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A study of the effects of sitosterol ingestion on the serum cholesterol concentrations of two genetically different strains of laboratory mice

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A STUDY OF THE EFFECTS OF SITOSTEROL INGESTION ON THE SERUM CHOLESTEROL CONCENTRATIONS OF TWO GENETICALLY DIFFERENT STRAINS OF LABORATORY MICE

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INTRODUCTION

A possible interrelationship between serum cholesterol level and atherosclerosis has long interested investigators in the fields of physiology and biochemistry. Cholesterol is found in very high concentrations in the plaques that occlude coronary arteries in man and laboratory animals. It is generally agreed that hypercholesteremia favors the appearance of atherosclerotic lesions.[1]

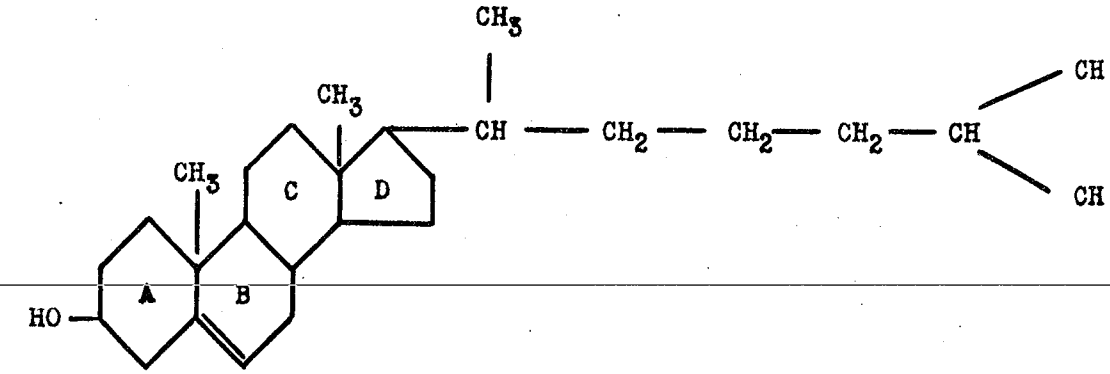
In recent years, much information has been accumulated regarding the factors which affect the serum cholesterol level. Exact and detailed biochemical mechanisms are still not clearly understood. However, it has been demonstrated that the serum cholesterol levels of man and several experimental animals can be lowered significantly by various dietary regimens and drug administrations.[2,3,4,5] One antihypercholesteremic agent that has aroused considerable interest is the sitosterol group.

The purpose of the present study is to investigate the effect of sitosterols from soya bean oil powder on the serum cholesterol levels and to evaluate that effect on the genetic differences of two strains of laboratory mice. These two strains of mice, produced previously by selective breeding, differ from each other in their serum cholesterol concentrations.

With the possible exception of certain bacteria and primitive algae, some type of sterol is found in all living organisms. Chemically, sterols are a class of solid alcohols; they have a structural ring system (1,2-cyclopentenoperhydrophenanthrene ring). Sterols are waxy substances derived from plant and animal tissues. Sitosterols are the most common sterols found in the plant kingdom, and cholesterol is the most common animal sterol.

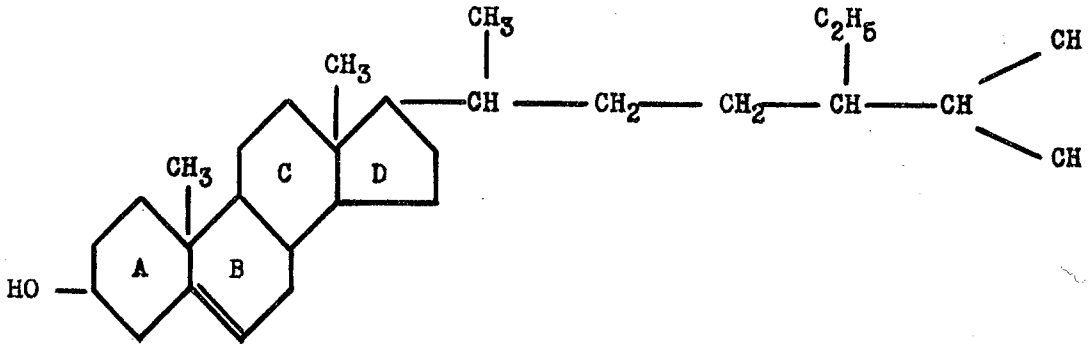
Sitosterol and cholesterol are closely related chemically. Both substances are white, odorless, tasteless, slightly waxy, crystalline solids; they are insoluble in water. The empirical formula for cholesterol is $C_{27}H_{45}OH$; the empirical formula of the sitosterols is $C_{29}H_{49}OH$. Great similarity exists between the structural formulas of cholesterol and the sitosterols. These formulas differ only in sitosterols having an ethyl group substituting for a hydrogen atom on carbon-24. Both molecules exhibit a free hydroxyl group on carbon-3. A comparison of the structural formulas of cholesterol and beta-sitosterols is presented in Figure 1.

