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[1] This study was part of a Northeast Regional Project (NE-37, Relationships between protein and other selected nutrients and their metabolism and utilization); a cooperative study involving agricultural experiment stations in the Northeast Region and supported in part by Regional funds.

Contents

RELATIONSHIPS OF PROTEINS, ESSENTIAL FATTY ACIDS AND CHOLESTEROL IN THE RAT AND THE MOUSE[1]

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INTRODUCTION

Dietary protein, fat and linoleic acid, an essential fatty acid, affect cholesterol levels in the blood serum and in various tissues (1, 2, 3)[2] The intimate relationship of the protein tissue of the arterial walls and deposits containing cholesterol in atherosclerosis, the relationship of linoleic acid and cholesterol in the transportation and utilization of cholesterol, and damage to protein tissue in an essential fatty acid deficiency led to a thorough investigation of these dietary relationships and their effects on tissue composition.

The symptoms of an essential fatty acid (EFA) deficiency were first described by Burr and Burr (4). Among the more common signs of an EFA deficiency are lesions of the skin, retardation of growth and interference with normal reproduction. In rats the scaly paws and a ringed tail are very apparent when an EFA deficient diet is fed. At present there is no specific metabolic function known for EFA, such as the role of pyridoxal phosphate as a cofactor for transaminases. An individual relationship is indicated by the similarity of EFA deficiency symptoms to those of a pyridoxine deficiency, and the reduction of the severity of these two deficiencies by the administration of either one (5). An uncoupling of oxidative phosphorylation occurs in an EFA deficiency, thereby reducing the efficiency of utilization of energy from oxidized metabolites (6). Sinclair (7) explained the abnormal accumulation of phospholipids and cholesterol esters in the epidermis of rats on the basis of abnormal forms of these lipids which occur in the absence of adequate linoleic acid.

While basic information is necessary concerning the relationship of EFA and protein and their effects on the well-being of the animal, possibly an even more important role of EFA is in their relationship

1 This study was part of a Northeast Regional Project (NE-37, Relationships between protein and other selected nutrients and their metabolism and utilization); a cooperative study involving agricultural experiment stations in the Northeast Region and supported in part by Regional funds.

2 Literature cited, page 50.

to serum cholesterol levels in the human being. The question of the significance of serum cholesterol levels has been discussed so widely that articles on the subject frequently appear in popular periodicals such as the **Readers Digest** ,(November, 1964). Since recent scientific literature on cholesterol-fat and cholesterol-fatty acid relationships is voluminous, a reference to a statement made earlier (but which is still valid) on cholesterol by Bronte-Stewart (8) that "the hypothesis that ischemic heart disease results from a disturbance in fat metabolism is based more on assumption than fact," and a recent review by Kummerow (9) will suffice.

Although the basic cause for atherosclerosis has not been discovered and while the effect of high cholesterol levels *per se* on the development of atherosclerosis has not been proven, it is nevertheless important that the effect of diet on cholesterol levels be determined because of the widespread occurrence of cholesterol in various tissues, e. g., the brain of a 65 kg. man contains 25 grams of cholesterol. Cholesterol is the precursor for many other steroid compounds which are essential to the well-being of the animal (10) so that cholesterol levels should occur within normal homeostatic levels in tissues as well as in the blood serum. Interference with cholesterol synthesis may also interfere with the levels of compounds for which cholesterol is the precursor.

Recent studies on 164 Kenya baboons immediately after they had been captured revealed the presence of lipid deposits in the arteries of about three-fourths of the adult animals (11). In their native habitat these animals subsist chiefly on a vegetable diet with negligible amounts of animal fat and protein from birds' eggs, small rodents and insects. The lipid deposits in their arteries resemble similar deposits in human beings. The mean of serum cholesterol levels of 108 baboons was 78 milligrams per cent, which is very low compared to the average level for human beings. The aortic intimal lipid deposits cannot be attributed to the consumption of excessive animal fat or to hypercholesterolemia. It is quite likely that the baboon will be used more frequently for the experimental investigation of atherosclerosis. This work also stresses the fact that although high cholest-osclerosis. This work also stresses the fact that although high cholesterol levels are overemphasized to the extent that margarines and drugs despite a low serum cholesterol level. The general public may be deceived into buying these products under the assumption that they will lessen the possibility of atherosclerosis.

Stare and Jessop (12) showed that in 500 pairs of brothers, one having emigrated to Boston and one having stayed behind in Ireland, the coronary rate was higher in the brothers living in Boston although the Irish brothers ate more animal fat, including twice as much butter. Rutstein (13) noted that blood from people fed meals rich in polyunsaturated oils caused a deposition of a greater amount of fat in artery cells grown in tissue culture than did blood from these people when they were fed butter. It is obvious that fat, protein, or linoleic acid by themselves or in combinations are not the direct causative agents of atherosclerosis.

Extensive experiments were undertaken in this laboratory to search for effects of various types and levels of dietary protein and dietary fat, especially linoleic acid, on such parameters of well-being as growth, appearance, tissue moisture and lipid content, tissue and serum cholesterol levels and lipid iodine numbers. Inbred mice were utilized in initial experiments and inbred rats were used in later experiments.

PROCEDURES

Animals

All mice and rats were purchased from Carworth Farms, New City, Rockland County, New York. Mice were of the CF No. 1 strain and the rats were of the CFN strain, which was obtained originally from the Wistar strain. In all cases except those specifically indicated, weanling animals of a narrow weight range were purchased.

Housing

Except for the first mouse experiment, all animals were housed in a temperature and humidity controlled environment. The mice were kept in cages of ten in some experiments and in individual cages in later experiments. All rats were housed individually. The animals had access to food and water at all times.

Diets

The diets were mixed with a Hobart Mixer. The components are listed below:

Rat and Mouse Diets

0.3% DL-Methionine or L-Cystine
2% Vitamin Mix
4% H.M.W. Salt Mix
4% Alphacel
0.2% Choline Chloride
Protein
Fat
Sucrose as needed to make 100%

Vitamin Mix

One hundred grams of vitamin mix contained:

20 mg. Vitamin A Acetate
0.10 mg. Calciferol
1.0 gm. Inositol
0.1 gm. Menadione
1.5 gm. Para-Aminobenzoic Acid
200 mg. Niacin
50 mg. Riboflavin
20 mg. Pyridoxine Hydrochloride
30 mg. Thiamine Hydrochloride
200 mg. Calcium Pantothenate
1 mg. Biotin
2.5 mg. Folic Acid
0.2 mg. Vitamin B_{12}
250 mg. Alpha Tocopherol
Powdered Sucrose to 100%

For mice the following was added:

4.0 gm. Para-Aminobenzoic Acid
1.0 gm. Inositol
250 mg. Ascorbic Acid

The proteins studied were: Vitamin-free Casein (Nutritional Biochemicals Corp.) which contains 92.5% crude protein and a maximum of 0.01% fat according to the manufacturer; soybean protein (Drackett or Promine) which contains over 90% crude protein and less than 0.5% fat; *Torulopsis utilis* (Lake States Yeast Corp.), a high protein yeast which contains 55% protein and 7% fat; and pea beans which had been extracted with ether. The fats were: lard, corn oil, linoleic acid, and hydrogenated peanut oil. An animal protein and fat and a plant protein and oil were thus included. Linoleic acid, an EFA, is not strictly speaking a fat, but is the hydrolysis product of a fat, and is included in the category of "crude fat."

Tissues

The animals were anesthetized with ether when blood was taken by heart puncture and then killed by decapitation. Where blood was not taken, the animals were stunned and killed by decapitation. The tissues indicated in the tables were removed immediately and placed in small tared beakers which were covered with aluminum foil or plastic film and weighed as soon as possible on a Mettler B-6 balance. Drying to constant weight was done in a vacuum oven at 90-100° C. The length of time necessary for drying depended on the total amount of moisture to be removed from the oven contents. The dried tissues were ground in a Micro Model Wiley Mill and extracted with 2:2:1 chloroform, ethanol and ethyl ether in semimicro Soxhlet extractors. Six hours were found sufficient for the extraction of the crude fat from the tissue. The solvent was evaporated to a low level in the extraction flasks and drying was completed in the vacuum oven. The crude fat was removed from the extraction flask with enough chloroform, ethanol, ether solvent to make a 10 ml. final volume. Samples for the determination of iodine numbers or cholesterol were taken from this extract. Where data are reported for the carcass, these include all tissues not removed for the other analyses mentioned in the tables of data which follow later.

Blood Free Amino Acids

One of the preliminary approaches employed in the study of relationships between protein and EFA metabolism concerned the level of free amino acids in blood and liver of the mouse as influenced by dietary conditions. The method of Kit (14), employing Dowex 50 hydrogen ion exchange resin was used to purify the amino acids from a trichloracetic acid extract of the tissues. The amino acids were then chromatographed on Whatman No. 1 paper by the descending procedure employing a butanol-acetic acid-water (8:2:5) solvent system. Colors were developed on the paper strips with ninhydrin by the method of Underwood and Rockland (15). The amino acid spots were then cut out and eluted from the paper and the color density measured spectrophotometrically. Since no qualitative or consistent quantitative differences in free amino acids were observable under any of the dietary regimes employed, this study was abandoned.

Nitrogen Balance

The nitrogen content of the feed, feces and urine, as determined by microkjeldahl analysis (16), was used to study the effect of fat and protein on the nitrogen retention of mice. The growing mice all gained weight over the five day trial period and all were in positive balance. The mice receiving 20% casein and 5% corn oil retained twice as much nitrogen as those receiving only 20% casein. The mice receiving 8% casein and 5% corn oil retained one and a half times as much nitrogen as those receiving only 8% casein.

Growth

All groups in an experiment were started at the same weight unless otherwise indicated. Weanling rats and mice were purchased from Carworth Farms and the groups were started at the same average weight. The animals were weighed weekly. Either the final weight, or the weight gain can be used for comparison of the effect of the diet on growth since the starting weights were so similar.

Crude Fat

The crude fat was extracted as indicated in the section on tissues.

Moisture

The moisture was removed as indicated in the section on tissues.

Ash

The ash content was determined by heating dried, extracted tissue samples in the muffle furnace to constant weight at 600° C.

Iodine Number

The iodine numbers were determined by the method of Hanus as found in the Methods of Analysis, AOAC (16).

Cholesterol

There are many methods for the determination of serum and tissue cholesterol, and the results obtained depend on the method used and to some extent on the analyst using them. Dr. Betty Hawthorne of the School of Home Economics, Oregon State College, Corvallis, Oregon, has sent referee samples to many stations cooperating in Regional Project W-44 and to a number of stations cooperating in Regional Project NE-37. In a referee sample of lyophilized serum (Hyland Serum Lot No. 369 El) this laboratory found 193 mg. %. The manufacturer reported 195 mg. %, and the average of all twenty-seven laboratories participating was 180 mg. %. The range of values reported for the twenty-seven laboratories was 148-208. In another referee sample distributed by Dr. Hawthorne, Maine reported 134 mg. % with a range of 130-136. The average for twelve laboratories was 124 with the range of 109-150. The ranges were relatively narrow at all stations but one.

Thus, it can be seen that there is a considerable variation among laboratories analyzing the same sample. The importance of the analyses lies in the difference in cholesterol levels due to different treatments in the same laboratory.

