0.05$) the serum HDL-C concentration of the rats regardless of the type of dietary protein (Table 22).

Serum HDL-C concentration was found to be low in breast cancer patients (71, 72). No animal studies have examined the serum HDL-C concentration while the animals developed tumors. The results on HDL-C concentration from the present study did not indicate that bearing DMBA-induced mammary tumor would result in lower HDL-C concentration. The results only suggest that feeding different dietary protein or imposing a 20% dietary restriction could lower serum HDL-C concentration regardless of the presence of tumors.

Serum TG concentrations were elevated ($p<0.05$) one week after the administration of sesame oil but not DMBA (Table 25). The reason for such change after the administration of sesame oil is not clear. A continual increase in the serum TG concentration was observed in the rats bearing tumors except those fed the wheat gluten diet (W-R) (Table 27). Rats that were fed the casein diet, ad libitum and received sesame oil also increased their serum TG concentrations significantly although these rats did not bear any tumors (Table 26). These results indicate that it was the free access of the casein diet which elevated the TG concentration in the serum. When the casein diet was consumed on the restricted basis (C-R group), the TG concentration in the serum of these rats was also elevated but not as high as those in the C-AL group (Table 27). By restricting the intake of the rats by 20%, the serum TG concentration was suppressed (Table 27). However, soy protein was most effective in suppressing the serum TG concentration when the rats did not receive DMBA (Table 26). It is possible that soy protein would suppress the TG concentration even though there was the presence of tumor. However, due to the small sample size in the present study no statistically significant difference was found (Table 26). At the time the study was designed, we did not expected such low percentage of rats in the soy protein and wheat gluten dietary groups to have tumors of > 1 cm in diameter 18 weeks after the administration of DMBA. One could extend the study beyond 26 weeks of age (18 weeks post DMBA administration) and waited until all the rats in these groups of rats had developed tumors to the size of greater than 1 cm in diameter. However, we would have to justify the age differences in the rats of the experimental groups in order to compare the effect of diet on the TG concentration. It is not known whether age has an effect on serum TG concentration. Since the TG concentration of the rats received sesame oil and bore no tumors exhibited the same pattern among the different dietary groups (Table 26), it is reasonable to suggest that feeding plant proteins to rats would lower serum TG concentration and bearing tumors is not a factor in influencing serum TG concentration. Feeding rats a diet containing animal protein (casein) certainly had a profound effect in elevating the serum TG concentrations (Table 26 and Table 27).

A significant negative correlation was found between the latent period and the serum HDL-C concentration ($r = -.4416$) which indicates the shorter the latent period the higher the HDL-C concentration at the time the tumor was greater than 1 cm in diameter. Latent period is defined as the time between the DMBA administration and the appearance of the first palpable tumor. Since the HDL-C concentration was not determined at the time the first tumor was palpable, it is difficult to interpret this negative correlation between the latent period and the HDL-C concentration when the tumor was already 1 cm in diameter. There was also a significantly negative correlation between the latent period and tumor diameter (tumor size). The shorter the latent period, the larger the tumor was when it was excised. The time when the rats were sacrificed and their tumors were excised was also significantly ($p<0.01$) negatively correlated to the size of the tumor.

These three negative correlations seem to point to the direction that the early the palpable tumor developed, the faster this palpable tumor grew. Different dietary treatments may influence the latent period thus influence the growth of the tumor.

Data presented in the present study are the first systematic determinations of the serum lipid concentration in rats induced by DMBA. No information on the same subjects is available in the literature. This investigator hopes that as time passes on, there will be more information available.

CHAPTER VI

SUMMARY AND CONCLUSION

The purpose of the present study was to investigate the serum lipid concentrations during DMBA-induced mammary carcinogenesis of rats fed diets of different types of protein with 20% dietary restriction. One hundred and thirty female Sprague-Dawley weanling rats were fed the AIN-76 diet, ad libitum until nine weeks of age. At 8 weeks of age, blood sera of 10 rats were analyzed for the concentrations of total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and triacylglycerol (TG). For the remaining 120 rats, 60 rats were intragastrically administered with DMBA (5 mg/100 g body weight) and 60 rats were given sesame oil alone.

At nine weeks of age, sera from ten DMBA and ten sesame oil-treated rats were analyzed for TC, HDL-C and TG. The remaining 50 DMBA- and 50 sesame oil-treated rats were randomly assigned to one of the five dietary treatments. Each dietary group consisted of 10 DMBA-treated and 10 sesame oil-treated rats. The C-AL group was fed the casein diet, ad libitum. Groups SPI-R, CS-R, W-R and C-R were fed diets containing soy protein isolate, defatted cottonseed flour, wheat gluten, and casein, respectively. These four groups of rats were also subjected to 20% dietary restriction, i. e. they were given 80% of the intake of the C-AL group.

When the first palpable tumor grew to 1-2 cm in diameter, the tumors were excised for pathological examination. At the same time, blood was drawn via cardiac puncture for the determination of serum TC, HDL-C, and TG concentrations.
The defatted cottonseed flour diet was only sufficient to carry the study until the rats

were 21 weeks of age. Our previous experience indicated that by the age of 21 weeks, all of the rats would have developed DMBA-induced mammary tumors. By the age of 21 weeks, five rats in the C-AL group and four in the cottonseed flour diet group (CS-R) group had developed tumor and were killed for the removal the tumors. Only one in each of the soy protein (SPI-R) and casein restricted (C-R) groups was killed and none in the wheat gluten group had developed tumor of 1 cm in diameter. The CS-R group had to be terminated at 21 weeks of age because of the lack of the diet and the remaining four groups were carried until 26 weeks of age. At termination, 90% of the rats that were fed the casein diet, ad libitum had tumors of > 1 cm in diameter while approximately 50% of the rats in each of the restricted groups had tumors of the same size. Among the four restricted groups fewer rats in the cottonseed flour diet and soy protein diet groups developed multiple tumors. All but one pathologically examined tumors were identified as adenocarcinomas.