Several isomers of sitosterol have been identified; of these beta-sitosterol is the most common. Although only a minor difference exists in chemical structures of cholesterol and beta-sitosterol, they differ greatly in metabolic behavior. Beta-sitosterol differs from



CHOLESTEROL

$C_{27}H_{45}OH$



SITOSTEROL

$C_{27}H_{49}OH$

Figure 1. Structural formulas of cholesterol and sitosterols.

cholesterol in that it is very poorly absorbed in the intestine.[6,7] The antihypercholesteremic effort of beta-sitosterol appears to be an interference with the absorption of cholesterol from the intestine.[8]

Several mechanisms have been postulated concerning the mode of sitosterol action in interfering with cholesterol absorption from the intestine. Pollack has advanced the theory that sitosterol and cholesterol form a 1:1 mixed crystal in the gut.[9] This admixture of cholesterol and sitosterol has a solubility which is only a small percentage of that of cholesterol alone. Since sitosterols act upon both endogenous and exogenous cholesterol, the effect of sitosterol is more efficacious than dietary restriction of cholesterol alone. The formation of a mixed crystal of sitosterols and cholesterol has been confirmed in vitro by Davis of the Lilly Research Laboratories.[10] The formation of such a stable, non-resorbable crystal in the gastrointestinal tract may result in making cholesterol relatively unavailable for absorption into the circulatory system.

The closely related chemical structures of sitosterol and cholesterol form the basis for the second postulate concerning the action of sitosterol in the inhibition of absorption of cholesterol from the intestine. It is believed that sitosterols interfere with cholesterol absorption

by competing for the enzyme necessary for the esterification and absorption of cholesterol. Sitosterols can be esterified in vitro by the same esterase that acts upon cholesterol as a substrate. Sitosterol can therefore tie up a portion of the available esterase in the intestine by the formation of insoluble sitosterol esters. This action prevents the formation of the esters of cholesterol which can be absorbed by the intestinal mucosa. Hernandez, working on rats, provided the main experimental work upon which this second postulate was based.[11]

REVIEW OF LITERATURE

Prior to 1929, the theory was held that plant sterols were absorbed in the intestine and then converted to cholesterol. R. Schoenheimer, in the first important biological work on plant sterols, disproved the above theory.[12] Schoenheimer also proved that sitosterols were not appreciably absorbed from the intestine. Schoenheimer fed experimental animals both cholesterol and plant sterols. He found that only cholesterol was present in an analysis of intestinal lymph.[13] Consistent with this finding was the report by Schoenheimer and others that plant sterols can be recovered unmodified and almost unreduced quantitatively from animal feces. When administered orally to an animal, plant sterols apparently were pharmacologically and nutritionally inert.

Peterson, in 1951, was the first to observe that the addition of plant sterol to the diet results in the lowering of serum cholesterol in white Leghorn chicks.[14] Peterson later compared the amount of atherosclerosis in the aortas of chicks fed a high cholesterol diet with that of chicks fed a high cholesterol diet plus sitosterols. Peterson found that the addition of the soy sterols significantly prevented cholesterol deposition in the aorta.[15]

In 1953, O. J. Pollack, working with cholesterol-fed rabbits, reported the preventative action of soy sterols on the development of hypercholesteremia and atherosclerosis.[16] Shipley, in 1955, confirmed Pollack's finding on rabbits and noted the antihypercholesteremic effect of sitosterols administered to human beings in double blind studies. Since these initial investigations, numerous studies and reports have confirmed the effectiveness of sitosterol in preventing and reducing hypercholesteremia in man and a variety of laboratory animals. Wilkinson, however, reported a failure of sitosterols to affect the serum cholesterol levels of human subjects.[17] Wilkinson's findings are inconsistent with those of the majority of investigators.