The method used in the results reported here is the ferric chloride method originated by Zlatkis et al. (17), and modified by Rosenthal and Jud (18) for use with as little as 0.05 ml. serum. This method was checked against the adaptation of the Lieberman-Burchard reaction (19) used previously in this laboratory and by recovery samples which gave a good recovery of added cholesterol. The rats were not fasted prior to sacrifice. The methods used in this laboratory follow

Serum Cholesterol

One-tenth ml. of serum was pipetted into a graduated centrifuge tube and 5.5 ml. of 1:1 95% ethanol-ether was added. The mixture was shaken with an offset rubber stopper attached to a stirring motor. The mixture was centrifuged at 4750 rpm for 20 min. in a Lourdes Model AB centrifuge and the volume was adjusted to 5.00 ml. (If the volume was less than 5.00 ml., enough solvent was added to bring the volume up to 5.5 ml. and recentrifuging was necessary.) Triplicate 1.0 ml. aliquots of the supernatant were carefully transferred to 15 x 125 mm. test tubes and the solvent was evaporated in a boiling water bath. Two ml. of glacial acetic acid were added to each tube. One and five-tenths ml. of color reagent (the color reagent was prepared just before use in the following proportion: 8 ml. of stock iron reagent [2.5 g. $FeCl_3 \bullet 6H_2O$ in 100 ml. 85% H_3PO_4] and sufficient concentrated H_2SO_4 to bring the volume up to 100 ml.) were added to each tube, and thorough mixing followed. The tubes were allowed to stand at room temperature for one half hour. The reagent blanks prepared from 2.0 ml. glacial acetic acid and 1.5 ml. color reagent were treated in a like manner. Standards were prepared from a solution containing 0.500 mg. cholesterol per ml. Standards of 0.050, 0.100 and 0.300 ml. were prepared in the same way as the samples. The optical density was read in a Beckman DU spectrophotometer at 560 millimicrons. With the volumes used, the mg. per cent serum cholesterol was calculated according to the following equation:

$$\text{Mg. \% serum cholesterol} = \text{average O. D.} \times \frac{5000}{\text{O.D. of standard per mg. of cholesterol}}$$

Extreme caution was required in making all measurements, and each analyst who used this method for serum or tissue cholesterols had to standardize his manipulations carefully.

Tissue Cholesterols

The lipid was extracted from the tissue in semimicro Soxhlet extractors and the solvent removal was completed in a vacuum oven. After weighing the lipid was removed from the flasks and diluted to 10.0 ml. The following aliquots were appropriate: liver 0.04 ml., heart 0.20 ml., brain 0.025 ml. and testes 0.10 ml. The aliquots, in duplicate or triplicate, were evaporated to dryness on the boiling water bath and the procedure used for the determination of serum cholesterol was followed. The results were calculated to a milligram per gram of lipid and a per gram of tissue basis.

RESULTS

The results for the many dietary groups follow. Tables are used whenever possible to present the data. The initial experiments utilizing mice are presented first.

Table I shows that the lack of fat in the diet for mice 1) lowers the adult weight, 2) leads to the formation of an enlarged fatty liver, 3) increases the degree of saturation of liver fat and 4) has a more deleterious effect than when the diet supplies a much lower percentage of protein but includes fat. A five-week supplement of 1 mg. per day of linoleic acid resulted in 1) a definite weight gain, 2) less fat in the livers when 8% casein was fed and 3) an improvement in the appearance of the mice. The percentage of fat plus the percentage of moisture in the liver gave a relatively constant value (75% to 79%).

TABLE I

Response of mice to casein and corn oil, after 11 weeks and 16 weeks

Diet	No. of Mice	Body Weight gm.	Liver Weight gm.	% Body Weight as Liver	% H_2O in Liver	% Fat in Liver Wet Wt.	% Ash from Liver Wet Wt.	% Protein in Liver Wet Wt.	Liver Fat Iodine No.
11 Weeks									
3% casein	5	20.3±1.4[1]	1.87±.03	9.3±.4	63± .7	15.5±1.0	1.0±.02	17.7± .5	69[2]
3% casein + 5% corn oil	4	28.9± .9	1.42±.24	4.9±.3	73±1.2	3.8± .5	1.2±.2	20.2±1.6	113
[illegible]% casein	5	25.3±1.2	2.04±.17	8.1±.6	64± .9	14.7±1.6	1.0±.04	19.2± .9	78
[illegible]% casein + 5% corn oil	4	34.9±1.1	1.57±.10	4.5±.3	70±1.5	4.8± .5	1.21±.04	23.5±1.0	118
5 Additional Weeks									
3% casein	4	20.5± .9	1.78±.05	8.7±.4	57±1.3	19.9±1.8		19±.8	68
3% casein + 1 mg/day linoleic acid	4	23.8±1.1	1.58±.02	6.7±.3	66± .9	9.7±1.2		20±.3	77
3% casein + 5% corn oil	4	30.0±1.2	1.21±.06	4.1±.2	69± .02	2.5± .2		22±.2	112
[illegible]% casein + 1 mg/day linoleic acid	5	29.0± .5	2.16±.03	7.5±.2	59±1.3	16.6±1.2		20±.4	73

[1] Standard error of the mean.
[2] Iodine number of combined liver fat from all animals in the group.

Effect of a Fat Free Diet on Reproduction of Mice

Twelve females and five males were used for breeding while on a 20% casein and no-fat diet. Seven of the females had offspring, two became pregnant and resorbed, and three apparently did not become pregnant. Thirty-seven of the young survived for five weeks on the fat-free diets. In the following seven weeks, 11 of 12 of these young died on this fat-free diet. Those offspring which were given 5% corn oil at the 8 and 20% casein levels for the seven weeks survived, but did not gain as much weight as the offspring of well-fed mothers.

Effect of Protein and Fat Combinations on Mice

Table II shows the effect of soybean protein in the diet for 12 weeks. The mice fed soybean protein gained about the same amount of weight whether they received corn oil, linoleic acid or no fat, while the mice gained less when fat was excluded from a diet supplying protein in the form of casein. Higher iodine numbers were observed in liver fat and carcass fat of animals receiving corn oil. Liver fat was increased when no fat, 0.5% stearic acid or 0.05% linoleic acid was fed and the feeding of 0.5% linoleic acid lowered this value somewhat, but not to that which occurred in the mice fed corn oil. Liver cholesterol values are presented in two ways: 1) as mg. cholesterol per gram of liver fat and 2) as mg. of cholesterol per gram of liver. This has been done to detect any correlation with the level of fat present as well as with the level of tissue. It should be noted that the ranking of the weights of cholesterol per gram of liver does not always parallel the ranking of the cholesterol in the liver fat due to the different levels of fat in the liver. It will be noted also that the highest liver cholesterol levels occur where no fat was fed, and where stearic acid, a saturated fatty acid, was fed. The unsaturated fatty acids are necessary for the transportation of cholesterol from the liver where it is synthesized from simple metabolites such as acetate (20, 21).

TABLE II

Response of mice fed various protein and fat combinations for 12 weeks

Diet	Mouse Wt., gm.	Liver Wt., gm.	% Body Wt. as Liver	% H_2O in Liver	% Fat in Liver, Dry Wt.	% Fat in Liver, Wet Wt.	Liver Fat Iodine No.	Carcass Fat Iodine No.	% H_2O in Carcass	% Fat in Carcass Dry Wt.	% Fat in Carcass Wet Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)
20% Soybean Protein +													
5% Corn Oil	32±1.1[1,2]	1.9±.1	5.9±.1	68±1	20±1	6.2±.2	85±5	70±2	64±1	40±2	14.0±1	75±16	14±2
No Fat	33±.8	2.2±.1	6.9±.2	66±1	30±1	10.0±.6	70±3	56±4	62±1	43±3	17.0±2	63±4	19±1
8% Soybean Protein +													
5% Corn Oil	23±1	1.1±.1	4.8±.1	70±1	20±1	6.0±12	123±6	73±4	59±1	48±2	20.0±1	74±5	14±1
0.5% Linoleic Acid	23±1	1.3±.1	5.8±.1	69±1	26±2	8.2±.7	71±6	65±1	62±1	41±2	16.0±1	107±15	30±5
0.05% Linoleic Acid	24±1	1.6±.1	6.7±.1	64±1	38±1	14.0±1	66±3	65±2	61±1	44±1	17.0±1	76±4	30±2
0.5% Stearic Acid	24±1	1.6±1	6.7±.3	63±1	37±2	14.0±1	61±4	64±2	63±1	40±3	15.0±1	95±19	35±8
No Fat	22±1	1.4±.1	6.5±.2	65±1	38±2	13.0±1	76±4	63±1	63±1	39±1	14.0±1	89±7	35±4
8% Casein +													
5% Corn Oil	29±1	1.5±.2	5.0±.2	69±1	18±1	5.7±.3	97±11	75±2	62±2	38±5	15.0±2	77±9	13±2
0.5% Linoleic Acid	24±1	1.3±.1	5.5±.1	66±1	29±1	9.6±.5	64±4	68±3	61±1	41±2	16.0±1	56±14	16±4
No Fat	19±1	1.6±.1	8.2±.2	62±1	42±2	16.0±1	64±5	63±3	66±1	25±3	8.7±1	131±20	49±5

1 Standard error of the mean.

2. **Eight mice per group.**

Effect of Different Proteins and Fats on Rats

The following data were all collected on rats, which, due to size, were considered more desirable experimental animals.

Table III shows that the response of rats to various dietary treatments was similar to that of mice receiving analagous dietary treatments. Less liver fat and less liver cholesterol were formed when corn oil or methyl linoleate was fed than when fat was omitted from the diet. The iodine numbers of the liver fat were higher with linoleic acid being fed and still higher when corn oil was fed. The iodine numbers for the carcass fat were also in this order. It is noteworthy that the rats receiving 8% dietary casein with no dietary fat had a higher carcass fat level on a dry weight basis than those fed methyl linoleate with 8% casein ($P < O. 10$).