There was no consistent changes in the concentrations of the serum total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and triaclyglycerol (TG) when the rats developed DMBA-induced mammary adenocarcinomas. There was no change in the TC concentrations of the rats except those consuming casein diet ad libitum which exhibited an elevation of TC concentration when they developed adenocarcinoma. In general, rats that were subjected to 20% dietary restriction and fed the plant protein diets showed a significant lower ($p<0.05$) serum TC concentration than those fed the casein diet, either ad libitum or restricted. In the groups of rats consuming the soy protein diet of restricted amount, there was a significant increase in the TC concentrations in those developed mammary adenocarcinomas compared to those received sesame oil and did not develop any tumors. Such effect was probably due to the profound cholesterol lowering effect of the soy protein.

Bearing mammary adenocarcinomas did not change the serum HDL-C concentrations of the rats. It was the feeding of a plant protein diet or the introducing of a 20% dietary restriction that lowered the HDL-C concentrations.

All the dietary groups except the W-R group had elevated TG concentration in comparison to the baseline value. Tumor-bearing did not have an effect on the TG concentration. However, dietary restriction could lower serum TG concentration of the rats.

A significant ($p<0.05$) negative correlation was found between the latent period and the HDL-C concentration or the size of the tumor. The reason(s) underlying the relationship is not clear. Further exploration of the mechanism is indeed warranted.

In conclusion, serum lipids such as TC, HDL-C, and TG concentrations are not directly affected by the development of DMBA-induced adenocarcinomas. Feeding a diet containing plant protein to rats subjected to 20% dietary restriction could influence the concentrations of serum lipids of the rats. The changes occurred in the serum lipids were independent of the development of the tumors.

REFERENCES

1. Anonymous (1993) Cancer Facts & Figures -1993 American Cancer Society, Inc., Atlanta, GA.

2. Kelsey, J. L. (1979) A review of the epidemiology of human breast cancer. Epidemiol. Rev. 1:74-109.

3. Wynder, E. L. & G. B. Gori, G. B. (1977) Contribution of the environment to cancer incidence: an epidemiologic exercise. J. Natl Cancer Inst 58:825-832.

4. National Academy of Sciences (1982) Committee on Diet, Nutrition, and Cancer, National Research Council, Diet, Nutrition and Cancer, National Academy Press, Washington, D. C.

5. Armstrong, B. & Doll, R. (1975) Environmental factors and cancer incidence and mortality in different countries, with special reference to dietary practices. Int. J. Cancer 15:617-631.

6. Carroll, K. K. & Khor, K. T. (1975) Dietary fat in relation to tumorigenesis. Prog. Biochem. pharm. 10:308-353.

7. Kolonel, L. N., Hankin, J. H., Lee, J., Chu, S. Y., Nomura, A. M. and Hinds, M. W. (1981) Nutrient intakes in relation to cancer incidence in Hawaii. Br. J. Cancer 44:332-339.

8. Farber, E. (1968) Biochemistry of carcinogenesis. Cancer Res. 28:1859-1869.

9. Fould, L. (1954) The experimental study of tumor progression. Cancer Res. 14:327-1742.

10. Clinton, S. K., Imrey, P. B., Alster, J. M., Simon, J. & Visek, W. J. (1988) The combined effects of dietary protein and fat intake during the promotion phase of 7,12-dimethlybenz(a)anthracene-induced breast cancer in rats. J. Nutr. 118:1577-1585.

11. Clinton, S. K., Imrey, B. P.,Alster, J. M., Simon, R. Truex, C. R. & Visek, W. J. (1984) The combined effects of dietary protein and fat on 7,12-dimethlybez(a)anthracene-induced breast cancer in rats. J. Nutr. 114:1213-1223.

12. Clinton, S. K., Alster, S. K., Imrey, P. B., Nandkumar, S.,Truex, C. R. & Visek, W. J. (1986) Effects of dietary protein, fat and energy intake during an initiation phase study of 7,12-dimethlybez(a)anthracene-induced breast cancer in rats. J. Nutr. 116:2290-2302.

13. Hawrylewicz, E. J., Huang, H. H., Kissane, J. Q. & Drab, E. (1982) Enhancement of 7,12-dimethlybenz(a)anthracene (DMBA) mammary tumorigenesis by high dietary protein in rats. Nutr. Rept. Int. 26:793-806.

14. Hawrylewicz, E. J. (1986) Fat-protein interaction, defined 2-generation studies. In: Dietary fat and cancer (Ip, C., Birt, D., Rogers, A., & Mettlin, C. eds.) Alan R. Liss, New York, pp. 403-433.

15. Hsueh, A. M., Shipley, R. & Park, H. S. (1992) Quality of dietary protein during initiation and promotion of chemical carcinogensis in rats. pp. 151-162. In: Dietary protein; How they alleviate disease and promote better health. (Liepa, G. U. ed.) Am. Oil Chem. Soc. Champaign, Illinois,

16. Handy, N. (1992) The combined effect of dietary protein quality and energy restriction on chemical carcinogenesis and the conjugating enzyme system. Ph D. Dissertation, Texas Woman's University.

17. Rose, G., Blackbum, H., Keys, A., Taylor, H. L., Kannel, W. B., et al. (1974) Colon cancer and blood cholesterol. Lancet 1:181-183.