The investigations dealing with sitosterols are almost entirely concerned with the reduction or prevention of hypercholesteremia due to dietary factors. The administration of sitosterols to strains of animals differing genetically in serum cholesterol levels has apparently not been attempted to any extent prior to this study. In 1957, Levkoff reported the treatment with sitosterol of two human subjects suffering from familial hypercholesteremia. The subjects were siblings, aged ten and eleven; both had serum cholesterol levels of over 300 mg/100 ml. The mother of the two youngsters had

exhibited a high level of serum cholesterol prior to her death from a stroke at age forty-seven. Two maternal uncles had died at ages fifty and fifty-one from occlusive vascular disease. In this study Levkoff found that, "The ingestion of beta-sitosterol, a sterol derived from plants, brought about a significant reduction in the concentration of cholesterol in the serum of two siblings with familial hypercholesteremia, form fruste."[18] Selection experiments on mice and chickens have produced distinct genetic strains differing in their serum cholesterol levels.[19,20]

MATERIALS AND METHODS

Experimental Mice

The experimental mice used in this study were received from Dr. J. David Carson of the University of the Pacific. A history of these mice is as follows:

In 1962 Carson began the selective breeding of a single strain of white laboratory mice at Utah State University. The trait upon which selection was based was the serum cholesterol concentration. One of the objectives of Carson's research was to diverge the genotypes within this single strain of mice into high and low abilities to synthesize serum cholesterol.

The initial project started with thirty mated pairs of white mice. Serum cholesterol determinations were made on the sixty parent mice as well as on their first generation offspring of 210 individuals. After determinations of their serum cholesterol levels, the mice of the first and of successive generations were mated at approximately eight weeks of age. Full-sib matings were disallowed; the closest inbreeding permitted was that of half-sibs.

By March of 1965 selective pressure based upon serum cholesterol determinations had been exerted upon six generations of mice. At this time the original, single

strain of white mice had diverged into two distinct genetic strains--one high and one low in serum cholesterol. The two strains differed significantly ($P < .01$) as a result of both individual and family selection over six generations. Mice from the second litter of the sixth generation were used as experimental animals in the present study. 102 mice were bled for serum cholesterol determinations on March 24, 1965.

The 102 mice which were tested represented three groups of closed breeding populations: line 1 (high serum cholesterol level), line 2 (low serum cholesterol level), and line 3 (random selection without regard to serum cholesterol level). Table I shows the individual serum cholesterol determinations of the thirty-seven line 1 (high serum cholesterol level) mice. Table II represents the individual serum cholesterol determinations of the forty-two line 2 (low serum cholesterol level) mice. Table III shows the individual serum cholesterol determinations of the line 3 mice which were randomly selected without reference to their serum cholesterol levels. For the purposes of this study, the test and control animals were selected from lines 1 and 2.

Equipment and Facilities

The mice were housed in plastic cages twelve inches long, seven inches wide, and five deep. Each cage held

TABLE I

MOUSE SERUM CHOLESTEROL DETERMINATIONS
LINE 1, HIGH CHOLESTEROL

Lot Number	Mice Tested	Mg. % Cholesterol Mouse A	Mouse B	Mouse C	Mean Cholesterol
MALE MICE					
663	2	136	151	---	143.5
665	3	151	136	137	141.3
667	2	130	133	---	131.5
671	3	123	161	157	147.0
673	2	---	120	136	128.0
677	2	133	119	---	126.0
681	3	161	138	139	146.0
Total	17			Mean	138.9
FEMALE MICE					
664	3	101	104	105	103.3
666	3	115	132	140	129.0
668	3	116	128	115	119.7
672	3	120	111	116	115.7
674	2	144	119	---	131.5
678	3	126	102	97	108.3
682	3	127	125	133	128.3
Total	20			Mean	118.8

NOTE: The 37 mice, both males and females, of line 1 had a mean serum cholesterol level of 128.0 mg. %.

TABLE II

MOUSE SERUM CHOLESTEROL DETERMINATIONS
LINE 2, LOW CHOLESTEROL

Lot Number	Mice Tested	Mg. % Cholesterol Mouse A	Mouse B	Mouse C	Mean Cholesterol
MALE MICE					
601	3	88	152	105	115.0
607	3	110	83	110	101.0
609	3	120	112	122	118.0
611	1	118	---	---	118.0
613	2	127	108	---	117.5
615	2	118	112	---	115.0
617	1	102	---	---	102.0
621	3	118	113	128	119.7
623	1	123	---	---	123.0
Total	19			Mean	114.2
FEMALE MICE					
602	2	89	---	71	80.0
608	2	81	83	---	82.0
610	3	93	93	95	93.7
612	3	98	80	85	87.7
614	2	105	114	---	109.5
616	3	101	106	98	101.7
618	2	97	91	---	94.0
622	3	111	98	112	107.0
624	3	106	135	109	116.7
Total	23			Mean	97.9

NOTE: The 42 mice, both males and females, of line 2 had a mean serum cholesterol level of 105.2 mg. %.