TABLE III

Response of rats fed various protein and fat combinations for 45 days

Diet	Rat Wt., gm.	Liver Wt., gm.	% Body Wt. as Liver	% H_2O in Liver	% Fat in Liver, Dry Wt.	% Fat in Liver, Wet Wt.	Liver Fat Iodine No.	Carcass Fat Iodine No.	% H_2O in Carcass	% Fat in Carcass, Dry Wt.	% Fat in Carcass, Wet Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)
20% Casein +													
5% Corn Oil	266±10[1,2]	11.3±.4	4.3±.6	66±1	17.5±1	5.9±.3	75±2	63±3	59±1	50±4	21±2	42±5	6.9±.3
0.5% Methyl Linoleate	253±5	11.0±.3	4.3±.1	66±1	20.0±1	6.7±.2	67±1	51±2	60±1	51±2	20±1	39±1	7.8±.2
No Fat	228±7	10.7±.4	4.6±.1	66±1	22.5±1	7.7±.4	62±1	47±1	61±1	49±2	19±1	45±4	10.1±1.1
8% Casein +													
5% Corn Oil	181±8	8.2±.2	4.6±.2	66±1	19.0±1	6.4±.5	78±3	61±2	60±0.1	48±3	19±1	42±3	7.6±.5
0.5% Methyl Linoleate	136±11	5.8±.3	4.3±.2	69±1	18.0±1	5.7±.2	76±1	54±1	62±2	42±3	16±1	49±3	8.8±.6
No Fat	142±10	6.6±.3	4.7±.1	65±1	29.0±3	10.0±1.2	64±2	48±2	62±1	50±3	19±1	48±5	14.2±2.7
8% Soybean Protein +													
5% Corn Oil	78±5	3.5±.2	4.4±.1	69±1	17.5±1	5.5±.3	85±3	67±2	64±1	40±2	14±1	46±3	7.8±.4
No Fat	75±7	3.3±.1	4.4±.1	69±1	20.0±2	6.1±.5	64±1	54±2	66±1	32±1	11±1	-44±3	8.9±.7

1. Standard error of the mean.
2. Six rats per group.

Peanut oil, hydrogenated to an iodine value of less than one, was used as a saturated triglyceride to determine the effect of a triglyceride *per se* on the fat and cholesterol percentages. While triglycerides with a high melting point are not efficiently digested and absorbed, some small differences between the no-fat diet and the hydrogenated peanut oil diet can be noted (Table IV). It can also be seen that the level of protein fed affects the liver fat and cholesterol data. At the 20% casein level the liver fat level was lower and the liver cholesterol level per gram of liver fat was higher when hydrogenated peanut oil was fed as compared to no fat. At the 8% casein level these effects were reversed. At the 8% casein level a mixture of 3% hydrogenated peanut oil and 2% corn oil produced growth superior to that obtained with 5% corn oil and the growth achieved with 4.5% hydrogenated fat plus 0.5% methyl linoleate equaled that obtained with 5% corn oil. While some utilization of the hydrogenated peanut oil probably occurred, the effects were not great.

TABLE IV

Effect of completely hydrogenated vegetable oils for seven weeks

Diet	Rat Wt., gm.	Liver Wt., gm.	% Body Wt. as Liver	% H_2O in Liver	% Fat in Liver, Dry Wt.	% Fat in Liver, Wet Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)
20% Casein +								
5% Corn Oil	295±9 [1,2]	13.1±.6	4.4±.1	66±.3	15.8±.4	5.4±.2	24.1±1.2	3.79±.16
3% Hyd. Peanut Oil + 2% Corn Oil	279±11	12.2±.6	4.3±.1	66±.2	16.0±.7	5.5±.3	28.1±1.4	**4.41±.13**
0.5% Me. Linoleate + 4.5% Hyd. Peanut Oil	268±10	11.6±.3	4.4±.2	66±.5	18.6±.8	6.3±.2	26.4±1.0	4.79±.19
5% Hyd. Peanut Oil	243±10	11.6±.7	4.7±.2	67±.5	19.9±1.0	6.7±.3	26.9±1.2	5.33±.19
No Fat	232±12	10.2±.5	4.4±.1	67±.6	24.1±1.4	7.9±.4	21.5±1.4	5.19±.50
8% Casein +								
5% Corn Oil	178±9	7.7±.4	4.4±.2	69±.6	**21.9±1.5**	6.7±.6	28.9±1.5	6.23±.22
3% Hyd Peanut Oil + 2% Corn Oil	206±9	**8.5±.4**	4.2±.1	69±.4	20.8±.9	6.4±.3	30.7±1.5	6.34±.20
0.5% Me. Linoleate + 4.5% Hyd. Peanut Oil	178±10	7.3±.5	4.1±.2	70±.3	20.7±.8	6.3±.3	30.3±1.7	6.24±.11
5% Hyd. Peanut Oil	163±8	7.2±.2	4.5±.2	68±.5	31.2±1.8	9.8±.7	20.7±1.1	6.37±.11
No Fat	160±8	6.9±.4	4.3±.2	69±.3	25.5±1.0	8.0±.4	32.4±1.4	8.29±.44

1. Standard error of the mean.
2. Six rats per group.

Since atherosclerosis is so frequently found in coronary arteries, information concerning heart weight, moisture, lipid and cholesterol content was collected. These data are included in Table V. With the soybean protein, lard and methyl linoleate combinations used, the fat content of the liver was highest on a low protein, no-fat diet and lowest on the 40% protein, no-fat diet. In general the heart fat was relatively constant. However, there was some indication that the heart fat level was slightly lower in animals receiving the high protein levels and no dietary fat. The liver cholesterol content was higher when 20 and 40% soybean protein was fed with methyl linoleate. In the case of the 8% soybean protein level the no-fat groups gave a slightly higher value. Heart tissue cholesterol levels were generally lower on a no-fat diet. The heart cholesterol level of the group fed 8% protein was higher than the levels of the groups fed 20% and 40% protein.

The level of methyl linoleate fed was quite high considering that the methyl alcohol released by the hydrolysis of this ester is toxic at high enough levels. The lower weights of the groups fed the 6% methyl linoleate with 20% and 8% soybean protein compared to those fed no fat show that growth was impaired by the high level of methyl linoleate. The rats fed 40% protein with the 6% methyl linoleate gained the same amount of weight as those fed no fat.

TABLE V

Effect of different levels of soybean protein, lard and methyl linoleate on the rat (8½ weeks)

Diet	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	% H_2O in Liver	% H_2O in Heart	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)
40% Soybean Protein +											
15% Lard	324±2.3 [1,2]	12.2±.30	.98±.025	64±.2	77±.3	15.7±.41	16.6±.95	74.0±4.3	11.5±.45	75.5±4.1	12.5±.30
6% Me. Lin. [3]	308±17.1	13.3±.49	.96±.069	64±.2	76±.3	16.3±.62	16.3±.15	77.0±4.1	12.5±.21	66.0±4.6	10.7±.72
No Fat	305±6.1	12.8±.50	.97±.014	66±.9	77±.02	14.4±.83	13.3±1.01	75.5±3.4	10.6±.28	68.0±4.6	8.8±.29
20% Soybean Protein +											
15% Lard	290±14.5	10.8±.49	.88±.019	65±.6	76±.3	15.1±.75	17.7±1.21	74.5±1.4	11.2±.50	73.5±4.4	12.8±.27
6% Me. Lin.	251±18.2	9.4±.54	.83±.060	66±1.2	76±.2	16.2±1.43	16.2±.60	83.0±4.2	13.5±.61	72.0±3.0	11.6±.33
No Fat	307±11.0	11.9±.64	.86±.020	66±.5	77±.2	16.4±.92	15.5±.86	63.5±4.0	9.8±.37	63.0±1.9	9.7±.29
8% Soybean Protein +											
15% Lard	100±2.9	4.4±.14	.43±.011	67±.4	77±.3	18.7±1.00	17.5±.81	64.0±2.6	11.6±.59	69.0±3.7	12.8±.64
6% Me. Lin.	65±9.2	2.8±.42	.28±.030	69±.5	77±.3	18.5±1.12	16.0±.95	76.0±6.0	14.5±1.21	82.0±6.9	13.1±.65
No Fat	115±8.2	4.9±.12	.49±.015	68±.4	78±.5	21.9±1.3	16.9±.93	68.5±4.5	14.9±.90	68.0±2.8	11.4±.52

1. Standard error of the mean.
2. Six rats per group.
3. Me. Lin = Methyl Linoleate.

Table VI includes serum cholesterol ratios where the no-fat group was 1.00 in addition to the other data. In this experiment the effect of the addition of fat to the diet was determined. The rats did not gain as well when 6% methyl linoleate was fed with 8% protein. Table V shows this also, but the gain on 8% casein and 6% methyl linoleate was much greater than on the 8% soybean protein plus the 6% methyl linoleate. The rats were able to grow much more on the 2% methyl linoleate. Liver fat was highest in the rats receiving no fat over the entire period while heart fat was lowest in this group. The liver cholesterol content was highest in rats receiving no fat while these rats had the lowest heart cholesterol content indicating that the transportation of cholesterol from the liver is impeded by the lack of fat in the diet. Methyl linoleate apparently aided this transport. No advantage of corn oil over lard in yielding lower cholesterol concentrations in the liver, heart or serum was found.

TABLE VI

Effect of 8% casein no-fat diets for six weeks followed by 10 weeks 3 days of other diets

Second Diet	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	% H_2O in Liver	% H_2O in Heart	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Ratio of Serum Cholesterol Levels[5]
8% Casein + 15% Lard	363±33[1,2]	10.9±.60	1.19±.05	65±.3	77±.5	19.1±.87	17.7±.95	71±3.5	13.4±.32	93±6.1	15.9±.26	**2.05±.07**
15% C. O.[3]	354±24	11.5±.74	1.18±.09	66±.5	77±.3	17.3±.72	18.6±.86	78±2.9	13.5±.54	102±6.8	18.4±1.4	**2.07±.13**
6% Me. Lin.[4]	267±3	10.0±.41	.93±.02	67±.5	77±.3	15.1±.46	18.1±1.9	99±2.0	14.8±.29	95±6.9	16.7±1.4	1.67±.11
5% C. O.	352±16	12.0±.87	1.04±.06	65±.4	76±.3	15.5±.96	17.8±.62	90±5.4	13.4±.48	82±4.3	14.3±.35	1.65±.07
2% Me. Lin.	336±24	11.2±.70	.98±.04	67±.3	76±.7	17.4±.79	16.7±.64	74±6.1	12.5±.73	64±.8	10.5±.19	1.44±.05
No Fat	274±5	9.8±.35	1.00±.03	65±.9	77±.2	30.2±2.5	15.7±.40	58±2.8	16.6±1.0	65±1.8	10.1±.10	1.00±.07

1. Standard error of the mean.
2. Six rats per group
3. C. O. = Corn Oil
4. Me. Lin. = Methyl Linoleate
5. Ratios of serum cholesterol levels to that of the group fed no fat as 1.00.

Table VII shows the effects of feeding the food yeast, *Torulopsis utilis*, to rats. Torula yeast, which contains 55% protein, is an excellent but relatively undeveloped source of protein for animals. Fatty livers did not result when yeast was used as a source of protein. This may have been due to the presence of residual fat (7%) or to this type of protein. The liver cholesterol values are quite similar for all groups while the heart cholesterol levels were higher for the rats receiving 16% yeast. The 16% yeast--5% corn oil diet resulted in the highest heart cholesterol levels. Linoleic acid did cause lower serum cholesterol levels. The extraction of the dietary yeast with a fat solvent resulted in a lower serum cholesterol value, but other cholesterol levels were close to those of rats fed unextracted yeast at the 16% dietary level.

