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EFFECTS OF DIETARY CHANGES ON THE TISSUE COMPOSITION OF RATS

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Many experiments utilizing mice and rats to study relationships of dietary protein and fat to tissue lipid and cholesterol have been done in this laboratory (1, 2, 3, 4). When dietary changes were made during some of the experiments, the final crude fat and cholesterol composition did not appear to reflect either initial or final diet consistently. These preliminary experiments indicated that further investigation of the effect of diet changes was desirable.

In nearly all nutrition experiments with animals the same diet is fed for the duration of the experiment. This is done to keep the number of variables as low as possible and to simplify conclusions. While the rat accepts the same diet continuously, the human will not unless forced by necessity or otherwise strongly motivated. In projecting the results of animal studies into possible effects on humans, this difference must always be considered.

If, as some investigators have indicated, atherosclerosis is initiated in humans at an early age (possibly the late teens or early twenties) (5), then the dietary contributions (if there are such) must also begin at an early age. Therefore, the early dietary history of the individual is important as well as the dietary habits at the time atherosclerosis manifests itself. Rat studies with periodic dietary changes would more closely resemble human dietary habits.

Day by day variations in diets are also needed in animal studies in an attempt to approximate the variety found in human dietary patterns. In this laboratory human diet composites were fed to rats in the same order they were given to human subjects (3). The resulting serum cholesterol levels were lower than those found with rats fed synthetic diets under the same experimental conditions. Whether or not this varied diet was more readily accepted by the rats was not determined. The following experiments were done to study the effects of both long term and day by day dietary changes of fat and protein on tissue fat and serum cholesterol levels.

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PROCEDURE

Male rats of the CFN strain were purchased from Carworth Farms, New City, Rockland County, New York. They were housed individually in a temperature and humidity-controlled environment. Each animal was weighed weekly and all groups in an experiment were started at the same average weight. The final body weights were used for comparison since all groups in an experiment were started at the same average weight.

The diets, in addition to the protein and fat, contained sucrose, cellulose, vitamins, minerals, methionine and choline as described in previous studies (1). The proteins, casein[3] and soybean protein[4] were incorporated in the diets at the 8, 20 or 40% levels while the fats, lard and corn oil, were fed at the 0, 2, 5, 15, 20, 20.6 and 25% levels.

Dietary changes were made between kinds and levels of proteins and fats. Several experiments involved a single dietary change after six to twelve weeks on the initial diet. The second diets were fed for periods varying from two to twenty-six weeks. One experiment employed a weekly alternation between the two fats and between no fat and corn oil, while in another, some diets were alternated biweekly.

In the final series of experiments, diets were alternated daily between various diets and fasting. Comparisons were made between groups of rats that consumed the same quantity of food while being fed every day and groups fed every other day.

The variations of the fat and protein components of the diet, the dietary change schedules, and the analyses made are indicated in the tables of results. Unless otherwise stated, the diets were fed *ad libitum*.

The methods of analysis for moisture, lipid and cholesterol used in these studies are described in a preceding publication (1).

Student's t-distribution table was used in obtaining probability values. When categories combining several groups were compared, data from individual animals were used to obtain probability values. Probability ratios greater than 0.05 are included to indicate trends.

RESULTS

The diets utilized for the experiments presented in table 1 provided approximately 40% of the calories as fat. Lard and corn oil were compared as were casein and soybean protein. A comparison was also made between 8 and 20% casein.

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[3] Vitamin-free Casein, Nutritional Biochemicals Corp., Cleveland, Ohio.
[4] Promine, Glidden Co., Chicago, Illinois.

TABLE 1

Effect of increasing of the dietary fat level on rat tissues[1]

Diet 20% Casein + 15% Corn Oil for 7 1/2 Weeks, Then the Following for 26 Weeks	Body Wt. gm.	Liver Wt. gm.	% H_2O in Liver	% Fat in Liver Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / 100 ml. Serum
A_1 20% Casein + 20.6% Corn Oil	500[2]	18.6±1.05[3]	69±.3	27.0±1.50	58±1.2	15.3±.52	187±4
B_1 20% Casein + 20.6% Lard	498	18.8±1.53	68±.5	25.0± .66	56±1.9	14.0±.22	183±11
C_1 20% Soybean Protein[4] + 20.6% Corn Oil	495	18.2± .57	69±.6	28.5± .27	57±1.4	16.8±.58	149±12
D_1 20% Soybean Protein + 20.6% Lard	499	18.4± .96	69±.2	27.7±1.12	57±4.5	15.0±.38	178±12
E_1 8% Casein + 20.6% Corn Oil	490	15.0± .65	69±.2	30.7± .34	54±1.8	16.4±.44	139±9

[1] Analyses of other tissues will be found in the Appendix, table 1A, page 23.

[2] Six rats per group.

[3] Standard error of the mean.

[4] Promine.

The rats all gained about the same amount of weight but the livers from the group fed a lower level (8%) of casein weighed less ($E_1 < C_1$, $P < 0.025$). These livers, however, contained the highest percentage of fat ($E_1 > C_1$, $P < 0.001$).

Dietary corn oil resulted in a lower serum cholesterol level than lard when fed with 20% soybean protein ($C_1 < D_1$, $P < 0.20$) but not when fed with 20% casein ($A_1 : B_1$). When 8% dietary casein was fed, the serum cholesterol level was lower than in the groups fed 20% casein ($E_1 < A_1$ or B_1, $P < 0.01$). Therefore, both the corn oil and the soybean protein were necessary for the lower serum cholesterol level when 20% protein was fed. It cannot be said that a lower dietary protein level results in a lower serum cholesterol level, but it can be stated that a lower dietary casein level results in a lower serum cholesterol level.

The milligrams of liver cholesterol per gram of liver fat were calculated to test the possibility of the cholesterol content being related to the amount of fat present. This experiment showed no significant difference in the concentration of cholesterol in liver fat. However, differences were found in later experiments.

The values of milligrams of cholesterol per gram of dry liver (fat included) have sufficiently low standard errors to allow more conclusions to be drawn than could be on the basis of liver fat levels. Corn oil resulted in higher liver cholesterol levels than lard when fed with 20% casein ($A_1 > B_1$, $P < 0.05$) and with 20% soybean protein ($C_1 > D_1$, $P < 0.025$). Soybean protein resulted in higher liver tissue cholesterol levels than casein when fed with lard ($D_1 > B_1$, $P < 0.05$) and corn oil ($C_1 > A_1$, $P < 0.10$).