TABLE III

MOUSE SERUM CHOLESTEROL DETERMINATIONS
LINE 3, RANDOM SELECTION WITHOUT REGARD TO
SERUM CHOLESTEROL LEVEL

Lot Number	Mice Tested	Mg. % Cholesterol Mouse A	Mouse B	Mouse C	Mean Cholesterol
MALE MICE					
631	3	118	130	140	129.3
633	1	128	---	---	128.0
637	3	144	126	161	143.7
639	3	123	128	123	124.7
641	3	121	140	121	127.3
Total	13			Mean	131.0
FEMALE MICE					
632	1	135	---	---	135.0
636	3	109	108	87	101.3
638	3	114	115	140	123.0
640	3	125	107	125	119.0
Total	10			Mean	116.5

NOTE: The 23 mice, both males and females, of line 3 had a mean serum cholesterol level of 124.7 mg. %.

only litter mates of the same sex; the number of animals in each cage varied from one to six. Cages were numbered in such a manner as to indicate parentage, generation, litter number, sex, and cholesterol level. The cages were covered with a grill which had an inverted overhead feeding area. The cages were stored on shelves in an air-conditioned animal room maintained at approximately twenty-five degrees centigrade. The shelves were built in the center of the room with space on both sides, and cages were placed on a single level only on each shelf. This placement permitted adequate ventilation and circulation of air. Bedding material was a commercial litter. Cages were washed weekly with a germicidal detergent, and fresh bedding material was provided at that time.

Diets

Tap water and Pureta Mouse Breeder Chow #D 5015 were provided ad libitum. The ingredients of this food was as follows: dried skim milk; ground wheat; brewer's dried yeast; vegetable oil; animal fat preserved with BHA; Vitamin A supplement; D activated plant sterol; 1.4% sterol; and 0. 13 % ferric ammonium citrate. As stated by the manufacturer, the guaranteed analysis of this food was: crude proteins--not less than 17.0%, crude fat--not less than 11.0%, crude fiber--not more than 2.0%, and ash--not more than 5.5%. This food was the diet upon which all

generations of Carson's experimental mice were maintained. It was the basic diet fed to the control animals and the basic diet to which the sitosterols were added for feeding the test group in this study.

The diet for the test animals in this study was prepared in the following manner: The Pureta chow was pulverized in a mechanical blender. Fifty grams of sitosterols were mixed in a mechanical pharmaceutical blender with 950 grams of pulverized chow. The substances were allowed to blend thirty minutes, a time sufficient to allow for complete and thorough mixing. The resultant mixture was dampened with a minimum of tap water and re-formed into pellets of the approximate size of the original Pureta chow. The pellets were dried overnight at a low oven temperature of 175 degrees Fahrenheit. Drying was continued in a low humidity, relatively warm room for three days. Neither mold nor deterioration was noted in these pellets during the course of the experiment. This method of preparing a diet incorporating sitosterols is a modification of that used by Herxman in his rat experiments.[21]

The prepared test pellets contained five per cent sitosterols by weight. Investigators in the field have found a lowering of serum cholesterol in experimental animals with sitosterols fed in concentrations as low as

two per cent.[22] There are no known contraindications to the use of sitosterols, and neither toxicity nor other adverse effects have been observed in administering sitosterols to human beings and various laboratory animals.[23] These findings would be expected because of the biochemical stability and inertness of the sitosterols.

The sitosterols used in this study (Lilly Lot Number 849905) were provided by Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, and were extracted from soya bean oil powder. The substance was stored under the conditions prescribed by the manufacturer until the test diet was compounded. The test diet pellets were stored under similar conditions until they were used in the experiment.

Collection of Serum Samples

A method devised by Carson was used for the collection of blood samples for analysis. Collecting tubes, approximately eighteen millimeters long and four millimeters in diameter were both alcohol and acetone cleaned. The mice were lightly anesthesized with ether, and the distal one-fourth to one-third of their tails was amputated with a very sharp scalpel. Approximately one-half millililiter of blood was collected directly into the capillary tube. Every effort was made to avoid contaminating the specimen with tissue fluid; as little

pressure as possible was exerted on the tail during the collecting period. The tubes were allowed to clot at room temperature for thirty minutes and were then plugged and centrifuged at 2200 R.P.M. for fifteen minutes. No problems were encountered with the separation of the serum from the cellular elements of the blood; hemolysis did not occur. After they were removed from the centrifuge, the samples were scored with a file at the point of juncture of the serum and the blood cells. The tubes were broken at the scored line, and the portion of the tube containing the serum was recapped at both ends and immediately frozen.