TABLE VII

Effect of the feeding of the yeast *Torulopsis utilis* to rats for 15 weeks

Diet	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	% H_2O in Liver	% H_2O in Heart	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. % Serum Cholesterol
40% Torula Yeast +												
5% Corn Oil	409±11[1,2]	15.2±.56	1.29±.09	67±.4	77±.4	15.1±.62	15.6±1.2	94±5.4	13.9±.6	69±7.5	11.6±.8	121±5.3
1% Linoleic Acid	356±16	13.0±.47	1.10±.06	68±.3	77±.3	14.1±.54	16.9±.04	86±2.5	12.4±.3	59±2.7	9.9±.4	81±3.6
No Fat	382±24	12.0±1.5	1.17±.04	69±.6	77±.3	15.3±1.10	15.1±.6	90±5.6	13.3±.9	61±.9	9.1±.7	91±6.0
16% Torula Yeast +												
5% Corn Oil	239±12	9.7±.43	.88±.05	68±.4	78±.1	16.1±.8	15.3±.07	75±4.0	12.1±.6	108±13	16.3±1.7	108±7.1
1% Linoleic Acid	207±5	9.2±.24	.79±.04	68±.3	77±.4	15.2±.8	19.2±1.2	78±7.1	11.4±.6	64±3.5	13.4±1.5	96±2.4
No Fat	222±16	9.6±.81	.78±.03	69±.4	78±.3	14.9±1.01	16.5±.09	82±3.4	12.0±.8	88±13	14.2±1.8	114±2.1
16% Extracted Torula Yeast	230±19	8.7±.91	.88±.07	70±.3	77±.2	15.3±.68	16.3±.08	84±6.0	12.9±1.2	68±6.3	13.0±2.4	84±6.5

1. Standard error of the mean.
2. Six rats per group.

Table VIII presents the data from some protein and lipid dietary combinations. Also, extracted soybean protein (with hot ethanol and ether) and unextracted soybean protein were compared. The extraction did not change any response appreciably. The results noted in previous tables on fatty livers and liver and heart cholesterol were observed in these animals, also. It is worthy of note that the serum cholesterol values were highest when corn oil was fed with either level of casein and with 8% soybean protein. However, with 20% soybean protein a lower serum cholesterol level was found with corn oil rather than with no dietary fat. The weights of the rats fed 8% casein were greater than those fed 8% soybean protein, while the weights resulting from the 20% soybean protein diets were greater than those resulting from 20% casein.

TABLE VIII

Effect of the feeding of different protein and fat combinations to rats for 13 weeks

Diet	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	% H_2O in Liver	% H_2O in Heart	% Fat in Liver Dry Wt.	% Fat in Heart Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. % Serum Cholesterol
8% Casein +												
5% Corn Oil	293±18[1,2]	11.3±.66	1.16±.03	69±.2	76±.5	21.0±2.3	20.1±.5	58±4.5	11.3±.18	41±1.1	8.5±.18	121±6
1% Linoleic Acid	287±15	10.3±.80	1.17±.03	68±.5	77±.6	24.2±2.1	17.1±1.2	54±3.9	13.7±.57	46±5.6	7.6±.17	93±5
No Fat	235±12	10.3±.42	1.14±.10	66±.2	77±.5	31.5±3.1	18.1±2.3	48±2.6	15.0±1.00	48±3.7	7.1±.25	72±3.5
8% Soybean Protein +												
5% Corn Oil	165±13	7.4±.58	.81±.04	69±.7	76±.4	17.2±.7	22.9±2.0	79±1.7	14.0±.54	46±6.9	10.0±.39	139±7
1% Linoleic Acid	202±16	7.9±.57	1.02±.06	69±.7	75±.7	15.4±1.4	25.9±2.1	72±7.8	13.0±.89	50±9.7	8.7±.58	95±5
No Fat	182±12	7.1±.59	.88±.06	68±.6	78±.4	22.6±.7	16.8±1.6	59±0.9	13.5±.43	58±6.4	8.2±.38	93±7
20% Casein +												
5% Corn Oil	411±12	12.7±.58	1.32±.05	67±.7	76±.4	15.5±.7	15.8±.8	82±3.3	12.6±.39	66±3.6	9.7±.38	119±5
No Fat	340±4	13.0±.46	1.21±.07	68±.9	77±.4	20.8±1.5	16.9±1.2	71±2.7	13.5±.41	56±6.1	7.5±.55	100±5
20% Soybean Protein +												
Corn Oil	422±14	14.6±.57	1.42±.08	69±1.1	78±.3	14.1±.8	13.4±.6	79±3.9	11.3±.24	62±4.8	8.4±.35	107±6
No Fat	409±12	14.6±.36	1.32±.05	68±.3	78±.4	20.0±.9	15.0±.6	57±4.0	11.2±.45	60±5.4	9.3±.85	127±3
20% Extracted Soybean Protein (15 Wks)	389±13	13.7±.36	1.32±.09	67±.3	77±.4	18.1±.4	17.8±2.2	67±2.9	12.1±.6	63±7.9	10.5±.7	121 ±7

1. Standard error of the mean.
2. Six rats in each group.

Table IX presents the results of pea bean diets on rats. Weanling rats lost weight and those rats on a previous diet of 8% casein and no fat also lost weight. The large amounts of gas found in the digestive tracts of the rats receiving the pea bean diet suggest that poor food consumption and digestion occurred. This probably accounts for the observed weight losses. Corn oil resulted in lower liver cholesterol levels but higher serum cholesterol levels.

TABLE IX

Effect of feeding extracted pea beans to rats

Diet	Group	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	% H_2O in Liver	% H_2O in Heart	% Fat in Liver, Dry Wt.	% Fat in Heart Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. % Serum Cholesterol
3 Weeks Plus 3 Days - Groups 1 and 2 lost 10 grams											
8% Pea Bean Protein + 5% Corn Oil	1	42.3±4.8[1,2]	1.89±.27		72.5±1.1		19.6±1.3		85±2	16±1	105±2
8% Pea Bean Protein No Fat	2	42.2±1.3	2.13±.17		69.0±.8		25.5±3.4		64±4	19±1	84±5
12 Weeks on 8% Casein Fat Free Diet - 6 Weeks on Pea Bean Diet											
8% Pea Bean Protein + 5% Corn Oil	3	156±7	5.24±.38	.63±.03	71.0±.5	78±.4	16.2±1.9	16.1±1.2			81±6
8% Pea Bean Protein No Fat		140±3	4.37±.14	.55±.01	72.0±.02	78±.02	17.8±1.2	15.4±.8			54±3

1. Standard error of the mean
2. Six rats in each group.

Table X shows the effects of adding an animal fat to the diet after a long period on an animal protein (casein) and a plant protein (soybean protein). The addition of 5% lard to the soybean protein diet for nearly nine weeks caused an insignificant rise in serum cholesterol levels, and an increase in heart fat. The addition of 5% lard to the 20% casein diet caused an increase in serum cholesterol, an increase in heart fat, a decrease in liver fat and a considerable increase in weight. The change from 20% soybean protein to 8% casein caused a drastic lowering of the serum cholesterol level, a liver which contained twice as much fat and more total cholesterol and a heart which contained more fat and cholesterol. Apparently cholesterol is not transported readily from the liver when casein is fed in the absence of dietary fat. The residual unextractable lipid which is contained in the soybean protein in small amounts may be enough to prevent the fatty livers.

TABLE X

Effect of the addition of dietary fat and a change in proteins on rats

Diet	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	% H_2O in Liver	% H_2O in Heart	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. % Serum Cholesterol
20% Soybean Protein No Fat - 24 Wks	405±19[1,2]	14.6±1.07	1.15±.01	71±.8	78±1.2	18.9±.88	15.4±.55	61±2.4	12.3±.69	67±4.7	10.3±.70	120±9.9
20% Casein - No Fat 24 Weeks	316±13	11.0±.16	.99±.01	68±.6	77±.6	30.3±2.17	16.0±.48	60±6.5	17.6±1.29	60±1.9	9.8±.29	71±6.6
20% Soybean Protein 24 Weeks, then 20% Soybean Protein + 5% Lard 8 Weeks, 5 Days	443±15	14.7±1.22	1.24±.09	68±.3	77±.3	19.8±.79	21.9±.88	69±1.4	13.3±.22	57±2.2	12.4±.21	126±7.8
20% Soybean Protein 24 Weeks, then 8% Casein 8 Weeks, 5 Days	425±10	14.2±.53	1.20±.04	65±1.8	77±.5	35.8±2.93	19.5±.71	46±2.4	17.6±.50	61±2.2	11.8±.17	70±3.8
20% Casein - 24 Weeks, then 20% Casein + 5% Lard 8 Weeks, 5 Days	437±11	14.2±.11	1.19±.03	68±.3	76±.8	26.6±1.31	20.0±.78	53±2.2	13.7±.31	60±4.7	11.7±.56	125±6.9

1. Standard error of the mean.
2. Six rats in each group.

Table XI presents the results occurring when soybean protein is compared to casein with no fat. The tissues chosen for study were liver, heart, muscle, brain and testis. Again the soybean protein gives a much higher body weight than the casein when no fat is fed. The liver fat was not higher in the group fed 40% casein with no fat, such as was the case when 20% and 8% casein were fed. This lack of fat accumulation in the liver may have been due to the extra bit of residual fat contained in the high dietary level of the vitamin-free casein or to the higher protein level itself. The heart cholesterol level was lower when no fat was fed as compared to the other groups. The serum cholesterol levels in the group fed corn oil were much higher than for all other groups. The serum cholesterol level of the group fed 40% soybean protein was lower than for any other group.

TABLE XI

Effect of different high protein diets for 26 weeks on rat tissues[1]

Diet	Body Wt., gm.	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	% Fat in Muscle, Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Fat	Mg. Cholesterol / gm. Brain (dry)	Mg. Cholesterol / gm. Testes Fat	Mg. Cholesterol / gm. Testes (dry)	Mg. % Serum Cholesterol
40% Casein + 5% Corn Oil	452[2]	17.1	15.7	32.7	42.6	21.5	82±2.3[3]	13.3±.67	80±4.5	12.6±.17	169±2.6	72.4+1.34	79±1.9	15.7±.42	137±7.5
40% Casein + 5% Lard	456	18.2	14.1	38.1	43.1	24.3	63±1.1	11.2±.03	89±4.1	12.5±.45	157±1.0	67.9±1.60	82±5.6	16.4±.66	101±8.0
40% Casein + 2% Linoleic Acid	453	17.6	16.8	39.9	42.2	22.9	77±3.1	13.4±.55	60±3.9	11.9±.16	156±1.7	66.4±1.64	74±4.0	16.6±.27	80±6.7
40% Casein + No Fat	286	18.9	17.8	28.0	43.9	22.6	75±1.0	13.7±1.08	56±2.0	10.0±.52	170±2.9	74.5±1.80	69±2.3	14.9±.49	75±14.0
40% Soybean Protein[4] - No Fat	431	16.2	15.5	33.9	45.5	20.7	81±2.1	13.5±.26	68±1.5	12.3±.17	162±6.2	67.8±1.92	80±3.9	16.2±.22	60±3.2

1. Additional data and standard errors of the mean for this experiment will be found in Table XI A, page 52, Appendix.
2. Six rats in each group.
3. Standard error of the mean.
4. Drackett

Table XII contains data from two feeding experiments, one involving Drackett Assay Protein (now produced by the Archer-Daniels-Midland Co.) for 26 weeks, and the second, Promine (Glidden Co.), for 27 weeks. These two soybean proteins, both containing over 90% protein, resulted in similar data in all but a few cases. There was more cholesterol in the hearts of the rats fed the Drackett protein and in three cases the serum cholesterol levels were higher when the Drackett was fed. In one case the serum cholesterol level was higher when Promine was fed. The liver fat was higher when Promine was fed. When corn oil, linoleic acid or no fat was fed, the Drackett resulted in higher serum cholesterol levels. When lard was fed the reverse was true. The results from the two different feedings of 8% casein were certainly as close as can be expected from two different experiments run in the same manner.