In another experiment, rats which weighed 189 grams at the beginning of the experiment were fed a high level (40%) of casein with 15% corn oil or lard and a low level (8%) of casein with 20% corn oil or lard after having been fed an 8% casein and no-fat diet for 10 weeks (table 2). The inclusion of corn oil in the diet for the remaining 26 weeks of the experiment resulted in a higher concentration of serum cholesterol than when lard was included. The difference due to corn oil or lard was greater with the higher level of dietary protein ($A_2 > B_2$, $P < 0.05$) than with the low level of protein ($C_2 > D_2$, $P < 0.10$).

The animals receiving the lower protein (8% casein) diets that included the more unsaturated fat, corn oil, exhibited higher cholesterol levels in the liver fat ($C_2 > D_2$, $P < 0.005$), liver tissue ($C_2 > D_2$, $P < 0.05$) and serum ($C_2 > D_2$, $P < 0.10$) than those with lard, the more saturated fat, in their diets.

Although a higher level of protein in the diet resulted in a higher serum cholesterol level, the change in the level of dietary fat had to be

TABLE 2

Effect of increasing the dietary fat and protein level on rat tissues[1]

Diet 8% Casein + No Fat for 10 Weeks, Then the Following for 26 Weeks	Body Wt.[2] gm.	Liver Wt. gm.	% H_2O in Liver	% Fat in Liver Dry Wt.	$\frac{\text{Mg. Cholesterol}}{\text{gm. Liver Fat}}$	$\frac{\text{Mg. Cholesterol}}{\text{gm. Liver (dry)}}$	$\frac{\text{Mg. Cholesterol}}{\text{100 ml. Serum}}$
A_2 40% Casein + 15% Corn Oil	497[3]	19.9±1.39[4]	69± .3	19.3±1.5	89±3.1	18.2± .56	206±3
B_2 40% Casein + 15% Lard	452	15.4±1.38	70± .4	20.5±1.28	87±3.5	17.7± .96	170±13
C_2 8% Casein + 20% Corn Oil	422	13.6±1.64	70±1.2	21.4± .87	90±2.1	19.2± .87	130±1
D_2 8% Casein + 20% Lard	486	17.1±1.18	70± .6	21.7±1.73	74±1.8	16.1± .91	111±9

[1] Analyses of other tissues will be found in the Appendix, table 2A, page 24.

[2] Starting weight of all groups was 189 gm.

[3] Four rats in the first group; three in each of the others.

[4] Standard error of the mean.

considered. Additional studies were initiated in which the level of one or both of these dietary constituents (protein, fat) were held constant.

The level and type of dietary protein (20% casein) and the fat level (25%) were held constant and dietary changes were made from corn oil to lard and from lard to corn oil in the next experiment which started with rats weighing approximately 200 grams (table 3).

After a change from lard to corn oil or corn oil to lard which had been fed 12 weeks, a lower level of liver fat was found after two additional weeks ($C_3 > E_3$, $P < 0.10$. $H_3 > J_3$, $P < 0.10$) These levels were raised after four additional weeks; however, only the change with the feeding of corn oil was significant ($K_3 > J_3$, $P < 0.05$). Here time is a factor in the conclusions which could be drawn.

The serum cholesterol levels resulting from feeding the lard diets were lower after 12 weeks ($H_3 < C_3$, $P < 0.10$) and 16 weeks ($I_3 < D_3$, $P < 0.05$) than those resulting from feeding corn oil diets at these times. This confirms similar findings reported in table 2. However, the lard (B_3) and corn oil (G_3) diets resulted in the same serum cholesterol levels after a six-week period.

After receiving the lard diet for 12 weeks one group of rats was fed the corn oil diet for two weeks (J_3) and another group for four weeks (K_3). The serum cholesterol level rose from 111 mg/100 ml at 12 weeks to 127 ($J_3 > H_3$, $P < 0.10$) after two weeks of the corn oil diet and to 142 ($K_3 > H_3$, $P < 0.001$) after four weeks of this diet. The corresponding data from the corn oil to lard changes were 125 mg/100 ml at 12 weeks, 147 after two weeks ($E_3 > C_3$, $P < 0.025$), and 142 at four weeks ($F_3 > C_3$, $P < 0.05$). This experiment shows that neither the concluding diet nor the beginning diet controlled the final cholesterol levels.

Four weeks on the corn oil diet were required for the serum cholesterol levels of rats that had been fed lard for 12 weeks (K_3) to reach the level of rats fed corn oil for 16 weeks (D_3). On the other hand, lard for four weeks (F_3) did not lower the serum level of rats fed corn oil for the previous 12 weeks (C_3). Where dietary changes were not made, the serum cholesterol levels of lard (B_3) and corn oil (G_3) groups were the same at six weeks, but lard resulted in lower levels at 12 ($H_3 < C_3$, $P < 0.10$) and 16 weeks ($I_3 > D_3$, $P < 0.05$). Once again lard resulted in lower levels. It should be emphasized that when the lard to corn oil change was made, the final diet, fed only one third as long, determined the final serum cholesterol level. When a corn oil to lard change was made, the first, not the last, diet determined the final level. Short-term experiments are often of doubtful value because of the influence of pre-experimental conditions.

TABLE 3

Effect of dietary changes of lard and corn oil on rats fed 20% casein[1]

Diet 20% Casein +	Weeks on Diets[2]	Body Wt.[3] gm.	Liver Wt. gm.	% H_2O in Liver	% Fat in Liver Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / 100 ml. Serum
A_3	0	221±15[4,5]	9.8± .80	71± .2	13.9± .39	72± .8	10.0± .29	77±3
B_3 25% Corn Oil	6	434±20	14.0± .92	69± .2	18.4±1.17	84±1.5	15.4± .72	128±2
C_3 Corn Oil	12	478±12	15.3± .59	69± .1	18.7± .58	79±1.5	14.5±2.23	125±4.5
D_3 25% Corn Oil	16	488± 6	15.2±1.31	70± .4	19.3± .52	86±1.8	17.3± .21	148±12
E_3 25% Corn Oil 25% Lard	12 2	480±14	16.4±1.28	68± .2	16.9± .62	83±4.6	13.7± .57	147± 6
F_3 25% Corn Oil 25% Lard	12 4	489± 9	14.7± .61	70± .3	17.9± .64	84±2.2	15.0± .44	142± 5
G_3 25% Lard	6	400± 9	13.6± .17	69± .2	19.3± .86	93±3.5	17.3± .42	129± 5
H_3 25% Lard	12	461± 7	14.2± .45	69± .3	18.3± .53	85±2.1	15.6± .58	111± 5
I_3 25% Lard	16	483± 9	14.5± .90	69± .4	18.1± .66	90±3.7	15.8± .45	118± 2
J_3 25% Lard 25% Corn Oil	12 2	453±15	13.5±1.33	69± .2	16.9± .54	76± .3	13.2± .60	127±6.5
K_3 25% Lard 25% Corn Oil	12 4	497	13.1± .88	70±0	18.7± .58	78± .5	14.6± .49	142± 4

[1] Analyses of other tissues will be found in the Appendix, table 3A, page 25.
[2] Previous diet was Eastern States Dog Ration.
[3] Starting weight of groups other than the control was 200 gm.
[4] Six rats per group.
[5] Standard error of the mean.