Cholesterol Determinations

The frozen serum samples were sent by fastest surface mail to Bio-Science Laboratories, 12330 Santa Monica Boulevard, Los Angeles, California 90025. Air mail was inadvisable because of possible leakage at high altitudes. Serum cholesterol because of its stability at room temperature could be transported to the laboratory in this manner. S. Lawrence Jacobs, Ph.D, Chief of the Special Projects Division at Bio-Science Laboratories, in a letter dated June 9, 1965, outlined the cholesterol procedure used in the sample determinations of this study as follows:

The method used for the determination of serum cholesterol was that in which steroids having the 3B-OH, 5-ENE grouping will give a purple chromophore in the presence of ferric ion, acetic and sulfuric acids. We use the autoanalyzer and employ this reaction to quantitate our cholesterol results.

Plan of the Experiment

Forty-eight mice, three to four months of age, were selected for the purposes of this study from the 102 mice whose serum cholesterol levels had previously been determined. The mice selected for study included twenty-four individuals from the line 1 (high serum cholesterol group) and twenty-four individuals from the line 2 (low serum cholesterol group). The control group consisted of twenty-four mice, and the test group of twenty-four mice. As far as possible, large litters with a nearly equal number of males and females were chosen for study. For example, litter number 665 was composed of six males, and litter number 666 was composed of six females. The two litters were full siblings produced at the same time by the same parents. Three males of litter number 665 and three females of litter 666 were placed in the control group; their litter-mates became part of the test group. Selecting litters of this composition allowed for the closest comparison of the effect of sitosterol administration on the serum cholesterol levels of closely related individuals within a genetic strain.

It also allowed for the comparison of differences between the two sexes.

The test diet containing the sitosterols was fed to the test animals for a period of four weeks. The control group was maintained on their regular laboratory chow for the same period. At the end of the test period, both groups of mice were bled, and their serum cholesterol concentrations determined. A period of four weeks was chosen for the administration of sitosterols to the test group. This length of time is consistent with testing periods selected by previous investigators using sitosterols in animal experimentations.[24,25,26]

Food and water consumption was measured before and during the test period. The purpose of these measurements was to determine the palatability of the test diet and to insure that a possible lowering of serum cholesterol was not due to lowered food consumption.

ANALYSIS OF DATA AND RESULTS

Food Consumption and Environment During Test Period

Two weeks prior to the start of the test period, the original 102 mice consumed an average of 3.2 grams of food daily per mouse during a forty-eight hour period of measurement. During the last week of the test period, the food consumption per mouse for the control group averaged 3.2 grams per day. For this same period, the test group consumed daily an average of 3.2 grams of food per mouse. Daily water consumption averaged 5.1 milliliters per mouse for both groups during the experiment. This result is consistent with the daily consumption of water as measured for a forty-eight hour period for the original 102 mice. This recording of the water consumption of the larger group of mice was made just prior to the beginning of the experiment. It can, therefore, be concluded that any lowering of food consumption due to the addition of sitosterols to the basic diet did not occur. Water consumption also remained consistent during the course of the study.

While the experiment was in progress, both the test and control mice appeared healthy; no deaths occurred in either group. Temperature and the other environmental conditions of the laboratory remained constant.

Cholesterol Determinations at the End of Test Period

On April 27, 1965, the diet containing the sitosterols was administered to the twenty-four animals of the test group; these mice were maintained on the test diet for a period of twenty-eight days. During this same period, the control group of twenty-four mice was fed the regular basic diet. Both groups of mice were bled for serum cholesterol determinations on May 25, 1965. There was an unavoidable delay in the arrival of the sitosterols from the research laboratory of Eli Lilly and Company. Therefore, it was not possible to initiate the experiment any closer to the March 24, 1965 bleeding date of the larger group of mice from which the animals of this study were chosen. However, the selection of the mice for this study was based on their genetic lines rather than on their individual cholesterol determinations. For example, in the original cholesterol determinations of the sixth generation, litter two mice, only three of the Lot number 666 were bled. In the present study, six of this same group of mice were used; three mice were used in the control group and three were used in the test group. These six mice did not necessarily include the three mice which were originally bled in March. Comparison in this study is made between control and test groups of mice with similar

genetic backgrounds. Table IV represents the individual serum cholesterol determinations of the control group of the line 1 mice (high serum cholesterol level) and the line 2 mice (low serum cholesterol) at the end of the experiment. Table V shows the individual serum cholesterol determinations of the test group of the line 1 and line 2 mice at the conclusion of the experiment.