TABLE XII

A comparison of Drackett (D) and Promine (P) soybean proteins at 12% and 10% levels with different dietary lipids. Included for comparison are two 8% casein-fed groups. The Drackett diets were fed for 26 weeks, the Promine for 27 weeks[1]

% Protein[2]	Diet: Lipid	Duration, Wks.	Body Wt., gm.	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	% Fat in Muscle, Dry Wt.	% Fat in Brain, Dry Wt.	% Fat in Testes, Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Fat	Mg. Cholesterol / gm. Brain (dry)	Mg. Cholesterol / gm. Testes Fat	Mg. Cholesterol / gm. Testes (dry)	Mg. % Serum Cholesterol
12 D	No Fat	26	410[3]	23.8	18.0	37.7	43.7	21.0	35±1.7	9.4±.27	70±3.8	12.4±.54	182±1.7	80.7±1.7	90±3.9	18.8±.60	137±7.9
12 P	No Fat	27	387	30.3	29.2	45.1	47.4	21.2	49±2.1	14.6±.57	28±1.6	8.3±.35	162±3.3	75.0±2.12	94±5.4	19.5±.56	108±8.2
12 D	5% Corn Oil	26	456	15.9	19.7	40.4	45.9	24.1	57±.8	8.8±.14	73±3.6	14.1±.40	172±5.1	80.8±1.34	83±2.0	19.7±.15	199±16.3
12 P	5% Corn Oil	27	489	21.4	20.0	38.7	46.8	23.3	63±4.5	13.7±.36	50±1.2	10.0±.43	176±4.7	82.5±2.69	90±5.1	20.7±.74	177±7.7
12 D	5% Lard	26	452	18.6	18.9	40.3	45.1	21.5	53±.9	10.2±.49	68±5.5	12.6±.21	172±7.2	76.8±3.77	83±4.9	17.7±.37	129±5.5
12 P	5% Lard	27	482	24.1	18.9	32.0	46.0	22.5	58±2.4	13.8±.17	50±3.0	9.5±.34	177±3.2	81.2±2.36	92±5.6	20.5±.69	172±4.7
12 D	2% Linoleic Acid	26	405	20.3	17.0	38.6	45.7	21.4	46±3.6	9.1±.23	70±1.6	12.4±.54	161±5.1	73.7±3.03	91±3.5	17.9±.14	161±7.8
12 P	2% Linoleic Acid	27	412	22.7	28.8	40.7	49.8	23.3	59±2.2	13.1±.36	33±2.0	9.3±.45	160±2.3	79.7±2.66	80±5.1	18.2±.50	107±5.8
10 D	No Fat	26	354	24.4	19.7	37.3	45.6	22.7	45±1.7	10.8±.34	62±5.4	12.3±.21	188±7.3	85.5±2.1	82±6.6	18.5±.22	124±4.0
10 P	No Fat	27	373	34.0	20.9	40.4	49.3	23.2	40±1.6	13.5±.61	42±4.0	8.6±.48	160±2.9	79.1±2.46	65±1.6	15.0±.21	119±4.3
8 C	No Fat	26	223	39.3	16.0	25.9	40.5	23.0	40±3.0	16.5±1.1	46±2.0	7.4±.23	156±3.0	63.1±2.84	75±1.3	16.7±.65	95±4.6
8 C	No Fat	27	221	36.1	18.1	29.8	45.8	25.2	41±2.1	14.7±.39	54±3.6	9.5±.27	174±3.2	79.4±0.87	62±6.4	14.8±.82	101±3.2

1. Additional data and standard errors of the mean for this experiment will be found in Table XII A, page 53 Appendix.
2. D=Drackett soybean protein
   P=Promine soybean protein
   C=Casein
3. Six rats per group
4. Standard error of the mean.

Table XIII compares results from groups of rats fed 10 and 12% Promine. The rats fed the 12% Promine gained more weight when corn oil or lard was fed but not when linoleic acid was fed. There was more heart fat when 12% Promine was fed with linoleic acid or no fat and the serum cholesterol was higher when corn oil was fed. Most of the data for these two close levels of dietary soybean protein are in good agreement. Those differences which do appear between the 10 and 12% levels for the same dietary lipid other than those mentioned above are not consistent.

TABLE XIII

A comparison of 10% and 12% promine with the different dietary lipids for 27 weeks[1]

Level of Promine	Dietary Lipid	Body Wt., gm.	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	% Fat in Muscle, Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes, Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Fat	Mg. Cholesterol / gm. Brain (dry)	Mg. Cholesterol / gm. Testes Fat	Mg. Cholesterol / gm. Testes (dry)	Mg. % Serum Cholesterol
10%	No Fat	373[2]	34.0	20.9	40.4	49.3	23.2	40±1.6[3]	13.5±.61	42±4.0	8.6±.48	160±2.9	79.1±2.46	65±1.6	15.0±.21	119±4.3
12%	No Fat	387	30.3	29.2	45.1	47.4	21.2	49±2.1	14.6±.57	28±1.6	8.3±.35	162±3.3	75.0±2.12	94±5.4	19.5±.56	108±8.2
10%	2% Linoleic Acid	435	25.6	19.4	41.0	50.1	24.1	45±2.8	12.5±.54	52±4.8	10.1±.72	153±5.4	76.7±2.58	78±4.3	18.6±.52	113±4.1
12%	2% Linoleic Acid	412	22.7	28.8	40.7	49.8	23.3	59±2.2	13.1±.36	33±2.0	9.3±.45	160±2.3	79.7±2.66	80±5.1	18.2±.50	107±5.8
10%	5% Corn Oil	422	19.9	18.7	41.2	50.5	21.0	60±1.6	11.9±.37	65±2.3	12.0±.29	167±4.3	84.1±1.38	92±5.4	18.8±1.01	151±5.9
12%	5% Corn Oil	489	21.4	20.0	38.7	46.8	23.3	63±4.5	13.7±.36	50±1.2	10.0±.43	176±4.7	82.5±2.69	90±5.1	20.7±.74	177±7.7
10%	5% Lard	425	22.3	20.2	33.0	48.4	22.3	55±1.2	12.1±.36	60±3.3	11.9±.29	170±4.8	85.4±2.51	81±2.9	18.0±.61	169±5.4
12%	5% Lard	482	24.1	18.9	32.0	46.0	22.5	58±2.4	13.8±.17	50±3.0	9.5±.34	177±3.2	81.2±2.36	92±5.6	20.5±.69	172±4.7

1. Additional data and standard errors of the mean will be found in Table XIII A, page 55, in the Appendix.
2. Six rats per group.
3. Standard error of the mean.

Table XIV presents data obtained when the diets of the rats were changed after 25 weeks and the new diet was fed for 20 more weeks. Three of the diets were fed for the entire 45-week period. The weights were not significantly different. There is a range in liver weights, but it does not correlate with any dietary factor, and the range in liver fat percentages (dry weight) does not correlate with the protein or fat in the diet. The muscle fat level was highest when an 8% casein--5% corn oil diet was changed to a 20% casein--15% lard diet and lowest when dog chow was changed to 20% casein--15% corn oil. When the change was made from a 20% casein --5% corn oil diet to an 8% casein and 15% lard or corn oil diet, the lard diet gave the highest muscle fat level. However, when the change was made from a 20% casein--5% lard diet to an 8% casein and 15% lard or corn oil diet, the corn oil gave the highest muscle fat level. Hence, the change in lipids can be considered responsible for the difference. The diet that the rats had for the last 20 weeks was not the sole factor in determining the muscle fat level as is seen by comparing the 8% casein--15% corn oil levels, *viz* 47.5, 33.5 and 43.5, and the levels obtained from the rats fed 8% casein and 15% lard as the final diet *viz* 44.7, 31.9 and 38.4. The diet fed for the first 25 weeks was not the sole factor either. It can be safely stated that the diet during the rat's entire life is important in the muscle lipid level.

There is no consistent pattern for the serum cholesterol levels according to the level of casein fed for the first 25 weeks or for the last 20 weeks, nor was there a correlation with lard or corn oil when it was fed. The serum cholesterol levels found in this experiment are higher than those normally found for rats which are not fed cholesterol. The fact that the rats were fed these diets for 45 weeks, which is of longer duration than most such dietary experiments, may have been a strong contributing factor along with the fact that the rats were not fasted prior to sacrifice, which according to Mayfield and Roehm (22) makes up to 1.7 fold difference in serum cholesterol levels.

(Turn to page 38 and 39 for table XIV)

TABLE XIV

Effect of dietary changes on rat tissues

Initial Diet 25 Weeks	Second Diet 20 Weeks	Body Wt., gm. Dry Wt.	% Fat in Liver Dry Wt.	% Fat in Heart Dry Wt.	% Fat in Muscle Dry Wt.	% Fat in Brain[1] Dry Wt.	% Fat in Testes Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Fat	Mg. Cholesterol / gm. Brain (dry)	Mg. Cholesterol / gm. Testes Fat	Mg. Cholesterol / gm. Testes (dry)	Mg. % Serum Cholesterol
8% Casein + 5% Corn Oil	20% Casein + 15% Lard	500[2]	25.9	16.6	55.1	46.2	29.1	70± 3.3[3]	18.1± .52	72± 2.9	11.8± .22	141± 2.1	65.2± 1.59	61± 5.3	17.2± .45	215± 13
8% Casein + 5% Corn Oil	8% Casein + 15% Corn Oil	479	27.0	15.5	47.5	46.1	24.6	70± 5.7	18.3± .51	82± 3.5	12.4± .22	156± 3.8	72.0± 2.03	68± 6.8	16.3± .57	199± 1
8% Casein + 5% Corn Oil	8% Casein + 5% Corn Oil	484	25.9	15.2	43.9	46.2	31.1	67± 4.1	17.0± .56	82± 1.7	12.3± .04	158± 6.9	73.0± 3.28	54± 3.2	16.7± .42	213± 25
20% Casein + 5% Corn Oil	8% Casein + 15% Lard	499	28.9	15.9	44.7	45.8	28.9	54± 2.5	15.3± .53	76± 1.7	12.0± .14	149± 4.4	67.9± 1.40	38± 2.8	10.9± .87	198± 6
20% Casein + 5% Corn Oil	8% Casein + 15% Corn Oil	495	31.4	14.3	33.5	46.0	21.9	57± 5.0	17.3± .60	94± 1.7	13.5± .31	158± 4.0	72.8± 2.02	87± 7.3	18.6± .64	194± 19
20% Casein + 5% Corn Oil	20% Casein + 5% Corn Oil	495	24.9	15.4	35.6	47.0	21.0	56± 2.9	13.8± .43	88± 1.9	13.6± .13	159± 5.6	74.9± 3.44	93± 6.3	19.3± 1.38	241± 18

1. Additional data and standard errors of the mean for this experiment will be found in Table XIV A, pp. 56 and 57 in Appendix.
2. Six rats per group.
3. Standard error of the mean.

TABLE XIV (Cont.)

Effect of dietary changes on rat tissues.