The rats fed the corn oil diets for 12 weeks had lower levels of cholesterol in their liver fat than those fed these same diets for six ($C_3 < B_3$, $P < 0.05$) or sixteen weeks ($C_3 < D_3$, $P < 0.025$). A similar trend was noted with the lard diets but only the differences between the six and twelve-week values are worthy of note ($H_3 < G_3$, $P < 0.10$). Changing lard diets to corn oil resulted in lower cholesterol levels in the liver fat ($J_3 < H_3$, $P < 0.005$; $K_3 < H_3$, $P < 0.01$) but changing corn oil diets to lard did not (C_3:E_3 or F_3).

After 16 weeks, corn oil diets resulted in higher liver tissue cholesterol levels than lard ($D_3 > I_3$, $P < 0.025$). However, after six of the sixteen weeks, the cholesterol levels in the livers of rats fed lard were greater than in those fed corn oil ($G_3 > B_3$, $P < 0.05$). Furthermore, when lard had been fed for 12 weeks and a change was made to corn oil, the tissue cholesterol level went down ($J_3 < H_3$, $P < 0.025$). A similar trend was noted when corn oil was changed to lard. Change itself could have been responsible. After four weeks on the new dietary fat, the liver tissue levels were about the same for the groups last fed lard or corn oil (F_3:C_3 and K_3:H_3). These results paralleled those found on the basis of liver fat.

A study of the effects of a weekly alternation of a lower level of corn oil and lard with a lower level of casein was made over 45 weeks (table 4).

The highest liver fat level occurred in the group fed lard ($A_4 > E_4$, $P < 0.025$) and a high level also occurred in the group fed no fat ($E_4 > C_4$, $P < 0.05$). Feeding corn oil alternated with either of these resulted in a level of liver fat similar to that observed when corn oil alone was fed (B_4, D_4: C_4).

In these studies, lard resulted in a lower serum cholesterol level than did corn oil ($A_4 < C_4$, $P < 0.01$), while no dietary fat resulted in the lowest serum level ($E_4 < B_4$, $P < 0.001$). The weekly alternation of lard and corn oil resulted in a lower serum cholesterol level than the continuous feeding of corn oil ($B_4 < C_4$, $P < 0.01$) and about the same level as occurred with the continuous feeding of lard (A_4:B_4). Corn oil alternated weekly with no dietary fat resulted in the highest serum cholesterol level ($D_4 > C_4$, $P < 0.20$), and resulted in a higher level than either alone ($D_4 > A_4$, $D_4 > E_4$, $P < 0.001$).

When lard was fed, the level of cholesterol in the liver fat was lower than when corn oil was fed for 45 weeks ($A_4 < C_4$, $P < 0.001$. When corn oil and lard were alternated the level was higher than that which resulted with lard alone ($B_4 > A_4$, $P < 0.20$) and lower than that from corn oil alone ($B_4 < C_4$, $P < 0.20$). Corn oil alternated with no dietary fat resulted in higher cholesterol levels in liver fat than the levels reached when these diets were fed without change ($D_4 > C_4$, $P < 0.10$;

TABLE 4

Effect of a weekly alternation of lard and corn oil fed with 8% casein for 45 weeks[1]

Diet 8% Casein +	Body Wt. gm.	Liver Wt. gm.	% H_2O Liver	% Fat in Liver Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / 100 ml. Serum
A_4 15% Lard	486	15.9± .77[2,3]	67± .9	37.2±1.79	36±1.1	13.8± .21	171±4
B_4 15% Lard and 15% Corn Oil alternated weekly	490	16.1± .85	68± .4	20.9± .69	42±3.7	11.2±1.33	164±7
C_4 15% Corn Oil	495	17.6± .85	69± .3	22.1± .73	50±2.5	10.4± .37	188±3
D_4 15% Corn Oil and No Fat alternated weekly	480	16.8±1.39	68± .6	21.9± .97	59±3.3	13.7± .76	197±4
E_4 No Fat	208	10.2± .66	66± .7	29.9±2.53	51±3.3	14.9± .28	109±8.5

[1] Analyses of other tissues will be found in the Appendix, Table 4A, page 26.
[2] Six rats per group.
[3] Standard error of the mean.

$D_4 > E_4$, $P < 0.20$). It must, of course, be considered that the rats receiving no dietary fat are about 40% of the size of the others.

Based on the liver tissue, lard resulted in a higher cholesterol level than did the corn oil ($A_4 > C_4$, $P < 0.001$). The highest liver tissue cholesterol level was shown by the group fed no fat ($E_4 > A_4$, $P > 0.025$). Corn oil resulted in lower levels when alternated with lard ($B_4 < A_4$, $P < 0.10$). Therefore, assuming it is desirable to have the lowest cholesterol level possible, the serum level, liver fat level and liver tissue level would allow lard, corn oil or no dietary fat to be considered the most desirable depending which parameter was selected.

The results of experiments utilizing two proteins, either casein or soybean protein, with variations of corn oil and no fat are given in table 5.

That the feeding schedule may effect the level of fat in the liver is shown by a comparison between the group fed casein with no fat for six weeks and casein with 15% corn oil for 20 weeks (E_5) and the group fed these two diets alternated biweekly (F_5). The group on the six and twenty-week schedule had a lower liver fat level than the group receiving the biweekly alternation ($E_5 < F_5$, $P < 0.05$) and also a lower liver fat level than the groups receiving no fat ($E_5 < A_5$, $P < 0.001$) or 15% corn oil ($E_5 < C_5$, $P < 0.10$) for the full 26 weeks.