18. Willams, R. R., Sorlie, P. D., Feinleib, M., McNamara, P. M., Kannel, W. B., et al. (1981) Cancer incidence by levels of cholesterol. J. Am. Med.Assoc. 245:247-252.

19. Carroll, K. K. (1975) Experimental evidence of dietary factors and hormone-dependent cancers. Cancer Res. 35: 3375-3383.

20. Bani, I. A., Williams, C. M., Boulter, P. S. and Dickerson, J. W. T. (1986) Plasma lipids and prolactin in patients with breast cancer. Br. J. Cancer 54:439-446.

21. Potischman, N., McCulloch, C. E., Byers, T., Houghton, L., Nemoto, T., Graham, S. & Campbell, C. T. (1991) Associations between breast cancer, plasma triglycerides, and cholesterol. Nutr. Cancer 15: 205-215.

22. Malarkey, W. B., Schroeder, L. L., Stevens, V. C., James, A. G. and Lanese, R. R. (1977) Twenty-four-hour preoperative endocrine profiles in women with benign and malignant breast disease. Cancer Res. 37:4655-4659.

23. Miller, S. R., Tartter, P. I., Papatestas, A. E., Slater, G. and Aufses, A. H. (1981) Serum cholesterol and human colon cancer. J. Natl. Cancer Inst. 67:297-300.

24. Howson, C. P., Kinne, D. and Wynder, E. L. (1986) Body weight, serum cholesterol, and stage of primary breast cancer. Cancer 58:2372-2381.

25. Boyd, N. F., McGuire, V. (1990) Evidence of association between plasma high-density lipoprotein cholesterol and risk factors for breast cancer. J. Natl. Cancer Inst. 82:460-468.

26. Alladi, S, Gilbert, R. & Shanmugasundaram, K. R. (1989) Lipids, lipoproteins and lipolytic activity in plasma with dietary protein changes. Nutr. Rept. Int'l. 40:653-661.

27. Hems, G. (1978) The contributions of diet and child-bearing to breast-cancer rates. Br. J. Cancer 37:974-982.

28. Hems, G. (1980) Association between breast-cancer mortality rates, child-bearing and diet in the United Kingdom, Br. J. Cancer 41:429.

29. Hirayama, T. (1978) Epidemiology of breast cancer with special reference to the role of diet. Prev. Med. 7:173-195.

30. Phillips, R. L. (1975) Role of life-style and dietary habits in risk of cancer among Seventh-Day Adventists. Cancer Res. 35:3513-3522.

31. Gaskill, S. P., McGuire, W. L., Osbome, C. K. & Stem, M. P. (1979) Breast cancer mortality and diet in the United States. Cancer Res. 39:3628-3633.

32. De Ward, F. (1975) Breast cancer incidence and nutritional status with particular reference to body weight and height. Cancer Res. 35:3351-3356.

33. Toniolo, P., Riboli, E., Protta, F.,Charrel, M & Cappa, A. P. M. (1989) Calorie-providing nutrients and risk of breast cancer. J. Natl. Cancer Inst. 81:278-286.

34. Adlercreutz H.; Mousavi Y. and Hockerstedt, K. (1992) Diet and breast cancer. Acta. Oncol. 31:175-181.

35. Tannenbaum A. (1940) The initiation and growth of tumors. I. Effects of underfeeding. Am. J. Cancer 38:335-350.

36. Tannenbaum, A. (1942) The genesis and growth of tumors. II Effects of caloric restriction per se. Cancer Res. 2:460-467.

37. Tannenbaum, A. (1945) The dependence of tumor formation on the composition of the caloric restricted diet as well as on the degree of restriction. Cancer Res. 5:616-625.

38. Chen, R. F., Good, R. A., Engelman, R. W., Hamada, N., Tanaka, A., Nonoyama, M. & Day, N. K. (1991) Suppression of mouse mammary tumor proviral DNA and protooncogene expression: Association with nutritional regulation of mammary tumor development. Proc. Natl. Acad. Sci., USA. 87:2385-2389.

39. Tucker, M. J. (1979) The effect of long-term food restriction on tumors in rodents. Int. J. Cancer. 23:803-807.

40. Tannenbaum, A. (1942) The genesis and growth of tumors. III. Effects of a high fat diet. Cancer Res. 2:468-475.

41. Ross, M. H. & Bras, G. (1971) Lasting influence of early caloric restriction on prevalence of neoplasms in the rat. J. Natl. Cancer Inst. 47:1095-1113.

42. Lavik, P. S. & Baumann, C. A. (1943) Further studies on the tumor-promoting action of fat. Cancer Res. 3:749-756.

43. Kritchevsky. D., Weber, M. M. & Klurfeld, D. M. (1984) Dietary fat versus caloric content in initiation and promotion of 7,12-dimethlybenz(a)anthracene-induced mammary tumorigensis in rats. Cancer Res. 44:3174-3177.

44. Klurfeld, D. M., Welch, C. B., Lloyd, L. M. & Kritchevsky, D. (1989) Inhibition of DMBA-induced mammary tumorigenesis by caloric restriction in rat fed high fat diets. Int. J. Cancer 43:922-925.

45. Kritchevsky, D., Welch, C. B. & Klurfeld, D. M. (1989) Response of mammary tumors to caloric restriction for different time periods during the promotion phase. Nutr. Cancer 12:259-269.

46. Biossonneault, G. A., Elson, C. E. & Pariza, M. W. (1986) Net energy effects of dietary fat on chemically induced mammary carcinogenesis in F344 rats. J. Natl. Cancer Inst. 76:335-338.

47. Davidson, H. B. & Carroll, K. K. (1982) Inhibitory effect on a fat free diet on mammary carcinogenesis in rats. Nutr. Cancer 3:207-215.