Initial Diet 25 Weeks	Second Diet 20 Weeks	Body Wt., gm. Dry Wt.	% Fat in Liver Dry Wt.	% Fat in Heart Dry Wt.	% Fat in Muscle Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Fat	Mg. Cholesterol / gm. Brain (dry)	Mg. Cholesterol / gm. Testes Fat	Mg. Cholesterol / gm. Testes (dry)	Mg. % Serum Cholesterol
20% Casein + 5% Lard	8% Casein + 15% Lard	472 [2]	29.4	16.3	31.9	47.5	21.3	55± 3.1	16.2± .51 [3]	63± 6.4	9.8± .23	188± 3.0	89.3± 2.11	83± 4.7	17.6± 64	160± 10
20% Casein + 5% Lard	8% Casein + 15% Lard	482	25.7	15.4	43.5	46.5	21.6	64± 5.2	16.3± .71	67± 2.8	10.2± .28	198± 4.9	92.2± 2 62	92± 1.0	19.8± .80	179± 8
20% Casein + 5% Lard	20% Casein + 5% Lard	500	26.2	16.3	38.5	43.8	20.8	50± 2.9	12.9± .09	59± 3.2	9.6± .18	197± 6.2	85.8± 1.19	82± 2.3	17.0± .07	188± 5
Dog Chow	20% Casein + 15% Lard	480	24.1	17.0	37.7	43.2	21.7	54± 2.7	12.9± .11	72± 1.3	12.3± 34	149± 3.1	64.3± 1.85	61± 5.2	13.2± 1.03	214± 8
Dog Chow	20% Casein + 15% Corn Oil	470	23.5	15.8	28.8	43.7	21.5	65± .8	15.2± 1.27	75± 4.7	12.5± .44	152± 4.2	66.3± 1.46	58± 3.3	12.6± .71	192± 11
Dog Chow	8% Casein + 15% Lard	459	29.5	18.5	38.4	45.3	23.2	58± 2.2	16.7± .86			147± 3.9	66.3± 1.96	69± 4.4	15.7± .82	187± 12

1. Additional data and standard errors of the mean for this experiment will be found in Table XIV A, pp. 56 and 57 in Appendix.
2. Six rats per group.
3. Standard error of the mean.

Table XV gives the data obtained from female rats fed 8% casein diets for 41 weeks. In one case the rats conditioned to a fat-free diet for 12 weeks were given corn oil and in another case linoleic acid was added after the 12 weeks. As with the males, the body weight was less when no lipid was fed, and linoleic acid allowed the rats to reach a weight similar to that obtained with corn oil. The females did not have a fatty liver when 8% casein was fed with no lipid. The liver lipid level for this group was 25.5% whereas Table XII shows 36 and 29% for two different groups of males fed 8% casein. For this group the cholesterol level per gram of dry liver was about the same as it was for groups receiving dietary lipid, whereas with the males, the cholesterol level per gram of dry liver was higher in animals receiving 8% casein with no lipid than it was for other male groups (Table VIII). The serum cholesterol level was lowest when no fat was included in the diet and highest when corn oil was included. The dietary changes made no significant differences in the data.

TABLE XV

The effect of dietary changes on the tissue data of female rats

Diet	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	Brain Wt., gm.	% H_2O in Liver	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	% Fat in Muscle, Dry Wt.	% Fat in Brain, Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Fat	Mg. Cholesterol / gm. Brain (dry)	Mg. % Serum Cholesterol
8% Casein No Fat for 12 Weeks, then																			
8% Casein + 5% Corn Oil for 29 Weeks	287± 8.4 [1,2]	10.5± .33	0.94± .02	1.69± .09	68± .5	75± 1.1	66± 2.1	76± .2	25.0± 1.78	18.2+ .97	40.4+ 6.34	52.2+ 1.36	56± 4.0	13.4+ .34	57± 4.3	12.4± .64	178± 7.4	92.3± 3.49	189± 13.8
8% Casein + 1% Linoleic Acid for 29 Weeks	312± 17.3	13.3± .66	1.12± .07	1.76± .08	68± .50	76± .7	62± 3.5	76± .3	27.7± 1.91	18.7+ .87	44.3+ 7.73	52.4+ 1.15	55± 2.8	15.0+ .75	51± 3.7	10.1± .18	178± 3.9	93.0± 2.67	134± 7.6
8% Casein No Fat for 41 Weeks	168± 16.0	7.2± .21	0.86± .09	1.54± .09	70± .9	77± .5	67± 2.7	76± 1.2	25.5± 2.82	19.3+ 1.11	34.7+ 6.48	52.9+ .30	56± 3.4	14.2+ 1.50	48± 1.8	9.2± .54	173± 3.9	92.1± 1.74	71± 8.9
8% Casein + 5% Corn Oil for 41 Weeks	293± 14.6	11.1± .80	0.97± .06	1.61± .09	68± .32	76± .4	61± 1.5	76± .3	24.4± 1.54	20.1+ 1.18	50.9+ 1.24	54.1+ 3.54	53± 2.6	12.8+ .43	52± .6	10.3± .48	—	—	196± 16.5
8% Casein 1% Linoleic Acid for 41 Weeks	279± 16.7	10.7± .38	0.96± .10	1.54± .01	69± .3	76± .3	58± 1.7	76± .3	28.1± 1.27	17.5+ .72	55.8+ 2.59	56.1+ 1.93	54± 2.0	15.0+ .47	54± 1.6	9.4± .17	—	—	154± 5.3

1. Standard error of the mean.
2. Six rats per group.

Table XVI presents data from rats which had been fed torula yeast for 24½ weeks after they had reached adulthood on a diet of dog chow. The torula yeast is 54.6% protein and, hence, the diets are 43.6%, 21.8% and 8.7% protein, levels near the protein levels found in the casein and soybean protein diets. The final weights after 24½ weeks of torula yeast were near those of rats fed casein or soybean protein. The serum cholesterol levels of the rats fed the two higher percentages of the yeast were lower than have been found for most rats of this age. Since the yeast contains 7% crude fat, these were not low fat diets. The rats fed the 16% yeast had the highest serum cholesterol level. This is not unexpected, since, as shown in Table VII, a similar diet yielded a serum level of 114 in 15 weeks while a diet similar to the second torula yeast diet, as shown in Table XVI, gave a serum cholesterol level of 91. Aging had an effect on cholesterol levels at the lower protein level but not the higher.

The torula yeast served as an excellent food material for the rats, and these data indicate that the rats gained as much weight as those fed soybean protein or casein and the tissue analyses were very similar to those of the rats fed casein or soybean protein. This relatively untapped source of economical protein will certainly be of considerable value when the world's source of food protein becomes more scarce due to population increases.

TABLE XVI

Effect of torula yeast on the tissue composition of rats. The rats were old when the experiment was begun.

Diet	Body Wt., gm.	Liver Wt., gm	Heart Wt., gm.	Brain Wt., gm	Testes Wt., gm.	% H_2O in Liver	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain	% H_2O in Testes	% Fat in Liver Dry Wt.	% Fat in Heart Dry Wt.
rula	432[1,2] ±12	14.2 ±.41	1.17 ±.04	1.60 ±.09	2.95 ±.11	69 ±.3	75 ±.1	69± 1.1	76± .2	86± .3	20.2 ±.46	17.5 ±.92
rula	455 ±10	14.1 ±.55	1.23 ±.08	1.77 ±.08	2.94 ±.13	68 ±.3	76 ±.1	67± .9	76± .1	86± .2	17.3 ±.49	16.5 ±.28
rula	483 ±10	16.8 ±.72	1.24 ±.04	1.72 ±.06	2.97 ±.09	67 ±.3	76 ±.1	66± +.5	76± .2	86± .2	19.8 ±1.44	M

Diet	% Fat in Muscle Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / gm. Heart Fat	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Fat	Mg. Cholesterol / gm. Brain (dry)	Mg. Cholesterol / gm. Testes Fat	Mg. Cholesterol / gm. Testes (dry)	Mg. % Serum Cholesterol
rula	36.9 2.6	M[3]	23.0 ±.77	84± 1.7	17.1± 1.11	78± 3.1	13.6 ±.29	152± 15	89.1± 1.81	87± 4	19.8 ±.32	93± 2.4
rula	35.1 2.74	46.3 ±.47	24.0 ±.99	71± 2.7	12.3± .46	70± 1.1	11.6 .08	189± 3.0	87.2± 1.63	80± 3.4	19.0 .45	89± 4.0
rula	39.8 2.52	48.3 ±1.44	24.9 ±1.22	57± 1.5	11.2± .76	M	M	179± 6.2	86.6± 2.15	81± 3.9	19.9 ±.43	152± 7.7

ndard error of the mean.
x rats per group.
M=missing.

DISCUSSION

The preceding results add to the large accumulation of data on cholesterol levels reported in the past several years in an effort to unravel the serum cholesterol--diet--atherosclerosis relationships. Since the rat utilizes cholesterol in much the same way that humans do, it is quite likely that any relationship between diet and tissue cholesterol levels would be reflected in rats as well as in humans. It is, of course, possible that the rat will differ from the human such as in the need for dietary ascorbic acid, but the similarities in metabolic functions in the human and the rat far outnumber the differences.

In this work we were able to demonstrate essential fatty acid deficiency symptoms when a fat-free diet was fed, and linoleic acid in the diet prevented the appearance of these symptoms. That linoleic acid is needed for transport of cholesterol was shown by the higher liver cholesterol levels in the rats receiving no fat. It is, of course, possible that the mere fact that there was more fat in the liver might also be responsible for the higher level of cholesterol. This fat in the fatty livers was synthesized from non-fat precursors as was the cholesterol, and the type of fatty acid found was more saturated as indicated by the iodine numbers. Only in the diets containing lard was there any appreciable dietary cholesterol. This dietary cholesterol made little difference as shown by the fact that lard and corn oil diets gave similar cholesterol levels in tissues and in serum.

When considering the vast amount of literature available on serum cholesterol levels as affected by diet, perhaps the most striking findings were that the serum cholesterol levels become quite high in older rats as indicated by the data presented in table XIV for rats 45 weeks on these synthetic diets as against the data presented in tables VIII and XI for shorter periods of time. The human also shows a higher serum cholesterol level with age (23). Many rat studies reported in the literature have been for a much shorter period of time, and, hence, lower serum cholesterol levels have been reported in these experiments.

Another finding worthy of note was that the addition of fat to the diets of animals fed 8% casein and no fat resulted in high serum cholesterol levels, while dietary changes with animals already receiving fat in the diet did not result in higher levels. Tables VI and XV show the change due to adding lipid to the diet after 8% casein and no fat was fed. Table X shows that this also occurred with 20% casein when lard was added, and table XIV shows that the rats receiving fat in the diet with 8% casein did not have an increase in serum cholesterol when a higher protein or fat level was fed.

Tables XII and XIII indicate the close agreement in data between two different soybean protein preparations at the same level and between two very close levels of the same soybean protein. Two different groups fed 8% casein are represented in table XII. The two groups had a one week difference in their time of the diet. The agreement in the data for the two casein groups was very good and results are as similar as can be expected from nutrition experiments. The data for the 10 and 12% Promine are also quite similar and the comparison between the Promine and Drackett soybean proteins indicates just a few more differences for data from comparable groups. The liver fat and heart fat levels were higher with the Promine so there apparently is an effect due to the peculiarities of the two products whose specifications are so similar.