No dietary fat resulted in lower serum cholesterol levels than 15% corn oil when either soybean protein or casein was fed. However, the lowest level when no fat was fed was shown by the group fed casein ($A_5 < B_5$, $P < 0.005$) while the lowest level with 15% corn oil was the one receiving soybean protein ($D_5 < C_5$, $P < 0.001$). The feeding of no fat for six weeks and 15% corn oil for 20 weeks resulted in similar serum cholesterol levels whether casein or soybean protein was the dietary protein ($E_5 : G_5$). Some influence can be attributed to the first six weeks of the diet, however, because soybean protein resulted in a higher level on the six and twenty arrangement than on the 26 weeks with corn oil ($G_5 > D_5$, $P < 0.025$), while the opposite trend resulted with similar casein treatments ($E_5 : C_5$). Biweekly alternation of corn oil and no fat resulted in a higher serum cholesterol level than either the corn oil or no fat diets fed with soybean protein for 26 weeks ($H_5 > D_5$, $P < 0.05$; $H_5 > B_5$, $P < 0.001$). However, when casein was the protein this biweekly alternation of diet tended to yield a lower serum cholesterol level than when corn oil was fed continuously ($F_5 : C_5$), but higher than when no dietary fat was fed ($F_5 > A_5$, $P < 0.005$). Thus a dietary fat change caused higher serum cholesterol levels with soybean protein, but not always with casein. Dietary protein was a factor in determining serum cholesterol levels. Age must be considered, also, because the 48-week old rats (table 4) fed no fat alternated with corn

Effect of dietary variations of 8% Soybean Protein[1], 8% Casein and Corn Oil[2]

Diet	Weeks on Diet	Body Wt. gm.	Liver Wt. gm.	% H_2O in Liver	% Fat in Liver Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / 100 ml. Serum
A_5 8% Casein and No Fat	26	250±15[3,4]	12.6± .87	66± .7	32.8±1.89	52±2.3	17.0± .89	80±4
B_5 8% Soybean Protein and No Fat	26	282±19	11.2± .64	66± .3	29.5± .52	47±1.3	13.5± .33	101±4
C_5 8% Casein and 15% Corn Oil	26	478±10	15.8± .50	67± .6	18.2± .76	100±2.4	17.0± .33	144±5
D_5 8% Soybean Protein and 15% Corn Oil	26	366±26	11.6±1.19	68± .6	20.3±1.06	88±3.9	17.7± .98	116±4
E_5 8% Casein and No Fat then 8% Casein and 15% Corn Oil	6 20	475± 8	16.2± .96	68± .1	16.7± .33	79±1.8	13.2± .36	129±11
F_5 8% Casein and No Fat alternated biweekly with 8% Casein and 15% Corn Oil	26	466±11	15.8± .62	68± .8	24.4±3.39	59±5.0	12.2± .84	130±12
G_5 8% Soybean Protein and No Fat, then 8% Soybean Protein and 15% Corn Oil	6 20	388±20	12.7± .58	67± .3	19.4±1.14	74±3.7	13.9± .23	134±5
H_5 8% Soybean Protein and No Fat alternated biweekly with 8% Soybean Protein and 15% Corn Oil	26	377±25	12.7± .71	68± .3	21.3±1.47	72±4.2	15.2± .46	144±10

[1] Promine.
[2] Analyses of other tissues will be found in the Appendix, Table 5A, page 27.
[3] Six rats per group.
[4] Standard error of the mean.

oil had higher serum cholesterol levels than the rats fed corn oil with casein ($D_4 > C_4$, $P < 0.20$).

The concentration of cholesterol in liver fat is lower in animals fed no fat as compared to those fed corn oil for 26 weeks ($A_5 < C_5$, $B_5 < D_5$, $P < 0.001$). The biweekly alternation of casein and no fat with casein and corn oil resulted in a lower level of cholesterol in the liver fat than the six and twenty alternation ($F_5 < E_5$, $P < 0.005$) or when corn oil was fed for the 26 weeks ($F_5 < C_5$, $P < 0.001$). With soybean protein, the biweekly alternation (H_5) and the six and twenty change (G_5) resulted in similar levels which were lower than when corn oil was fed with soybean protein for 26 weeks ($G_5 < D_5$, $P < 0.005$). The highest level of liver fat cholesterol was in the groups fed 8% casein with corn oil for the 26 weeks ($C_5 > D_5$, $P < 0.05$).

When casein was fed with no fat, higher tissue cholesterol levels resulted than when soybean protein was fed with no fat ($A_5 > B_5$, $P < 0.005$). On the other hand, casein and corn oil alternated biweekly with casein and no fat resulted in lower liver tissue cholesterol levels than did soybean protein under these conditions ($F_5 < H_5$, $P < 0.025$).

In the next experiment, daily changes in the diet, to which the rats had free access, were made to assess the effect of alternate fast days at two levels of protein (table 6). When a diet containing 20% casein and 15% corn oil was fed daily for eight weeks the rats weighed 352 grams (F_6), while alternate day feeding of this diet resulted in rats weighing 205 grams (A_6). The liver weights of these rats, fed daily was twice that of those fed every second day ($F_6 > A_6$, $P < 0.001$).

The level of fat in the livers was one-fourth less ($F_6 < A_6$, $P < 0.025$) in the rats fed every day.

Corresponding daily alternations of no food and corn oil resulted in higher serum cholesterol levels in the groups fed 8% casein as compared to the corresponding ones fed 20% casein ($B_6 > A_6$, $P < 0.005$; $D_6 > C_6$, $P < 0.005$). The change from a higher to a lower corn oil level in the change pattern resulted in a lower serum cholesterol level with the groups receiving both 8 and 20% casein ($D_6 < B_6$, $P < 0.01$; $C_6 < A_6$, $P < 0.025$).

The lowest level of cholesterol in the liver fat occurred in the low protein group fed the lowest levels of corn oil on alternate days ($D_6 < C_6$, $P < 0.001$). The highest level among the groups fed on alternate days occurred with the high protein, highest corn oil diet ($A_6 > C_6$, $P < 0.025$). The rats fed daily, which were considerably larger, had a much higher level of cholesterol in their liver fat ($F_6 > A_6$, $P < 0.001$).

The liver tissue cholesterol level of the group fed daily (F_6) was not significantly higher than that of the groups fed every second day

TABLE 6

Effect of daily alternations of diet during eight weeks

Diet	Body Wt. gm.	Liver Wt. gm.	% H_2O in Liver	% Fat in Liver Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / 100 ml. Serum
A_6 1. 20% Casein + 15% Corn Oil 2. Fast Alternated Daily	205±11[1,2,3]	7.17± .40	69± .4	20.0±1.09	48±1.6	8.9± .71	101±3.5
B_6 1. 8% Casein + 15% Corn Oil 2. Fast Alternated Daily	152±10	7.31± .42	71± .5	21.5±1.48	42±1.2	9.5± .25	129±6
C_6 1. 20% Casein + 15% Corn Oil 2. Fast 3. 20% Casein + 2% Corn Oil 4. Fast Alternated Daily	167± 9	7.03± .53	70± .2	23.5± .76	42±1.4	9.8± .24	90±2
D_6 1. 8% Casein + 5% Corn Oil 2. Fast 3. 8% Casein + 2% Corn Oil 4. Fast Alternated Daily	144± 5	6.84± .42	71± .2	23.9± .59	32±1.1	8.0± .37	102±5
E_6 1. Dog Chow 2. Fast Alternated Daily	204± 8	7.53± .42	70± .2	21.2±1.12	47±1.1	10.3± .37	M[4]
F_6 20% Casein + 15% Corn Oil	352± 9	14.3± .98	68± .3	15.5±1.13	75±3.7	10.6± .38	M

[1] Six rats per group.
[2] Standard error of the mean.
[3] Starting weights averaged 69 gm.
[4] Missing.