48. Cohen, L. A., Rose, D. P.and Wynder, E. L. (1993) A Rationale for dietary intervention in postmenopausal breast canser patients: an update. Nutr. Cancer 19:1-10.

49. Tannenbaum, A. & Silverstone, H. (1949) The genesis and growth of tumors. IV. Effects of varying the proportion of protein (casein) in the diet. Cancer Res. 9:162-173

50. Ross, M. H. & Bras, G. (1973) Influence of protein under-and overnutrition on spontaneous tumor prevalence in the rat. J. Nutr. 103:944-963.

51. Shay, H., Gruenstein, M. & Shimkin, M. B. (1964) Effect of casein, lactalbumin, and ovalbumin on 3-methylcholanthrene-induced mammary carcinoma in rats. J. Natl. Cancer Inst. 33:243-253.

52. Clinton, S. K., Truex, C. R. & Visek, W. J. (1979) Dietary protein, aryl hydrocarbon hydroxylase and chemical carcinogenesis in rats. J. Nutr. 109:55-62.

53. Schulsinger, D. A., Root, M. M. and Campbell, T. C. (1989) Effect of dietary protein quality of development of alfatoxin B1-induced hepatic preneoplastic lesions. J. Natl. Cancer Inst. 81:1241-1245.

54. Lee, H. P., Gourley, L., Duffy, S. W., Esteve, J., Lee, J. & Day, N. E. (1991) Dietary effects on breast-cancer risk in Singapore. Lancet 337:1197-1200.

55. Barnes S., Grubbs, C., Setchell, K. D. R. & Carlson, J. (1990) Soybeans inhibit mammary tumors in models of breast cancer. In: Pariza, M. W., Aeschbacher, H., Felton, J. S., Sato, S. eds. (1990) Mutagens and Carcinogens in the Diet. New York, N. Y.: Wiley Liss; 239-253.

56. Hawrylewicz, E. J., Huang, H. H., & Blair, W. H. (1991) Dietary soybean isolate and methionine supplementation affect mammary tumor progression in rats. J. Nutr. 121:1693-1698.

57 Vahouny, G. V., Chalcarz, W., Satchithanandam, S., Adamson, I., Klurfeld, D. M. & Kritchevsky, D. (1984) Effect of soy protein and casein intake on intestinal absorption and lymphatic transport of cholesterol and oleic acid. Am. J. Clin. Nutr. 40:1156-1164.

58 Vahouny, G. V., Adamson, I., Chalcarz, W., Satchithanandam, S., Muesing, R., Klurfeld, D. M., Tepper, S. A., Sanghvi, A. & Kritchevsky, D.(1985) Effects of casein and soy protein on hepatic and serum lipids and lipoprotein lipid distributions in the rat. Atherosclerotis 56:127-137.

59. Radcliffe, J. D. (1989) A comparison of the effect of dietary casein and cottonseed isolate on serum and hepatic lipids in the rat. Nutr. Rept Int'l. 40:821-825.

60. Boyd, N. F., McGuire, V., Fishell, E. et al. (1989) Plasma lipids in premenopausal women with mammographic dysplasia. Br. J. Cancer 59:766-771.

61. American Institute of Nutrition (1977) Report of the AIN and hoc committee on standards for nutritional studies. J. Nutr. 107:1340-1348.

62. American Institute of Nutrition (1980) Second report of the AIN and hoc committee on standards for nutritional studies. J. Nutr. 110:1726.

63. National Research Council (1978) Nutrient Requirements of Laboratory Animals, No. 10, 3rd rev. ed., pp. 7-38. National Academy of Sciences, Washington, D. C.

64. Bucolo, G. & David, H (1973) Quantitative determination of serum triglycerides by the use of enzymes. Clin. Chem. 19:476.

65. Allain, C. A., Poon, L. S., Chan, C. S., Richmond, G.W. & Fu, P. C. (1974) Enzymatic determination of total serum cholesterol. Clin. Chem. 20:470.

66. Warnick, G. R. & Alberts, J. J. (1978) A comprehensive evaluation of the heparin-manganese precipitation procedure for estimating high density lipoprotein. J. Lipid Res. 19:65-76.

67. Yoo, J. S. H. & Hsueh, A. M. (1985) Amino acid(s) fortification of defatted glandless cottonseed flour; I. Lysine fortification. Nutr. Rept. Int'l. 31:147-155.

68. Krebs, H. A. (1964) The metabolic fate of amino acids. In: Mammalian Protein Metabolism. Munro, H. N., and Allison, J. B. (eds), Academic Press, New York and London, 1:5:125-173.

69. Sinha, D. K., Gebhard,R. L. & Pazik, J. E. (!988) Inhibition of mammary carcinogenesis in rats by dietary restriction. Cancer Letters 40:133-141.

70. Barner, C. A. (1993) Quality of dietary protein and dietary restriction during promotion on chemically-induced mammary carcinogenesis and serum lipids concentrations in rats. Ph D. dissertation, Texas Woman's University.

71. Dilman, V. M., Berstein, L. M., Ostroumova, M. N., Tsxrlina,Y. V. and Goluber, A. G. (1981) Peculiarities of hyperlipidaemia in tumor patients. Br. J. Cancer 43:637-643.

72. Barclay, M., Skipski, V. P. Terebus-kekish, O. Greene, E. M. Kaufman, R. J. and Stock, C. C. (1970) Effect of cancer upon high-density and other lipoproteins. Cancer Res. 30:2420-2430.