Dietary changes during a long growth period as illustrated in table XIV show that the entire dietary history of the rat must be considered in trying to attach significance to the diet and its relationship to lipid levels. The fact that the same finishing diets gave different muscle lipid levels means that the starting diet also influenced the muscle lipid content of the rats. Female rats showed this as well as males. A corn oil diet fed throughout 45 weeks at the same level of protein resulted in higher serum cholesterol levels than when a dietary change was made to the same level of protein and a higher level of corn oil. There are, therefore, indications that the previous dietary history may influence results although the final diet was fed for 20 weeks, which is considered about 19% of the rat's life. The fact that previous dietary history has an influence on some data must always be considered in interpreting data.

When all of the serum cholesterol levels are tabulated according to levels and kinds of fats and 40%, 20% or 8% casein, regardless of age, no significant difference can be shown due to protein level alone. A consistent difference due to soybean protein at the 8, 10 and 20% dietary level is also lacking. There is no significant difference between the corn oil and lard fed groups regardless of the protein fed. The corn oil groups averaged slightly higher. More serum cholesterol formed when 15% corn oil or lard was fed than when 5% corn oil or lard was fed. Serum cholesterol levels were significantly lower when linoleic acid or no fat was fed instead of lard or corn oil. The fat-free diets resulted in lower levels than did the diets containing linoleic acid, but the difference was not significant.

When the serum cholesterol levels are arranged according to age regardless of the protein or fat fed to the rats, the 48-week old rats have a significantly higher level than those 29 or 30 weeks old, which are in turn significantly higher than those 16 weeks of age. This relationship (the increase of serum cholesterol levels with age) is similar to that which is observed in humans and, as mentioned previously, points out that cholesterol studies with rats should be done for a long period of time and dietary comparisions should be made with animals of approximately the same age.

The percentage of moisture in the brain, heart and testis tissue varied only within narrow limits while that of the liver and muscle varied more. Where the lipid content was highest, the moisture percentage was lowest.

The fat content of the livers varied considerably. The older the rat the higher the liver fat content and the rats receiving no dietary fat were higher in liver fat than those receiving linoleic acid, lard or corn oil. The linoleic acid, lard or corn oil resulted in about the same liver fat levels. The heart fat content varied only slightly regardless of dietary treatment, and there is no readily apparent relationship between diet and heart fat content, brain fat content and testis fat content. Further data analysis is necessary to detect any subtle differences which may exist.

The tissue cholesterol results were calculated per gram of dry tissue and per gram of fat. Calculated either way the animals receiving no dietary fat had the highest liver cholesterol levels. The liver cholesterol levels were slightly higher for rats fed corn oil compared to those fed lard. Rats over half a year old had higher liver cholesterol levels than younger rats regardless of the diet. Rats fed 40% casein had higher liver cholesterol levels than those fed 20% or 8% casein regardless of the fat fed.

Younger rats had lower heart cholesterol values. No significant trends could be observed in the relationship of heart cholesterol and heart fat levels. Consistent differences attributable to diet have not been found in the brain and testis cholesterol data.

It must be emphasized that the rats were not fasted prior to sacrifice in any of these experiments. Mayfield and Roehm (22) showed that in variety of dietary treatments both serum and liver lipid cholesterol levels were up to 1.7 times higher in unfasted rats as compared to fasted rats receiving the same treatment. These workers also found that certain statistical differences were noted with the non-fasted rats that were not noted with the fasted rats. The reverse could also be true. When these experiments were initiated long before the appearance of the Mayfield and Roehm article, it was felt that the non-fasting state more nearly represented the rats average condition than did the fasting state. Hence, all experiments were conducted with the sacrifice of the rats in the non-fasted state, but as nearly as possible at the same time of the day. In the comparison of these results with those found in the literature for rats fasted at sacrifice, the higher results are found here. However, in most cases the relative effect on levels due to treatment should be the same.

Further research was conducted on the effect of dietary changes of fat and protein levels on fat and cholesterol levels of serum and tissues and will be reported later.

SUMMARY

Rats and mice were fed several proteins and fats at different levels and the effects on body composition were determined. The proteins included casein (animal protein), soybean protein, torula yeast and pea bean protein (plant proteins); the fats were lard (animal fat), corn oil (plant oil) and linoleic acid (an essential fatty acid).

The following tissues were removed in one or more experiments for analysis for moisture, fat and cholesterol: liver, heart, brain, muscle and testis. Serum was obtained for cholesterol analysis in most of the experiments.

Dietary protein (8 or 20% casein) or fat (corn oil, linoleic acid and no fat) did not result in any detectable differences in levels of serum free amino acids or liver free amino acids in mice.

All mice remained in positive nitrogen balance on the protein and fat combinations.

Iodine numbers of tissue fat were higher when mice or rats received dietary corn oil as compared to no fat. When linoleic acid was fed the iodine numbers were higher than when no fat was fed but lower than with dietary corn oil. Differences in iodine numbers with different dietary fats were not as great when soybean protein was fed as compared to casein.

When the weight of liver was compared to the total weight of the rat or mouse, the percentage of body weight as liver was higher when no fat was fed due to the formation of fatty livers. Dietary linoleic acid prevented the formation of fatty livers.

The rats grew faster on 20% or higher dietary protein levels, but after longer periods of time, e. g. 26 to 45 weeks, the rats reached nearly the same weight if fat was included in the diet. Linoleic acid allowed faster growth than no fat, but not the same growth as dietary lard or corn oil. Lard and corn oil were equivalent in growth promotion.

The moisture percentage of the brain, heart and testis tissue varied only within narrow limits while that of the liver and muscle varied more. When the lipid content was highest, the moisture content was lowest.

The fat content of the livers varied considerably. The older the rat the higher the liver fat content and the rats receiving no dietary

fat were higher in liver fat than those receiving linoleic acid, lard or corn oil. The linoleic acid, lard or corn oil diets resulted in nearly the same liver fat levels. The heart fat content varied only slightly regardless of dietary treatment, and there was no apparent relationship between diet and heart fat content, brain fat content and testis fat content.

The tissue cholesterol results were calculated per gram of dry tissue and per gram of fat. The animals receiving no dietary fat had the highest liver cholesterol levels. The liver cholesterol levels were slightly higher for rats fed corn oil compared to those fed lard. Rats over half a year old had higher liver cholesterol levels than younger rats regardless of diet. Rats fed 40% casein had higher liver cholesterol levels than those fed 20% or 8% casein, regardless of the fat fed.

Younger rats had lower heart cholesterol levels. No significant trends could be observed in the relationship of heart cholesterol and heart fat levels. Consistent differences attributable to diet have not been found in the brain and testis cholesterol data.

Serum cholesterol data was obtained on unfasted rats, a condition which more nearly represents the normal state. Older rats had higher serum cholesterol levels than younger ones. Dietary lard and corn oil result in similar serum cholesterol levels. Absence of fat from the diet or inclusion of linoleic acid in the diet generally resulted in lower serum cholesterol levels.

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APPENDIX

Tables of additional data and standard errors of the means supplementing those found in the text.

TABLE XI A

Effect of high protein diets for 26 weeks on rat tissues

Diet	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	Brain Wt., gm.	Testes Wt., gm.	% H_2O in Liver	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain	% H_2O in Testes	% Fat in Liver Dry Wt.	% Fat in Heart Dry Wt.	% Fat in Muscle Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes Dry Wt.
40% Casein + 5% Corn Oil	452± 12±[1,2]	16.0± 0.65	1.32± 0.05	1.32± 0.02	3.19± 0.18	68± 0.3	76± 0.2	68± 1.6	77± 0.2	87± 0.5	17.1± 1.30	15.7± 0.80	32.7± 3.77	42.6± 0.61	21.5± 1.66
40% Casein + 5% Lard	456± 15	15.7± 0.60	1.44± 0.16	1.32± 0.28	3.21± 0.07	68± 0.5	76± 0.1	66± 0.7	78± 0.2	85± 1.1	18.2± 1.63	14.1± 0.28	38.1± 1.79	43.1± 0.92	24.3± 3.71
40% Casein + 2% Linoleic Acid	453± 14	14.5± 0.47	1.31± 0.08	1.20± 0.14	3.44± 0.06	69± 0.3	76 ± 0.4	65± 1.4	76± 0.9	86± 0.4	17.6± 0.78	16.8± 2.60	39.9± 2.36	42.2± 0.81	22.9± 1.37
40% Casein + No Fat	286± 19	11.4± 1.88	1.05± 0.07	1.64± 0.07	2.58± 0.53	68± 1.0	76± 0.2	70± 0.2	77± 0.3	88± 1.2	18.9± 0.83	17.8± 0.62	28.0± 1.48	43.9± 1.09	22.6± 0.89
40% Soybean Protein[3] + No Fat	431± 19	13.7± 0.51	1.17± 0.07	1.21± 0.04	3.29± 0.10	69± 0.3	76± 0.3	66± 1.4	76± 0.2	86± 0.3	16.2± 0.67	15.5± 1.60	33.9± 2.74	45.5± 3.05	20.7± 0.81

1. Standard error of the mean.
2. 6 rats in each group
3. Drackett

TABLE XII A

A comparison of Drackett (D) and Promine (P) soybean proteins at 12% and 10% levels with different dietary lipids. Included for comparison are two 8% casein fed groups. The Drackett diets were fed for 26 weeks, the Promine for 27 weeks.

% Protein[1]	Diet Lipid	Duration, Wks.	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	Brain Wt., gm.	Testes Wt., gm.	% H_2O in Liver	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain	% H_2O in Testes	% Fat in Liver, Dry Wt.	% Fat in Heart, Dry Wt.	% Fat in Muscle Dry Wt.	% Fat in Brain, Dry Wt.	% Fat in Testes, Dry Wt.
12 D	No Fat	26	410± 22.6[2,3]	14.3± .37	1.29± .13	1.64± .11	2.81± .11	69± 1.5	76±.5	66±2.4	77±.2	86±.2	23.8± 1.69	18.0± 1.38	37.7± 1.63	43.7± 1.03	21.0± 62
12 P	No Fat	27	387± 9.9	10.2± .73	1.02± .04	1.53± .07	1.91± .32	67± .5	76±.6	66±2.4	76±.3	86±.8	30.3± 2.01	29.2± 1.06	45.1± 3.90	47.4± .75	21.2± 1.39
12 D	5% Corn Oil	26	456± 8.2	15.9± .41	1.18± .06	1.74± .05	2.91± .16	69± .5	75±.5	63±2.2	79±1.4	86±.2	15.9± 0.58	19.7± 1.26	40.4± 1.56	45.9± 0.67	24.1± .26
12 P	5% Corn Oil	27	489± 10.7	15.3± .68	1.24± .08	1.63± .07	3.25± .19	67± .2	75±.9	69±3.4	76±.3	87±.7	21.4± 1.11	20.0± .71	38.7± 5.77	46.8± 1.00	23.3± 1.02
12 D	5% Lard	26	452± 13.0	12.9± .21	1.20± .07	1.62± .10	2.65± .10	70± .4	76±.6	66±.9	77±.2	85±1.3	18.6± .68	18.9± 1.80	40.3± 1.40	45.1 .83	21.5 1.08
12 P	5% Lard	27	482± 14.4	13.9± 1.03	1.18± .07	1.68± .07	2.80± .44	67± .2	75.±.4	67±1.5	76±.3	86±1.1	24.1± .86	18.9± .74	32.0± 3.61	46.0± 1.63	22.5± 1.95

1. D=Drackett soybean protein.
   P=Promine soybean protein.
   C=Casein.
2. Standard error of the mean.
3. Six rats per group.