The difference which was so pronounced on the basis of liver fat is not apparent when studied on the basis of concentration in liver tissue.

In the preceding experiment no attempt was made to control the amount of food eaten by the different groups, so that the comparison of dietary patterns was influenced by different food intakes. The food intake was controlled in the experiments presented in table 7. Where fasting was done on alternate days, the group fed 8% casein and 5% corn oil diet was allowed to eat *ad libitum* on its feeding days. The amount that this group ate was fed to the other groups on the following day and the other groups fasted on the days that the control group was feeding. Where no dietary changes were made, half the amount eaten by the control group was fed daily. Thus all the groups presented in table 7 received the same amount of food.

All groups that were fed daily (I_7, J_7, K_7, L_7) gained more weight ($P<0.05$) than the corresponding groups (A_7, C_7, E_7, G_7) fed on alternate days even though the same quantity of food was consumed by all groups. In fact, all groups that consumed their food on alternate days, with the exception of the high protein (20% casein) high unsaturated fat (15% of corn oil) group (G_7), were of lower weight than any of the groups fed daily ($H_7<J_7$, $P<0.20$). Of the rats fed on alternate days or those fed every day, those on the high protein, high-fat diets (20% casein and 15% fat, G_7, H_7 and L_7) gained the most weight.

The groups of rats fed 20% casein on alternate days had a lower percentage of liver fat than those fed 8% casein on alternate days (E_7, F_7, $G_7<A_7$, B_7, C_7, D_7, $P<0.025$). This difference did not occur in the rats fed daily (K_7, L_7: I_7, J_7). The level and kind of fat in the diet did not affect the differences which occurred with the alternate day feeding.

Of the groups fed on alternate days, the one fed the diets containing 8% casein and 15% corn oil (C_7) had the highest level of serum cholesterol ($C_7>G_7$, $P<0.005$) and the group fed 20% casein and 5% lard (F_7) had the lowest level ($F_7<E_7$, $P<0.05$). The other groups fed on alternate days were similar with the exception of the groups fed 20% casein and 15% lard (H_7) or corn oil (G_7) which tended to be slightly higher ($H_7>D_7$, $P<0.20$). The rats fed daily (I_7—L_7) had similar cholesterol levels. Differences which were apparent in the data presented from previous experiments (tables 3, 4 and 5) were not shown in this experiment. Both the controlled food intake and the relatively short period of time (9 weeks) probably affected results.

The concentration of cholesterol in the liver fat was affected by the level of protein and the method of feeding. Of the rats on the alternate day feeding schedule, those fed the low protein (8% casein) had a lower level of cholesterol in the liver fat than those fed the high protein (20% casein diets) (A_7-$D_7<E_7$-H_7, $P<0.005$). This dif-

Diet[1]	Body Wt.[2] gm.	Liver Wt. gm.	% H_2O in Liver	% Fat in Liver Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	100 ml. Serum
A_7 1. 8% Casein + 5% Corn Oil 2. Fast Alternated Daily	133±4[3,4]	6.4± .23	72± .5	19.5± .81	57.3± .89	11.1± .32	95±2.0
B_7 1. 8% Casein + 5% Lard 2. Fast Alternated Daily	131±6	6.7± .37	71± .3	19.2±1.10	58.2±1.97	11.1± .46	95±6.0
C_7 1. 8% Casein + 15% Corn Oil 2. Fast Alternated Daily	119±4	5.5± .29	72± .2	20.1± .51	54.2±1.73	10.8± .95	133±4.0
D_7 1. 8% Casein + 15% Lard 2. Fast Alternated Daily	136±4	5.8± .32	71± .3	21.1±1.09	54.9±2.60	11.5± .50	97±3.0
E_7 1. 20% Casein + 5% Corn Oil 2. Fast Alternated Daily	160±7	7.3± .58	71± .2	17.6± .63	64.1±1.50	11.2± .83	92±3.1
F_7 1. 20% Casein + 5% Lard 2. Fast Alternated Daily	140±3	6.4± .34	71± .4	17.1± .35	65.0±1.70	11.2± .39	78±4.0
G_7 1. 20% Casein + 15% Corn Oil 2. Fast Alternated Daily	182±7	7.7± .27	71± .2	17.8± .72	66.4±2.00	11.8± .36	109±4.0
H_7 1. 20% Casein + 15% Lard 2. Fast Alternated Daily	171±4	7.4± .41	70± .3	17.5± .48	61.0±1.20	10.8± .50	108±7.0
I_7 8% Casein + 5% Corn Oil	183±3	7.9± .57	70± .2	21.3± .66	57.7±9.00	12.6± .53	128±3.0
J_7 8% Casein + 15% Corn Oil	182±5	7.0± .23	70± .5	21.7±1.43	61.4±1.40	13.3±1.03	129±3.0
K_7 20% Casein + 5% Corn Oil	184±2	8.3± .34	69± .7	21.6±1.09	43.0±2.30	9.2±1.22	121±6.0
L_7 20% Casein + 15% Corn Oil	242±6	9.4± .42	69± .2	20.2± .72	54.0±2.20	10.8± .99	126±5.0

[1] Where a daily alternation was made in the diet, the amount of food offered all groups was the same and was determined by the first group. The last four groups

[2] Starting weights averaged 43 grams.
[3] Six rats per group.
[4] Standard error of the mean.

erence was reversed in the groups fed daily; the rats receiving the high protein diets had a lower level of cholesterol in the liver fat than those fed the low protein diets (K_7, $L_7 < I_7$, J_7, $P < 0.10$). At the lower protein level with alternate day feedings, the level of the fat in the diet affected the concentration of cholesterol in the liver fat. The groups receiving 5% fat (corn oil or lard) had a higher level of cholesterol in liver fat than those receiving 15% fat (A_7, $B_7 > C_7$, D_7, $P < 0.05$). The feeding of 8% casein and 15% corn oil every second day resulted in a lower liver fat cholesterol level than the daily feeding of the same amount of this diet ($C_7 < J_7$, $P < 0.01$). On the other hand 20% casein and 5 or 15% corn oil resulted in lower levels with daily feeding (K_7, $L_7 < E_7$, G_7, $P < 0.001$).

The cholesterol levels in the liver tissue were similar for all groups with alternate day feeding. The groups fed daily showed similar levels but larger standard errors. The higher protein diet reduced the cholesterol level in the liver (K_7, $L_7 < I_7$, J_7, $P < 0.001$).