APPENDICES

APPENDIX A

Animal Care and User Committee Approval

APPROVED

No 1992-11 (For Office Use Only)

USE OF VERTEBRATE ANIMALS
TWU ANIMAL RESEARCH FACILITY

Project Title:
Relationship of protein-quality/dietary restriction to carcinogenesis, hepatic glutathione S-transferase activity, and serum lipids concentrations

Investigators/Instructors (Indicate Principal Investigator/Instructor with an asterisk)

Andie M. Hsueh* and Jean Rim

Department Nutrition and Food Sciences Phone Ext. 2636

Proposed Duration of Project: From 7/1/92 to 8/31/95

Funding Source or Proposed Funding Source Texas Food and Fiber Commission

Project Classification (check)
A. Grant Proposal (external source)____________
New Proposal or Pilot Project ____X____ Modification of Ongoing Grant ________
Competitive Renewal ____________ Grant Supplement ____________
Noncompetitive Continuation (indicate significant changes only) ____________
B. Local Research ____________ funding source ____________
C. Thesis/Dissertation Project ____________
D. Course ______

By whom was (will) peer review accomplished?
TWU Selection Committee of Texas Food & Fiber Commission Advisory Committee

Previously assigned Animal Project No. If application is other than a New Proposal or Pilot Project ____________

Date Received by ACUC: 5-18-92
Review Board Action : Date 6-1-92
Approved ✓ Approved Contingent ____ Disapproved _____ Returned for Revision ________
Remarks:

Additional Review Required? NO ✓
YES _____ Safety _______ Radiation ______ Biohazard ________
Signature of ACUC Representative Linda Uphouse

Date Received by Safety/Radiation/Biohazard Committee: ____________

Review Board Action: Date: ____________
Approved _____ Approved Contigent _____ Disapproved _____ Returned for Revision ________

Remarks:

Signature of Safety/Radiation/Biohazard Representative ____________

APPENDIX B

Proximate Analysis of Diets

POPE *Testing* LABORATORIES, Inc.
CONSULTING ANALYTICAL CHEMISTS
AND TESTING ENGINEERS

FOODS, FEEDS, DAIRY PRODS.
WATER, MISCL. ANALYSES
COTTON SEED PRODUCTS
PACKING HOUSE PRODUCTS

P. O. BOX 903
DALLAS, TEXAS 75221
AC 214 7-2-8491

OFFICIAL CHEMISTS
WEIGHERS AND INSPECTORS
NATL. COTTONSEED PRODUCTS ASS'N.
REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

March 19, 1992

Rec'd.: 3-13-92
P.O. No.: 41498-2-0015

Texas Woman's University
Dept. of Nutrition and Food Sciences
P.O. Box 24134
Denton, Texas 76204

Report of Tests on: Casein

Sample No.	Moisture	Protein (N x 6.38)	Fat (Acid Hydrol.)	Fiber	Ash	Carbo-hydrates
Casein #1	4.4%	91.3%	0.3%	0.1%	0.9%	3.0%
Casein #2	4.5	91.1	0.3	0.3	0.6	3.2

****

Respectfully submitted,

POPE TESTING LABORATORIES, INC.

Leon Hunter

Lab No. 28505-06 Incl.

POPE *Testing* LABORATORIES, Inc.

CONSULTING ANALYITICAL CHEMISTS
AND TESTING ENGINEERS

FOODS, FEEDS, DAIRY PRODS
WATER, MISCL. ANALYSES
COTTON SEED PRODUCTS
PACKING HOUSE PRODUCTS

P. O. BOX 903
DALLAS, TEXAS 75221
AC 214 742-8491

OFFICIAL CHEMISTS
WEIGHERS AND INSPECTORS
NATL. COTTONSEED PRODUCTS ASS'N.
REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

March 19, 1992

Rec'd.: 3-13-92
P.O. No.: 41498-2-0015

Texas Woman's University
Dept. of Nutrition and Food Sciences
P.O. Box 24134
Denton, Texas 76204

Report of Tests on: Diets (Wheat Gluten and Soy Protein Isolate)

	Sample No.	Moisture	Protein (N x 6.38)	Fat (Ether Extr.)	Fiber	Ash	Carbo-hydrates
SpI	#3	5.3%	88.8%	0.5%	0.3%	4.5%	0.6%
SpI	#4	5.1	89.0	0.4	0.2	4.0	1.3
WG	#5	7.9	79.8	0.8	0.3	1.0	10.2
WG	#6	8.0	80.4	0.7	0.4	0.9	9.6

****

Respectfully submitted,

POPE TESTING LABORATORIES, INC.

Leon Hunter

Leon Hunter

Lab No. 28501-04 Incl.

POPE *Testing* LABORATORIES, Inc.

CONSULTING ANALYITICAL CHEMISTS
AND TESTING ENGINEERS

P O BOX 903
DALLAS, TEXAS 75221
AC 214 742 8401

OFFICIAL CHEMISTS
WEIGHERS AND INSPECTORS
NATL COTTONSEED PRODUCTS ASS'N
REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

April 2, 1992

Rec'd.: 3-30-92
P.O. No.: 41498-2-0014

Texas Woman's University
Nutrition and Food Sciences Department
P. O. Box 24134
Denton, Texas 76204-2134

Report of Tests on: Diets

	Sample No.	Moisture	Protein	Fat	Fiber	Ash	Carbohydrates	Calories Per 100 Grams
	1	2.9%	18.0%	9.6%	2.3%	4.2%	63.0%	410
	2	4.2	19.9	10.1	2.6	4.4	58.8	406
CS	3	4.6	18.1	11.0	3.5	4.3	58.5	405
C-AL	4	3.1	18.0	9.8	1.8	2.7	64.6	419
WG	5	4.1	20.5	10.1	1.6	2.4	61.3	418
SPI	6	3.2	18.0	10.0	1.5	3.3	64.0	418
C-AL	7	3.2	18.0	9.9	1.8	2.4	64.7	420
WG	8	3.8	19.9	10.0	1.6	3.0	61.7	416
WG	9	3.0	18.2	9.8	2.1	2.7	64.2	418
SPI	10	3.3	17.9	10.1	2.3	3.2	63.2	415
C-AL	11	3.1	18.1	9.7	1.9	3.0	64.2	417

****

Respectfully submitted,

POPE TESTING LABORATORIES, INC.