Precision of Methodology

The precision of the methodology used by Bio-Science was tested by submitting duplicate samples of twenty-two mouse serums. The differences between the two sets of data were shown not to be significant when tested by the Wilcoxan signed rank test.[27]

Statistical Analyses

The means of the serum cholesterol determinations, plus or minus one standard error, of the various groupings of test and control animals are summarized in Table VI. The means for the strain differences in each instance show higher means for the line 1 (high serum cholesterol) mice than for the line 2 (low serum cholesterol) mice. The mean serum cholesterol determinations for the female mice in the various groups are consistently lower than those of the male mice. However, an exception to this difference is found between the males and females of the line 1 mice; the means, in this case, are almost the same. A lower

TABLE IV

MOUSE SERUM CHOLESTEROL DETERMINATIONS

CONTROL GROUP

Lot Number	Mice Tested	Mg. % Cholesterol Mouse A	Mouse B	Mouse C	Mean Cholesterol
LOW STRAIN MALES					
609	2	112	127	---	143.5
613	3	140	115	134	129.7
621	1	107	---	---	107.0
Total	6			Mean	122.5
LOW STRAIN FEMALES					
612	3	118	116	100	111.3
614	1	110	---	---	110.0
622	2	95	102	---	98.5
Total	6			Mean	106.8
HIGH STRAIN MALES					
665	3	127	132	148	135.7
671	3	124	140	122	128.7
Total	6			Mean	132.2
HIGH STRAIN FEMALES					
666	3	123	127	129	126.2
672	3	139	143	137	139.6
Total	6			Mean	133.0

TABLE V

MOUSE SERUM CHOLESTEROL DETERMINATIONS

TEST GROUP

Lot Number	Mice Tested	Mg. % Cholesterol Mouse A	Mouse B	Mouse C	Mean Cholesterol
LOW STRAIN MALES					
609	2	117	120	---	118.5
613	2	133	137	---	135.0
621	2	101	108	---	104.5
Total	6			Mean	119.3
LOW STRAIN FEMALES					
612	3	99	105	101	101.7
614	1	120	---	---	120.0
622	2	95	102	---	98.5
Total	6			Mean	103.7
HIGH STRAIN MALES					
665	3	132	137	125	131.3
671	3	131	138	123	130.7
Total	6			Mean	131.0
HIGH STRAIN FEMALES					
666	3	101	134	110	115.0
672	3	124	114	111	116.3
Total	6			Mean	115.7

TABLE VI

MOUSE SERUM CHOLESTEROL DETERMINATIONS

MEANS ± ONE STANDARD ERROR

Group	n	mg. per 100 ml.
Control	24	123.6 ± 2.9
Test	24	117.4 ± 2.9
Control, Low Strain	12	114.7 ± 3.9
Control, High Strain	12	132.0 ± 2.5
Test, Low Strain	12	111.5 ± 4.0
Test, High Strain	12	123.2 ± 3.5
Control, Low Strain, Males	6	122.5 ± 5.4
Control, Low Strain, Females	6	106.8 ± 3.8
Control, High Strain Males	6	132.2 ± 4.1
Control, High Strain Females	6	133.0 ± 3.2
Test, Low Strain, Males	6	119.3 ± 5.7
Test, Low Strain, Females	6	103.7 ± 3.6
Test, High Strain, Males	6	131.0 ± 2.5
Test, High Strain, Females	6	115.7 ± 4.7

mean is found for every test group in comparison with its respective control group.

Figure 2 is a modified Dice Leraas diagram representing a graphic estimation of the significances of differences of several means. In this type of diagram, if the two standard error interval for one sample includes the observed mean for another sample, the two means may not be significantly different. Such an overlap is not observed in the comparisons of strain differences. However, the mean of the control, high cholesterol males coincides with the upper limit of the two standard error intervals for the low cholesterol, control males. Considering sex differences, only the means of the control, line 1, male and female mice are found to be included in the other's two standard error interval. This overlapping does not occur in the control males and females of the line 2 mice, nor does it occur in test groups of line 1 and line 2 males and females. Of the four categories used for a comparison of test and control animals, three test groups have their means incorporated within the two standard error rectangle of their respective control groups. Only the line 1 females show a significant difference between the control and test groups.