In another similarly designed experiment, adult rats, weighing 180 grams, were fed an amount of food limited by the amount consumed by the group fed 8% casein 5% corn oil diet on alternate days (table 8). The rats fed on a daily basis gained more weight than the corresponding groups fed the same amount of food but on an alternate day basis (E_8, $F_8 > A_8$, B_8; $P < 0.001$). The high-protein, high-fat diet resulted in the greatest growth of the groups receiving the alternate day feeding schedule ($D_8 > C_8$, $P < 0.001$). The results agree with those found for weanling rats (table 7).

More cholesterol was found in liver fat ($P < 0.001$), in the liver tissue ($P < 0.005$) and in serum ($P < 0.001$) when rats were fed daily (E_8, $F_8 > A_8$-D_8). The higher level of corn oil resulted in higher levels of serum cholesterol both when the diets were fed on alternate days ($B_8 > A_8$; $D_8 > C_8$, $P < 0.025$) and when fed daily ($F_8 > E_8$, $P < 0.20$).

DISCUSSION

Cholesterol levels in the serum, and to a lesser extent in the tissue, are still receiving much attention despite the lack of a direct cause and effect relationship between cholesterol levels and the occurrence of atherosclerosis. Therefore, it is mandatory that the significance of changes in cholesterol levels be interpreted cautiously. Not only is the cause-effect relation still without establishment, but the many factors which contribute to a change in cholesterol levels must be considered.

A large amount of scientific literature on statistical relationships between cholesterol levels and protein, carbohydrate, fat, minerals, vitamins and other possible dietary components has been published.

TABLE 8

Effect of fasting on alternate days on adult rats fed casein and corn oil for 9 weeks

Diet[1]	Body Wt.[2] gm.	Liver Wt. gm.	% H_2O in Liver	% Fat in Liver Dry Wt.	Mg. Cholesterol / gm. Liver Fat	Mg. Cholesterol / gm. Liver (dry)	Mg. Cholesterol / 100 ml. Serum
A_8 1. 8% Casein + 5% Corn Oil 2. Fast Alternated Daily	257±9[3,4]	11.8± .59	M[5]	21.3±1.09	47.1±2.0	10.0± .48	115±1
B_8 1. 8% Casein + 15% Corn Oil 2. Fast Alternated Daily	272±14	12.0±1.02	M	18.8± .70	60.7±3.3	11.3± .58	129±7
C_8 1. 20% Casein + 5% Corn Oil 2. Fast Alternated Daily	284±2	12.2± .40	70± .3	19.6± .68	51.5±3.8	10.0± .59	88±6
D_8 1. 20% Casein + 15% Corn Oil 2. Fast Alternated Daily	323±3	13.2± .40	70± .2	19.1± .40	60.5±4.2	11.6± .94	118±9
E_8 8% Casein + 5% Corn Oil	327±7	12.0± .47	70± .3	19.3± .54	80.5±5.7	15.5± .98	136±4
F_8 8% Casein + 15% Corn Oil	367±9	13.1± .93	69± .3	18.7± .75	70.7±2.2	13.3± .86	152±11

[1] Where a daily alternation was made in the diet, the amount of food offered was the same and was determined by the first group. The last two groups were fed half this amount each day.
[2] Starting weight averaged 180 grams.
[3] Six rats per group.
[4] Standard error of the mean.
[5] Missing.

The work presented in this manuscript concerns itself with rat studies. While the rat does not develop atherosclerosis except under extreme conditions, the metabolism of cholesterol in the rat is likely to closely parallel that in humans. The utilization of feeding of cholesterol, sodium cholate, or thiouracil to raise cholesterol levels was not done and the cholesterol levels were not fasting levels (and, hence, are higher (6) than the fasting levels). The number of dietary variables was kept low—casein and soybean were sources of protein and lard and corn oil as sources of fat. The length of the experiment, the age of the rats, and dietary changes were varied.

The results showed that the length of time that the experiment was run and the length of time of the dietary change affected the results. The shortest period—nine weeks—is longer than in many experiments reported in the literature. The longest period—45 weeks—is about 40% of the life span of the rat.

Both the level and the kind of protein affected the level of cholesterol in the serum and liver so that when two proteins and two fats were studied at two different levels of each, a very complex set of variables was being considered. Caution is necessary when generalizations are made on the basis of one series of experiments.

The change from one dietary protein or fat to the other was not consistently reflected in the data from the group fed the beginning diet or the group fed the final diet. Therefore, the pre-experimental diets in short-term experiments are of great importance.

A factor which cannot be readily evaluated is the effect of change itself. Liver cholesterol levels went down after two weeks when a dietary change was made from corn oil to lard or from lard to corn oil. In each case the level had risen again after four weeks of the new diet. This change in the level of liver cholesterol was not attributable to the influence of the corn oil or the lard when other factors were held constant.

In those cases where lard and corn oil were fed consistently and the data compared, lard resulted in the lower serum cholesterol level in five cases, resulted in the same level as corn oil in two cases and resulted in a higher level in just one case. When a daily alternation with a fast was done, lard resulted in lower serum cholesterol levels in two cases and the same levels in two cases. Corn oil is not superior in the maintenance of a lower serum cholesterol level in the rat.

Daily alternations of fasting and 8% casein with 15% corn oil in the diet resulted in a lower serum cholesterol level than the daily feeding of the same amount of food when the experiment was begun with adult animals, but the level was nearly the same when weanling rats were used to start the experiment. Here the age of the animals affected the results.

The ultimate of variability was shown using the data from table 4. These data showed that whether lard or corn oil reduced cholesterol levels depended on which carcass component, serum, liver fat or liver, was selected for examination. Hence, the carcass component used to evaluate cholesterol levels is an important factor. Serum cholesterol levels do not necessarily reflect the tissue cholesterol levels. In previous experiments it was shown that cholesterol levels in the brain were higher while the serum cholesterol levels were lower in rats that were stressed by physical exercise (2).

In conclusion, it must be emphasized that cholesterol levels are affected by many factors such as age, dietary fat and protein kinds and levels, method of feeding and the tissue under consideration. Considerable caution must be used in the interpretation of results from experiments where cholesterol level changes are the major data.

SUMMARY

Rats were fed two proteins, casein and soybean protein, and two fats, corn oil and lard, at several concentrations. Changes in the levels and kinds of fat and protein were made to evaluate the effect of dietary change. Changes were made daily, weekly, and after several weeks.

The following results were reported: weights of the complete rat, livers, brains and hearts; the mositure and fat content of livers, hearts, brains and muscles; and the cholesterol levels of the livers, hearts, brains and the blood sera. The data representing the cholesterol levels of serum, liver fat and liver was not attributable to the influence of the corn oil or the lard when other factors were held constant.