Leon Hunter

Leon Hunter

Lab No. 29160-70 Incl.

POPE *Testing* LABORATORIES, Inc.
CONSULTING ANALYITICAL CHEMISTS
AND TESTING ENGINEERS

FOODS, FEEDS, DAIRY PRODS
WATER, MISCL. ANALYSES
COTTON SEED PRODUCTS
PACKING HOUSE PRODUCTS

P. O. BOX 903
DALLAS, TEXAS 75221
AC 214 742-8491

OFFICIAL CHEMISTS
WEIGHERS AND INSPECTORS
NATL. COTTONSEED PRODUCTS ASS'N.
REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

August 13, 1992

Rec'd.: 8-11-92
P.O. No.: 30910-2-0070

Texas Woman's University
Nutrition and Food Sciences Dept.
P.O. Box 24134
Denton, Texas 76204

Report of Tests on: Diets

	Sample No.	Moisture	Protein	Fat	Fiber	Ash	Carbo-hydrates	Calories Per 100 Grams
	#1	3.0%	17.8%	9.7%	2.6%	4.6%	62.3%	408
	#2	3.0	18.0	9.6	2.1	3.0	64.3	416
	#3	4.0	19.8	9.9	1.8	4.3	60.3	410
	#4	2.9	18.2	9.8	2.0	3.9	63.2	414
	#5	3.7	19.8	9.9	1.8	4.8	60.0	408
	#6	2.9	17.4	9.8	2.5	3.0	64.4	415
C-AL	#7	2.9	18.1	9.5	2.6	3.1	63.8	413
WG	#8	3.0	18.0	9.6	3.0	2.5	63.9	414

****

Respectfully submitted,

POPE TESTING LABORATORIES, INC.

Leon Hunter

Lab No. 35457-64 Incl.

POPE *Testing* LABORATORIES, Inc.

CONSULTING ANALYITICAL CHEMISTS
AND TESTING ENGINEERS

FOODS FEEDS DAIRY PRODS
WATER MISCL ANALYSES
COTTON SEED PRODUCTS
PACKING HOUSE PRODUCTS

P. O. BOX 903
DALLAS, TEXAS 75221
AC 214 742-8491

OFFICIAL CHEMISTS
WEIGHERS AND INSPECTORS
NATL. COTTONSEED PRODUCTS ASS'N.
REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

September 3, 1992

Rec'd.: 8-31-92
P.O. No.: 30910-2-0070

Texas Woman's University
Dept. of Nutrition & Food Sciences
P.O. Box 24134
Denton, Texas 76204

Report of Tests on: Diets

	Sample No.	Moisture	Protein	Nitrogen	Fat	Fiber	Ash	Carbo-hydrates	Calories Per 100 Grams
WG	1	3.7%	19.4%	3.10%	10.0%	2.4%	2.7%	61.8%	415
WG	2	3.5	19.6	3.14	9.9	1.7	2.7	62.6	418

****

Respectfully submitted,

POPE TESTING LABORATORIES, INC.

Leon Hunter

Lab No. 36190-91 Incl.

POPE *Testing* LABORATORIES, Inc.

CONSULTING ANALYITICAL CHEMISTS
AND TESTING ENGINEERS

FOODS, FEEDS, DAIRY PRODS.
WATER, MISCL. ANALYSES
COTTON SEED PRODUCTS
PACKING HOUSE PRODUCTS

P. O. BOX 903
DALLAS, TEXAS 75221
AC 214 742-8491

OFFICIAL CHEMISTS
WEIGHERS AND INSPECTORS
NATL. COTTONSEED PRODUCTS ASS'N.
REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

November 23, 1992

Rec'd.: 11-13-92
P.O. No.: 19-0381012
P-4344

Texas Woman's University
Nutrition and Food Sciences Department
Box 24134, TWU Station
Denton, Texas 76204

Report of Tests on: Diets

Sample No.	Moisture	Protein	Fat	Fiber	Ash	Carbo-hydrates	Calories Per 100 Grams
C-AL 1	3.3 %	17.8 %	9.5 %	2.2 %	2.1 %	65.1 %	417
C-AL 2	2.8	17.9	10.0	1.7	2.9	64.7	420

****

Respectfully submitted,

POPE TESTING LABORATORIES, INC.

Leon Hunter

Lab No. 39375-76 Incl.

POPE *Testing* LABORATORIES, Inc.

CONSULTING ANALYTICAL CHEMISTS
AND TESTING ENGINEERS

FOODS, FEEDS, DAIRY PRODS.
WATER, MISCL. ANALYSES
COTTON SEED PRODUCTS
PACKING HOUSE PRODUCTS

P. O. BOX 903
DALLAS, TEXAS 75221
AC 214 742-8491

OFFICIAL CHEMISTS
WEIGHERS AND INSPECTORS
NATL. COTTONSEED PRODUCTS ASS'N.
REFEREE CHEMISTS
AMERICAN OIL CHEMISTS SOCIETY

December 14, 1992

Rec'd.: 12-11-92
P.O. No. 19-0381012
P-5437
Dr. Andi Hsueh

Texas Woman's University
Nutrition and Food Sciences Department
Box 24134
Denton, Texas 76204

Report of Tests on: Diets

Number	Moisture	Protein	Nitrogen	Fat	Fiber	Ash	Carbo hydrates	Calories Per 100 Grams
C-AL 1	2.8 %	18.3 %	2.9 %	9.9 %	1.8 %	2.9 %	64.3 %	420
WG 2	3.3	19.5	3.1	10.1	2.5	2.7	61.9	417
WG 3	3.6	19.5	3.1	10.1	2.1	3.0	61.7	416
C-AL 4	3.1	18.0	2.9	9.6	2.3	2.8	64.2	415

****

Respectfully submitted,

POPE TESTING LABORATORIES, INC.