A three factor design for the analysis of variance is presented in Table VII. This form of variance analysis

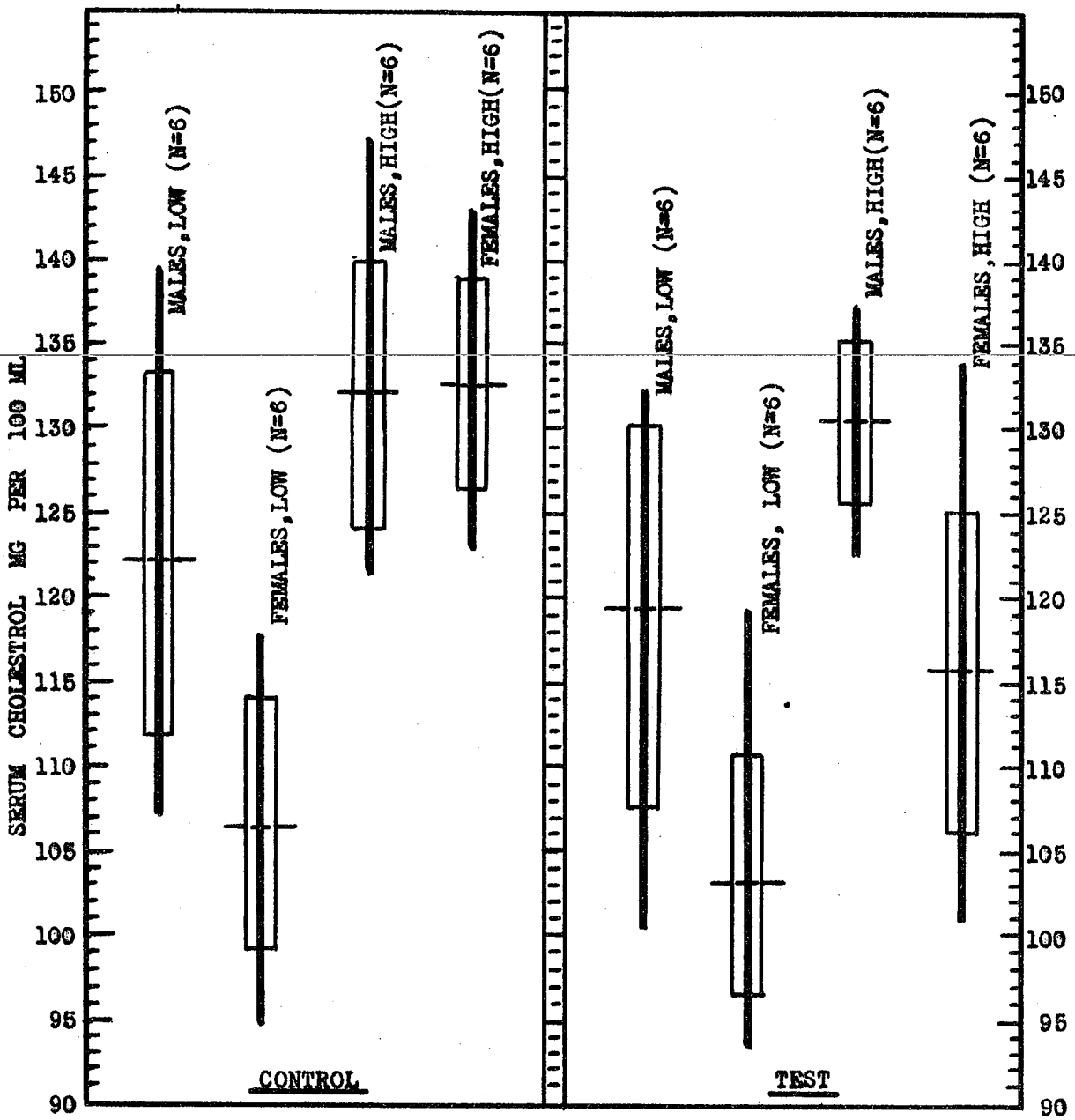


Figure 2. Graphic estimation of the significances of differences of several means of mouse serum cholesterol determinations.