In those cases where lard and corn oil were fed consistently and the data compared, lard resulted in the lower serum cholesterol level in five cases, resulted in the same level as corn oil in two cases and resulted in a higher level in just one case. When a daily alternation with a fast was done, lard resulted in lower serum cholesterol levels in two cases and the same levels in two cases. Corn oil is not superior to lard in the maintenance of a lower serum cholesterol level in the rat.

Daily alternations of fasting and 8% casein with 15% corn oil in the diet resulted in a lower serum cholesterol level than the daily feeding of the same amount of food when the experiment was begun with adult animals, but the level was nearly the same when weanling rats were used to start the experiment. Here the age of the animals affected the results.

The ultimate of variability was shown using the data from table 4. These data showed that drawing a conclusion as to whether lard or corn oil reduced cholesterol levels depended on which carcass component, serum, liver fat or liver tissue, was selected for consideration.

Cholesterol levels were affected by factors such as age, dietary fat and protein kinds and levels, the method of feeding, the length of time and the tissues studied.

Dietary changes gave results which were not consistently typical of the beginning or the final diets.

In most cases lard resulted in lower cholesterol levels than did corn oil.

In most cases rats fed the same amount of food on alternate days had lower cholesterol levels than those fed this amount of food in daily feeding.

LITERATURE CITED

1. Radke, F. H., H. De Haas, E. K. Gabrielson and M. E. Norburg. Relationships of proteins, essential fatty acids and cholesterol in the rat and the mouse. Maine Agricultural Experiment Station Bulletin T 21, 1966.

2. Radke, F. H., H. De Haas and S. C. Jacobs. The effect of stress on the formation of tissue lipid from dietary protein. Maine Agricultural Experiment Station Bulletin T 16, 1965.

3. Tucker, R. E., ed., Dietary protein relationships to serum lipids. Rhode Island Agricultural Experiment Station Bulletin 385, 1966.

4. Radke, F. H., M. E. Norburg and E. K. Gabrielson. Effect of dietary protein and fat on the deposition of C^{14} from *Rhodospirillum rubrum* in rats conditioned to a low protein—fat-free diet. Maine Agricultural Experiment Station Bulletin T 1, 1962.

5. Enos, W. F., J. C. Beyer and R. H. Holmes. Pathogenesis of coronary disease in American soldiers killed in Korea. J. Am. Med. Assoc. *158*, 912 (1955).

6. Mayfield, H. L. and R. R. Roehm. Effect of fasting on serum and liver lipid levels in the rat. J. Nutrition *75*, 265 (1961).

Effect of increasing of the dietary fat level on rat tissues

Diet 20% Casein + 15% Corn Oil for 7 1/2 weeks, then the following for 26 weeks	Body Weight, gm.	Heart Weight, gm.	Muscle Weight, gm.	Brain Weight, gm.	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain
A_1 20% Casein + 20.6% Corn Oil	500[1]	1.76±0.04[2]	3.62±0.23	1.34±0.16	77±0.5	65±1.1	76±0.8
B_1 20% Casein + 20.6% Lard	498	1.73±0.09	2.94±0.19	1.46±0.16	77±0.4	68±2.7	77±0.5
C_1 20% Soybean Protein[3] + 20.6% Corn Oil	495	1.73±0.06	3.16±0.22	1.56±0.11	77±0.4	66±3.4	77±0.3
D_1 20% Soybean Protein + 20.6% Lard	499	1.57±0.05	2.27±0.27	1.61±0.08	77±0.2	68±1.3	77±0.2
E_1 8% Casein + 20.6% Corn Oil	490	1.58±0.05	2.34±0.26	1.54±0.08	77±0.2	70±2.5	77±0.2

20% Casein + 15% Corn Oil for 7 1/2 weeks, then the following for 26 weeks	% Lipid in Heart, Dry Wt.	% Lipid in Muscle, Dry Wt.	% Lipid in Brain, Dry Wt.	Mg. Cholesterol / gm. Heart Lipid	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Lipid	Mg. Cholesterol / gm. Brain (dry)
A_1 20% Casein + 20.6% Corn Oil	17.0±1.94	45.9±4.74	46.2±1.72	79±3.6	12.2±0.41	135±3.4	58.7±3.45
B_1 20% Casein + 20.6% Lard	18.4±1.23	37.7±8.58	43.9±0.48	65±4.4	11.9±0.31	156±3.4	68.3±1.40
C_1 20% Soybean Protein[3] + 20.6% Corn Oil	18.0±0.98	33.6±6.30	44.3±0.74	87±3.0	14.7±0.31	158±3.7	69.9±1.81
D_1 20% Soybean Protein + 20.6% Lard	17.5±0.76	32.5±3.63	45.0±0.73	65±2.1	10.7±0.29	155±3.5	71.9±2.85
E_1 8% Casein + 20.6% Corn Oil	16.8±0.95	22.0±5.17	45.2±0.67	69±1.6	11.5±0.39	186±1.2	84.4±1.94

[1] Six rats per group.
[2] Standard error of the mean.
[3] Promine.

TABLE 2A

Effect of increasing the fat and protein level on rat tissues

Diet 8% Casein + No fat for 10 weeks, then the following for 26 weeks	Body Weight[1], gm.	Heart Weight, gm.	Muscle Weight, gm.	Brain Weight, gm.	% H_2O in Heart	% H_2O in Muscle	% H_2O in Brain
A_2 40% Casein + 15% Corn Oil	497[2]	1.68±0.13[3]	3.07±0.74	1.58±0.14	77±0.3	69±2.0	78±0.3
B_2 40% Casein + 15% Lard	452	1.50±0.11	2.50±0.03	1.44±0.21	77±0.7	70±1.0	78±0.4
C_2 8% Casein + 20% Corn Oil	422	1.58±0.08	2.89±0.01	1.64±0.21	78±0.4	69±1.2	78±0.7
D_2 8% Casein + 20% Lard	486	1.49±0.09	2.58±0.19	1.55±0.15	78±0.4	73±1.2	77±0.6

8% Casein + No fat for 10 weeks, then the following for 26 weeks	% Lipid in Heart, Dry Wt.	% Lipid in Muscle, Dry Wt.	% Lipid in Brain, Dry Wt.	Mg. Cholesterol / gm. Heart Lipid	Mg. Cholesterol / gm. Heart (dry)	Mg. Cholesterol / gm. Brain Lipid	Mg. Cholesterol / gm. Brain (dry)
A_2 40% Casein + 15% Corn Oil	17.8±0.79	34.0±5.54	43.2±0.86	75±2.1	13.3±0.37	170±5.6	73.6±3.77
B_2 40% Casein + 15% Lard	15.3±1.10	28.4±3.44	43.0±0.69	80±9.5	12.0±0.73	187±5.5	80.6±3.68
C_2 8% Casein + 20% Corn Oil	17.5±0.49	32.3±3.09	43.5±0.75	74±2.1	13.0±0.59	181±1.7	78.6±0.87
D_2 8% Casein + 20% Lard	16.0±0.15	19.8±3.95	43.5±0.87	74±3.3	11.9±0.46	171±3.3	74.3±2.69

[1] Starting weight of all groups was 189 gm.
[2] Four rats in the first group, three in each of the others.
[3] Standard error of the mean.

TABLE 3A

Effect of dietary changes of lard and corn oil on rats fed 20% casein

Diet 20% Casein +	Weeks on Diet[1]	Body Weight[2], gm.	Heart Weight, gm.	% H_2O in Heart	% Lipid in Heart Dry Wt.	Mg. Cholesterol / gm. Heart Lipid	Mg. Cholesterol / gm. Heart (dry)
A_3	0	221±15[3,4]	0.88± .06	78±0.2	15.7±0.24	61±2.1	9.8± .15
B_3 25% Corn Oil	6	434±20	1.36± .08	77±0.1	14.9±0.59	64±1.2	9.9± .11
C_3 25% Corn Oil	12	478±12	1.54± .14	76±0.9	19.9±2.87	62±1.9	10.8± .69
D_3 25% Corn Oil	16	488± 6	1.30± .06	77±0.3	15.7±0.60	65±2.2	10.1± .35
E_3 25% Corn Oil 25% Lard	12 2	480±14	1.32± .02	77±0.2	16.4±0.36	60±1.6	9.9± .11
F_3 25% Corn Oil 25% Lard	12 4	489± 9	1.38± .08	76±0.7	20.9±2.48	54±3.8	10.7± .52
G_3 25% Lard	6	400± 9	1.41± .07	76±0.4	16.4±1.44	57±4.6	9.0± .34
H_3 25% Lard	12	461± 7	1.35± .06	77±0.6	16.4±1.17	63±3.4	10.1± .42
I_3 25% Lard	16	483± 9	1.25± .04	76±0.7	18.3±2.12	59±3.9	9.6± .30
J_3 25% Lard 25% Corn Oil	12 2	453±15	1.27± .07	77±0.5	17.5±1.67	57±3.1	9.8± .34
K_3 25% Lard 25 %Corn Oil	12 4	497± 0	1.31± .07	77±0.3	15.4±0.61	64±2.2	9.8± .18

[1] Previous diet was Eastern States Dog Ration.
[2] Starting weight of groups other than the control was 200 gm.
[3] Six rats per group.
[4] Standard error of the mean.

TABLE 4A

Effect of a weekly alternation of lard and corn oil fed with 8% casein for 45 weeks

Diet 8% Casein +	Body Weight, gm.	Heart Weight, gm.	Brain Weight, gm.	% H_2O in Heart	% H_2O in Brain	% Lipid in Heart Dry Wt.	% Lipid in Brain, Dry Wt.
A_4 15% Lard	486[1,2]	1.29±0.04	1.92±0.10	76±0.2	76±0.7	13.3±1.80	40.8±3.60
B_4 15% Lard and 15% Corn Oil Alternated weekly	490	1.26±0.05	1.92±0.07	76±1.3	75±0.5	14.5±0.96	43.9±2.44
C_4 15% Corn Oil	495	1.30±0.09	2.01±0.05	75±0.8	75±0.2	17.8±3.84	45.9±4.52
D_4 15% Corn Oil and No Fat Alternated weekly	480	1.32±0.07	1.91±0.06	77±0.1	74±0.9	14.4±2.04	43.2±1.30
E_4 No Fat	208	0.90±0.09	1.72±0.02	76±0.5	76±0.4	14.9±0.87	45.2±4.57

Diet 8% Casein +	Mg. Cholesterol gm. Heart Lipid	Mg. Cholesterol gm. Heart (dry)	Mg. Cholesterol gm. Brain Lipid	Mg. Cholesterol gm. Brain (dry)	
A_4 15% Lard	76±3.7	11.0±0.30	177±9.1	86.0±3.14	
B_4 15% Lard and 15% Corn Oil Alternated weekly	75±1.5	10.5±0.53	170±3.0	77.1±2.05	
C_4 15% Corn Oil	73±3.7	10.6±0.46	172±4.5	80.4±2.86	
D_4 15% Corn Oil and No Fat Alternated weekly	87±7.1	11.0±0.86	171±1.3	77.2±1.29	
E_4 No Fat	56±3.1	7.9±0.55	174±3.9	71.4±2.87	

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

TABLE 4A
Effect of a weekly alternation of lard and corn oil fed with 8% casein for 45 weeks

Diet 8% Casein +	Body Weight, gm.	Heart Weight, gm.	Brain Weight, gm.	% H_2O in Heart	% H_2O in Brain	% Lipid in Heart Dry Wt.	% Lipid in Brain, Dry Wt.
A_4 15% Lard	486[1,2]	1.29±0.04	1.92±0.10	76±0.2	76±0.7	13.3±1.80	40.8±3.60
B_4 15% Lard and 15% Corn Oil Alternated weekly	490	1.26±0.05	1.92±0.07	76±1.3	75±0.5	14.5±0.96	43.9±2.44
C_4 15% Corn Oil	495	1.30±0.09	2.01±0.05	75±0.8	75±0.2	17.8±3.84	45.9±4.52
D_4 15% Corn Oil and No Fat Alternated weekly	480	1.32±0.07	1.91±0.06	77±0.1	74±0.9	14.4±2.04	43.2±1.30
E_4 No Fat	208	0.90±0.09	1.72±0.02	76±0.5	76±0.4	14.9±0.87	45.2±4.57

Diet 8% Casein +	Mg. Cholesterol gm. Heart Lipid	Mg. Cholesterol gm. Heart (dry)	Mg. Cholesterol gm. Brain Lipid	Mg. Cholesterol gm. Brain (dry)
A_4 15% Lard	76±3.7	11.0±0.30	177±9.1	86.0±3.14
B_4 15% Lard and 15% Corn Oil Alternated weekly	75±1.5	10.5±0.53	170±3.0	77.1±2.05
C_4 15% Corn Oil	73±3.7	10.6±0.46	172±4.5	80.4±2.86
D_4 15% Corn Oil and No Fat Alternated weekly	87±7.1	11.0±0.86	171±1.3	77.2±1.29
E_4 No Fat	56±3.1	7.9±0.55	174±3.9	71.4±2.87

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