Leon Hunter

Lab No. 40305-08 Incl.

APPENDIX C

Histopathological Reports of Tumors Examined by the
Texas Veterinary Medical Diagnostic Laboratory, Bryan, Texas

Telephone
409/845-3414

Accession #: C93144209

TEXAS VETERINARY MEDICAL DIAGNOSTIC LABORATORY
Drawer 3040, College Station, Texas 77841-3040

FINAL REPORT - VETERINARIAN'S COPY

Date Shipped:
Date Received: 05/24/93

Vet. Acct. Number: 16238
Vet. Phone Number: (817) 898-2656

Owner Name:
Rim, Jean C.
Dept of Nutrition & Food Sc.

Veterinarian's Name:
TEXAS WOMAN'S UNIVERSITY
P.. BOX 22906
C. BARNER - #19-0381012
DENTON TX 76204-0906

Prelim Report Dates:
Telephone/Fax Dates:
Final Date: 05/26/93

ASSIGNMENTS:

CS =	TOX:	BAC:	CPT:	PAR:	HIS: F	SER:	VIR:	NEC:	THR:	RAB:
AM =	TOX:	BAC:	CPT:	PAR:	HIS:	SER:	VIR:	NEC:	THR:	RAB:

SPECIMENS SUBMITTED:
35 rat mammary tumors. /mb

TESTS REQUESTED:
histopath

SPECIES: Exotic
BREED: RAT
SEX: Unknown
AGE: Unknown
WT: Unknown

#ANIMALS IN GROUP:
#ANIMALS SICK:
#ANIMALS DEAD:
DATE OF DEATH LOSS:
ILLNESS DURATION: Unknown

CONCLUSION:
See lab results, coordinator's comments and/or conclusions at end of report.

COORDINATOR: Dr. Fiske

CHARGES

Necrop:		Bact:		Cl Path:		Parasit:	
Path:	$350.00	Serol:		Toxic:		Virol:	
Therio:		Ph/Fax:		Ctn Rtn:		Pick-up:	
Bus:		Other:				TOTAL:	$350.00

The fee for the Services of the Texas Veterinary Medical Diagnostic Laboratory are listed above. This charge doesn't include professional service fees by your Veterinarian or costs of preparing, packaging, and shipping of the specimens.

BILLED TO VETERINARIAN - You are advised to consult your Veterinarian for his analysis of this report and for any treatment that might be indicated.

Accession #: C93144209

*****HISTOPATHOLOGY REPORT**
5/26/93

05/25/93 70/35

CAL GROUP (10)	
CAL-1DA	Mammary adenocarcinoma
CAL-2DA	" "
CAL-3DA	" "
CAL-4DA	" "
CAL-5DA	" "
CAL-1DB	" "
CAL-2DB	" "
CAL-3DB	" "
CAL-4DB	" "
CAL-5DB	" "
CS-R GROUP (4)	
CS-R-2DA	Mammary adenocarcinoma
CS-R-3DA	" "
CS-R-1DB	" "
CS-R-4DB	" "
SPI-R GROUP (7)	
SPI-R-1DA	Mammary adenocarcinoma
SPI-R-3DA	" "
SPI-R-5DA	" "
SPI-R-1DB	" "
SPI-R-2DB	" "
SPI-R-3DB	" "
SPI-R-4DB	" "
W-R GROUP (6)	
W-R-1DA	Mammary adenocarcinoma
W-R-2DA	" "
W-R-3DA	" "
W-R-4DA	" "
W-R-5DA	" "
W-R-1DB	Mammary fibroadenoma
W-R-5DB	Mammary adenocarcinoma
C-R GROUP (8)	
C-R-1DA	Mammary adenocarcinoma
C-R-2DA	" "
C-R-3DA	" "
C-R-4DA	" "
C-R-1DB	" "
C-R-2DB	" "
C-R-4DB	" "
C-R-5DB	" "

COMMENT: All specimens except W-R-1DB were mammary adenocarcinomas. All of the adenocarcinomas fit the following general description with the only difference being the proportions of the components.

***HISTOPATHOLOGY (CONTINUED):

The tumors are nonencapsulated but well-demarcated. They are expansive rather than invasive. The consist of tubular, acinar, papillary, microcystic and cystic structures formed by large generally cuboidal epithelial cells averaging 1-3 cells show excessive nucleus:cytoplasm ratio and have hyperchromic nuclei with generally inconspicuous nucleoli. There is mild aniso-karyosis. Cells generally have good polarity though in some areas the pile upon one another. The areas between organized epithelial structures are filled with a stroma of solid sheets of similar cells. The tumor are vaguely subdivided into partial lobules by fine fibrovascular septa which are infiltrated with variable numbers of lymphocytes and plasma cells.
Specimen W-R-1DB is a mammary fibroadenoma which is well demarcated and noninvasive. It consists of clusters of small tubules formed by well-differentiated uboidal epithelial cells with approximately 1 mitotic figure per hpf. The tubular clusters are contained within an extensive stroma with moderate numbers of well-differentiated fibrocytes.