TABLE XII A (Cont.)

% Protein[1]	Diet: Lipid	Duration, Wks.	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	Brain Wt., gm.	Testes Wt., gm.	% H_2O in Liver	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain	% H_2O in Testes	% Fat in Liver Dry Wt.	% Fat in Heart Dry Wt.	% Fat in Muscle Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes Dry Wt.
12 D	2% Linoleic Acid	26	405± 10.0	13.5± .68	1.19± .12	1.74± .11	2.82± .09	69±.7	76±.3	65±1.7	77±0	86±0	20.3± 1.31	17.0± .52	38.6± 2.70	45.7± .44	21.4± 1.06
12 P	2% Linoleic Acid	27	412± 5.3	11.8± .11	1.05± .04	1.60± .02	2.64± .18	68±1.2	75±.4	65±3.0	76±.3	86±.7	22.7± 1.22	28.8± 1.35	40.7± 4.98	49.8± 1.54	23.3± .97
10 D	No Fat	26	354± 11.8	12.3± .60	1.03± .05	1.75± .41	2.77± .06	69±.3	76±.4	69±.5	77±.2	86±.4	24.4± 1.11	19.7± 1.61	37.3± .89	45.6± .85	22.7± 1.42
10 P	No Fat	27	373± 15.1	12.1± .75	1.08± .06	1.56± .10	2.66± .15	65±.5	76±2.4	65±.8	75±1.0	86±.3	34.0± 1.25	20.9± 1.43	40.4± 2.19	49.3± 1.01	23.2± 1.34
8 C	No Fat	26	223± 19.0	10.4± .88	0.84± .05	1.66± .05	1.20± .10	66±1.1	76±.3	70±.2	75±.2	85±1.7	39.3± 3.37	16.0± .58	25.9± 1.65	40.5± 2.01	23.0± .79
8 C	No Fat	27	221± 8.6	10.5± .54	1.05± .08	1.56± .05	1.35± .09	64±.3	77±.3	68±1.1	76±.3	86±.7	36.1± 1.72	18.1± 1.25	29.8± 2.05	45.8± 1.00	25.2± 2.26

1. D=Drackett Soybean protein.
   P=Promine soybean protein.
   C=Casein.
2. Standard error of the mean.
3. Six rats per group.

TABLE XIII A

A comparison of 10% and 12% Promine with the different dietary lipids for 27 weeks

Level of Promine	Dietary Lipid	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	Brain Wt., gm.	Testes Wt., gm.	% H_2O in Liver	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain	% H_2O in Testes	% Fat in Liver Dry Wt.	% Fat in Heart Dry Wt.	% Fat in Muscle Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes Dry Wt.
10%	No Fat	373 ± 15.1[1,2]	12.1 ± 0.75	1.08 ± 0.06	1.56 ± 0.10	2.66 ± 0.15	65 ± 0.5	76 ± 2.4	65 ± 0.8	75 ± 1.0	86 ± 0.3	34.0 ± 1.25	20.9 ± 1.43	40.4 ± 2.19	49.3 ± 1.01	23.2 ± 1.34
12%	No Fat	387 ± 9.9	10.2 ± 0.73	10.2 ± 0.04	1.53 ± 0.25	1.91 ± 0.32	67 ± 0.5	76 ± 0.6	66 ± 2.4	76 ± 0.3	86 ± 0.8	30.3 ± 2.01	29.2 ± 1.06	45.1 ± 3.90	47.4 ± 0.75	21.2 ± 1.39
10%	2% Linoleic Acid	435 ± 24.3	13.2 ± 1.05	1.07 ± 0.08	1.69 ± 0.26	2.98 ± 0.29	65 ± 0.8	76 ± 0.3	64 ± 2.0	76 ± 0.2	86 ± 0.4	25.6 ± 0.92	19.4 ± 0.16	41.0 ± 4.64	50.1 ± 0.12	24.1 ± 0.84
12%	2% Linoleic Acid	412 ± 5.3	11.8 ± 0.11	1.05 ± 0.04	1.60 ± 0.02	2.64 ± 0.18	68 ± 1.2	75 ± 0.4	65 ± 3.0	76 ± 0.3	86 ± 0.7	22.7 ± 1.22	28.8 ± 1.35	40.7 ± 4.98	49.8 ± 1.54	23.3 ± 0.97
10%	5% Corn Oil	422 ± 13.7	12.1 ± 0.67	1.03 ± 0.07	1.70 ± 0.29	2.58 ± 0.15	65 ± 1.7	76 ± 0.4	65 ± 2.0	76 ± 0.4	86 ± 0.3	19.9 ± 0.63	18.7 ± 1.12	41.2 ± 4.21	50.5 ± 0.68	21.0 ± 2.02
12%	5% Corn Oil	489 ± 10.7	15.3 ± 0.68	1.24 ± 0.08	1.63 ± 0.07	3.25 ± 0.19	67 ± 0.2	75 ± 0.9	69 ± 3.4	76 ± 0.3	87 ± 0.7	21.4 ± 1.11	20.0 ± 0.71	38.7 ± 5.77	16.8 ± 1.00	23.3 ± 1.02
10%	5% Lard	425 ± 19.0	12.5 ± 0.89	1.04 ± 0.03	1.46 ± 0.08	2.94 ± 0.13	66 ± 0.4	75 ± 0.3	66 ± 1.9	76 ± 0.3	86 ± 0.3	22.3 ± 1.12	20.2 ± 0.99	33.0 ± 4.33	48.4 ± 1.87	22.3 ± 1.21
12%	5% Lard	482 ± 14.4	13.9 ± 1.03	1.18 ± 0.07	1.68 ± 0.35	2.80 ± 0.44	67 ± 0.2	75 ± 0.4	67 ± 1.5	76 ± 0.3	86 ± 1.1	24.1 ± 0.86	18.9 ± 0.74	32.0 ± 3.61	46.0 ± 1.63	22.5 ± 1.95

1. Standard error of the mean.
2. Six rats per group.

TABLE XIV A

Effect of dietary changes on rat tissues.

Initial Diet 25 Weeks	Second Diet 20 Weeks	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	Brain Wt., gm.	Testes Wt., gm.	% H_2O in Liver	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain	% H_2O in Testes	% Fat in Liver, Dry Wt.	% Fat in Heart Dry Wt.	% Fat in Muscle Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes Dry Wt.
8% Casein + 5% Corn Oil	20% Casein + 15% Lard	500[1]	17.0± .52[2]	1.23± .06	1.71± .16	3.11± .17	69±.6	76±.4	60±1.9	76±.2	86±.2	25.9± 1.75	16.6± .60	55.1± 4.78	46.2± .47	29.1± 1.86
8% Casein + 5% Corn Oil	8% Casein + 15% C. Oil	479	14.9± .66	1.24± .06	1.81± .07	2.60± .79	70±.5	77±.3	62±2.4	76±.2	87±.3	27.0± 2.02	15.5± .89	47.5± 4.97	46.1± .48	24.6± 1.55
8% Casein + 5% Corn Oil	8% Casein + 5% C. Oil	484	15.3± .96	1.27± .04	1.63± .17	3.00± .12	70±.7	76±.2	65±1.2	76±.2	86±.4	25.9± 1.63	15.2± .20	43.9± 3.92	46.2± .54	31.1± 1.41
20% Casein + 5% Corn Oil	8% Casein +15% Lard	499	15.1± 1.31	1.24± .10	1.71± .10	2.75± .14	69±.4	76±.2	62±8.8	76±.2	87±.3	28.9± 1.87	15.9± .35	44.7+ 3.98	45.8± 1.14	28.9± 2.59
20% Casein + 5% Corn Oil	8% Casein +15% C. Oil	495	16.8± 1.27	1.36± .08	1.73± .12	2.90± .19	70±.7	77±.5	68±2.2	76±.5	86±.2	31.4± 2.36	14.3± .10	33.5+ 5.44	46.0± .93	21.9± 1.36
20% Casein + 5% Corn Oil	20% Casein + 5% C. Oil	495	17.9± .73	1.33± .04	1.81± .10	2.89± .31	70±.4	76±.3	67±2.2	76±.3	87±.5	24.9± 1.04	15.4± .39	35.6+ 6.80	47.0± .64	21.0± 1.21

1. Six rats per group.

2. Standard error of the mean.

TABLE XIV A (Cont.)

Initial Diet 25 Weeks	Second Diet 20 Weeks	Body Wt., gm.	Liver Wt., gm.	Heart Wt., gm.	Brain Wt., gm.	Testes Wt., gm.	% H_2O in Liver	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain	% H_2O in Testes	% Fat in Liver Dry Wt.	% Fat in Heart Dry Wt.	% Fat in Muscle, Dry Wt.	% Fat in Brain Dry Wt.	% Fat in Testes Dry Wt.
20% Casein + 5% Lard	8% Casein +15% Lard	472± 18 [1,2]	14.0± .42	1.19± .06	1.76± .06	2.95± .13	70± .6	76± .7	68± 2.0	75±.2	86±.2	29.4± 1.16	16.3± 1.55	31.9± 5.69	47.5± .46	21.3± .70
20% Casein + 5% Lard	8% Casein +15% C. Oil	482± 18	13.3± 1.46	1.23± .03	1.75± .13	2.91± .09	70± .5	77± .4	67± 1.8	76±.6	86±.3	25.7± 1.15	15.4± .75	43.5± 8.61	46.5± .97	21.6± .79
20% Casein + 5% Lard	20% Casein + 15% C. Oil	500	18.1± .26	1.29± .11	1.87± .14	3.21± .08	70± 0	77± .4	66± 3.0	77±0	86±.4	26.2± 1.37	16.3± .81	38.5± 4.82	43.8± 1.34	20.8± .65
Dog Chow	20% Casein + 15% Lard	480± 13	16.2± 1.24	1.37± .09	1.62± .11	3.15± .19	70± .2	76± .2	65± 1.5	77±.7	86±0	24.1± 1.21	17.0± .58	37.7± 6.64	43.2± .72	21.7± .88
Dog Chow	20% Casein + 15% C. Oil	470± 13	13.9± .98	1.29± .05	1.92± .07	3.12± .15	70± .5	77± .3	69± 2.6	76±.2	86±.2	23.5± 1.90	15.8± 1.10	28.8± 5.69	43.7± .67	21.5± .62
Dog Chow	8% Casein + 15% C. Oil	459± 18	12.8± 1.25	1.32± .07	1.90± .05	2.71± .15	70± .4	77± .8	66± 2.4	76±0	87±.3	29.5± 2.20	18.5± 2.52	38.4± 5.75	45.3± .88	23.2± 1.60

1. Standard errors of the mean are included.
2. Six rats per group.