Vertical lines -- observed ranges
Horizontal lines -- means
Rectangle -- area encompassed by two standard errors on each side of the mean

TABLE VII

MOUSE SERUM CHOLESTEROL DETERMINATIONS

THREE FACTOR ANALYSIS OF VARIANCE

Source	df	MS	F	P
Main Effect of Treatment	1	463	4.0	.051
Main Effect of Strain	1	2655	23.0	.01
Main Effect of Sex	1	1576	13.6	.01
Interaction of Treatment and Strain	1	111	1.0	not sign.
Interaction of Strain and Sex	1	212	1.8	not sign.
Interaction of Treatment and Sex	1	195	1.7	not sign.
Treatment, Strain and Sex Interaction	1	197	1.7	not sign.
Deviations	40	116		
Total	48			

was chosen for this study because every level of each factor appears with every level of all other factors. Formulas used in the computations were taken from Simpson, Roe, and Lewontin.[28]

While not so precise nor pertinent to the problem as the three factor analysis of variance, a summary of t-tests is presented in Table VIII. This computation does not have the refinement of the three factor analysis of variance but does offer an approximation for testing the differences between samples.

TABLE VIII

MOUSE SERUM CHOLESTEROL DETERMINATIONS

SUMMARY OF T-TESTS

Group	t	d	P
Treatment, High Strains	1.9	18	not sign.
Treatment, Low Strains	0.7	21	not sign.
Strains	4.0	41	.01
Sex	3.4	41	.01

DISCUSSION

Sitosterols have been administered to man and many laboratory animals. The drug has been given to animals exhibiting pronounced hypercholesteremia due to additives to the diet. In these cases, the sitosterols resulted in a significant lowering of serum cholesterol. The drug has also been administered as an additive to a diet that without the sitosterols produced pronounced hypercholesteremia in the control group of animals. In this line of experimentation, sitosterols effectively prevented a rise in serum cholesterol.

Behr, Baker, and Penney working with laboratory mice found that their control group with no diet supplements had a mean serum cholesterol concentration of 107 ± 5.6 milligrams per 100 milliliters (5.6 = 1 standard deviation). A test group fed the basic diet of the control group plus one per cent cholesterol and one-half per cent cholic acid had at the end of a three weeks period a mean cholesterol of 350 ± 63 milligrams per 100 milliliters.[29] In contrast to this dietetically induced hypercholesteremia, the line 1 (high serum cholesterol) mice of this study had a mean serum cholesterol of 133.0 milligrams per 100 milliliters. Selective breeding through six generations did not produce hypercholesteremia as markedly elevated

as did the addition of dietary factors to the diet of laboratory mice that were not genetically selected. It might be expected that the pronouncedly elevated serum cholesterol due to dietary supplements might be more responsive to sitosterols than would be a lesser increase in serum cholesterol due to genetic or other factors.

The present study indicates that the effect of sitosterols on the serum cholesterol levels of the mice used in this experiment was inconclusive. The P value for treatment was .05 and did not approach the significances of differences between strains and sexes which were both highly significant at $P < .01$. Although not statistically supported, the experiment indicates that sitosterols would be more effective in lowering the serum cholesterol concentrations of high strain mice than those of low strain mice. The t-test values for control against treatment for the high strain mice approaches $P < .05$ while the comparative t-test value for the low strain mice is clearly without significance.

Further investigations along the lines of this study would be of value. The biochemical mechanisms and the method of genetic transmission of strains of animals differing genetically in their serum cholesterol levels should be of interest as little is known in these areas.

The use of a statistically larger group of mice might answer conclusively the questions left unanswered in this study.

SUMMARY

A control diet and a treatment diet containing five per cent sitosterols were fed to two groups of laboratory mice for a period of four weeks. Both the control and the treatment group included two strains of mice produced previously by selective breeding and differing from each other in serum cholesterol concentrations. Routine records were kept of environmental conditions and food consumption during the course of the experiment. The results are summarized as follows:

1. Strain differences between line 1 (high serum cholesterol) and line 2 (low serum cholesterol) were highly significantly different. The administration of sitosterols did not alter the strain differences.

2. Sex differences were highly significantly different at the end of the test period. The administration of sitosterols did not alter the sex differences in serum cholesterol levels.

3. The control and test groups of mice showed a significant difference in the three factor analysis of variance. The t-tests showed the effect of sitosterol administration was significant on the high serum cholesterol mice and was clearly without significance on the cholesterol levels of the low serum cholesterol mice.

4. On the basis of this study, it is not possible to conclude that sitosterols affect significantly the serum cholesterol levels of the two genetically different strains of laboratory mice. The experiment indicates that sitosterol ingestion is without effect on the serum cholesterol level of the genetically low strain of mice and is inconclusive in effect on the serum cholesterol concentrations of the genetically high strain of mice.

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