CONCLUSION: Mammary adenocarcinoma (34 specimens), mammary fibroadenoma (1 specimen).
--DR. FISKE/mm

APPENDIX D

Report on the Fasting Serum Amino Acid Concentrations from the Genetic Screening and Counseling Service in Denton, Texas

Genetic Screening & Counseling Service
3600 E. McKinney, Denton, Tx. 76201
817/383-3561

Client Twin Specimens
DOB Rat Sera
Lab No. 3-8-93

Serum Amino Acid Levels (um/ml)

Amino Acid	Rat #1	Value #2	#3	#4	#5
Taurine	0.45	0.44	0.39	0.35	0.38
Aspartic Acid	0.07	0.07	0.07	0.25	0.06
Threonine	0.88	0.65	0.57	0.60	0.80
Serine	0.50	0.48	0.46	0.49	0.47
Glutamic Acid	0.45	0.41	0.37	0.30	0.36
Glycine	0.35	0.37	0.37	0.34	0.33
Alanine	0.88	0.87	0.82	0.79	0.84
Valine	0.30	0.26	0.23	0.23	0.25
Cystine	ND	ND	ND	ND	ND
Methionine	0.07	0.07	0.07	0.06	0.07
Isoleucine	0.16	0.14	0.13	0.13	0.14
Leucine	0.26	0.23	0.20	0.21	0.22
Tyrosine	0.10	0.08	0.09	0.07	0.09
Phenylalanine	0.10	0.09	0.09	0.08	0.09
Ornithine	0.19	0.16	0.13	0.18	0.15
Lysine	0.90	0.78	0.69	0.75	0.76
Histidine	0.06	0.05	0.05	0.05	0.05
Arginine	0.22	0.24	0.25	0.16	0.21
Hydroxyproline	0.03	0.03	0.02	0.03	0.03
Proline	0.30	0.28	0.24	0.24	0.30
Citrulline	0.09	0.09	0.09	0.08	0.09
α aminobutyric acid	0.13	0.08	0.08	0.08	0.10
Tryptophan	0.04	0.05	0.05	0.06	0.06

ND: none detected
TR: trace

Comments:

Genetic Screening & Counseling Service

3600 E. McKinney, Denton, Tx. 76201
817/383-3561

Client: TWU Specimens

DOB: Rat Sera

Lab No.: 3-8-93

Serum Amino Acid Levels (um/ml)

Amino Acid	Rat #6	Value #7	#8	#9	#10
Taurine	0.44	0.40	0.41	0.41	0.49
Aspartic Acid	0.06	0.07	0.07	0.06	0.07
Threonine	0.64	0.66	0.54	0.60	0.71
Serine	0.47	0.50	0.46	0.49	0.49
Glutamic Acid	0.34	0.35	0.40	0.38	0.48
Glycine	0.32	0.34	0.38	0.37	0.36
Alanine	0.83	0.78	0.86	0.84	0.92
Valine	0.28	0.23	0.23	0.24	0.26
Cystine	ND	ND	ND	ND	ND
Methionine	0.08	0.07	0.08	0.07	0.07
Isoleucine	0.16	0.13	0.12	0.13	0.15
Leucine	0.25	0.21	0.21	0.21	0.24
Tyrosine	0.10	0.08	0.09	0.08	0.09
Phenylalanine	0.10	0.09	0.09	0.09	0.10
Ornithine	0.17	0.19	0.11	0.17	0.18
Lysine	0.75	0.76	0.65	0.77	0.83
Histidine	0.05	0.05	0.05	0.05	0.05
Arginine	0.23	0.19	0.27	0.19	0.22
Hydroxyproline	0.03	0.03	0.03	0.03	0.03
Proline	0.29	0.27	0.26	0.25	0.27
Citrulline	0.09	0.08	0.10	0.09	0.09
α amino butyric acid	0.13	0.09	0.05	0.07	0.11
Tryptophan	0.07	0.07	0.07	0.07	0.06

ND: none detected
TR: trace

Comments:

Genetic Screening &
Counseling Service
3600 E. McKinney, Denton, Tx. 76201
817/383-3561

Client Twu Specimens
DOB Rat Sera
Lab No. 3-8-93

Serum Amino Acid Levels (um/ml)

Amino Acid	Rat #11	Value #12	#13	#14	#15
Taurine	0.46	0.38	0.48	0.35	0.35
Aspartic Acid	0.07	0.07	0.08	0.07	0.06
Threonine	0.64	0.90	0.82	0.60	0.50
Serine	0.44	0.50	0.60	0.44	0.52
Glutamic Acid	0.43	0.32	0.38	0.29	0.29
Glycine	0.30	0.29	0.43	0.35	0.42
Alanine	0.72	0.77	0.87	0.69	0.71
Valine	0.25	0.24	0.24	0.22	0.19
Cystine	TR	TR	TR	TR	ND
Methionine	0.08	0.08	0.07	0.05	0.05
Isoleucine	0.15	0.12	0.13	0.12	0.11
Leucine	0.21	0.20	0.22	0.20	0.18
Tyrosine	0.09	0.07	0.08	0.07	0.06
Phenylalanine	0.09	0.08	0.09	0.08	0.07
Ornithine	0.11	0.12	0.19	0.22	0.13
Lysine	0.73	0.75	0.74	0.57	0.50
Histidine	0.04	0.05	0.05	0.05	0.05
Arginine	0.22	0.18	0.14	0.07	0.16
Hydroxyproline	0.02	0.03	0.03	0.03	0.03
Proline	0.25	0.33	0.27	0.23	0.23
Citrulline	0.07	0.09	0.09	0.10	0.10
α amino butyric acid	0.12	0.07	0.07	0.07	0.04
Tryptophan	0.06	0.06	0.05	0.05	0.04

ND: none detected
TR: trace

Comments: