EFFECT OF 2, 2, 9, 9 - TETRAMETHYL - 1, 10 - DECANEDIOL (CI - 720) ON BLOOD, EGG YOLK, AND EXCRETA CHOLESTEROL, BLOOD TRIGLYCERIDES, FEED CONSUMPTION, EGG PRODUCTION, AND EGG COMPONENT WEIGHTS

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ABSTRACT

EFFECT OF 2,2,9,9-TETRAMETHYL-1,10-DECANEDIOL (CI-720) ON BLOOD, EGG YOLK, AND EXCRETA CHOLESTEROL, BLOOD TRIGLYCERIDES, FEED CONSUMPTION, EGG PRODUCTION, AND EGG COMPONENT WEIGHTS

by

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CI-720 (2,2,9,9-tetramethyl-1,10-decanediol; supplied by the Parke-Davis Company) was administered to Single Comb White Leghorn laying hens to determine if the drug would produce the antilipemic effect (of decreased free and total plasma cholesterol, and plasma triglycerides) that have been reported for the rat, mouse, and rhesus monkey.

All hens were in their first year of lay, caged individually, and had feed and water administered ad libitum. Three hens were used in each of the four treatments of experiment 1, which consisted of feed and capsule administration of the drug, plus the respective controls. In the second experiment, eight hens were used in the control and in each of the two drug treatments.

In this study, administration of CI-720 during experiment 1 resulted in declines in total (133 to 114 mg/dl ± 5.6 pooled SEM) and free (107 to 79 mg/dl ± 5.7 pooled SEM) plasma cholesterol, which were significant as calculated by the split plot statistical analysis. These differences

were not significant statistically when calculated as a percentage, using the hen as her own control. In experiment 2, the total plasma cholesterol was significantly decreased by CI-720 (155; 111; 101 mg/dl ± 5.3 pooled SEM, for treatments of 0, 2600, and 5200 ppm of the diet, respectively). Free plasma cholesterol was lowered by the drug (85; 50; 38 mg/dl ± 5.7 pooled SEM, for the same respective treatments). During both experiments, drug treatment caused a significant decrease in the percent free of total plasma cholesterol, *versus* the control hens.

Plasma triglycerides were not determined in the first experiment, but were significantly decreased by the drug in experiment 2. Treatments of 0, 2600, and 5200 ppm drug *via* the diet resulted in means of 1524; 613; 228 mg triglycerides/dl plasma ± 96 pooled SEM, for respective treatments.

The total yolk cholesterol was increased in the first experiment, but was not changed in the second.

Hen body weight was not altered in experiment 1, but was lowered in experiment 2 by the drug. A related parameter, percent change in body weight, showed no difference between treatment means during the first experiment, but during the second experiment the drug treated hen's body weight varied significantly more than control hens.

The percent egg production was lowered very significantly (to zero in some cases) in both experiments by CI-720. During the second experiment, the drug treated hens changed

in the amount of weekly percent egg production very significantly more than control hens. There was no difference between the four treatment means for this latter parameter during the first experiment.

Comparisons of the total egg weight, component weights, and their percent of total egg weight were not significantly different among treatments during either experiment, although the eggs from drug treated hens of experiment 2 tended toward lower egg weight and yolk weight than those from the control hens.

The statistical analysis showed that the drug lowered the feed consumption during both experiments toward or below the maintenance level of 70 g feed consumption/hen/day.

There was no difference between the four treatment means of excreta cholesterol, although the actual amount of excreta cholesterol was possibly low.

Before any concrete statements about the degree of CI-720 effectiveness are made, a study using a pair-feeding technique should be accomplished. The reason for this lack of complete confidence in the indicated statistical results of the drug effect, is due to the lowered feed consumption for most of the drug treated hens. With lowered feed intake one would expect blood lipids, as well as egg production and body weight to decline. Probably the drug effect is confounded with the lowered feed consumption.

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to my family and parents

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INTRODUCTION

The compound 2,2,9,9-tetramethyl-1,10-decanediol (CI-720), has been found to be an effective antilipemic agent (decreases plasma triglycerides and cholesterol) in rats, mice, and rhesus monkeys (Parke-Davis, 1974). Because of these results, the researchers at Parke-Davis have proposed that CI-720 be used in humans to treat hyperlipemias II, III, IV, and V of the Fredrickson classification. These classes are outlined in a bulletin by the World Health Organization (1973). The basis of this classification is the use of blood levels of triglycerides and cholesterol as a method of detecting and defining defects in lipid metabolism.

Although much information exists which fails to support the much commercialized saturated fatty acid/cholesterol theory of atherosclerosis etiology (Pinckney and Pinckney, 1973; Kaunitz, 1975), these data are generally disregarded (Stare et al., 1974; Palmer, 1975). Hence, the degree of cholesterol involvement in atherosclerosis is still unresolved.

Nevertheless, there is a small portion of the human population that does experience various pathologies of

lipid metabolism. These people may benefit from CI-720 or other antilipemic compounds.

In the research reported in the present paper, CI-720 was administered to Single Comb White Leghorn laying hens in order to determine if the antilipemic effects of this drug, as observed by the Parke-Davis scientists, were also manifested in the chicken. Other parameters of interest were total yolk cholesterol, feed consumption, egg production, egg component weights, and excreta cholesterol levels.

REVIEW OF LITERATURE

Cholesterol

1. Effect of CI-720

No research on the antilipemic effect of CI-720 has been completed except that with the rat, mouse, and rhesus monkey. Based on the findings from these species, Parke-Davis Company researchers reported that CI-720 was about ten-fold as active in decreasing plasma triglycerides as was clofibrate® (Imperial Chemical Industries Inc., Ltd.). At high levels (150 mg CI-720/kg body weight), CI-720 caused plasma cholesterol to be lowered in the monkey and rat (Parke-Davis, 1974).

The mechanism of action of CI-720 as an antilipemic drug is unknown. This drug has not been found to increase the fecal cholesterol content. *In vivo*, CI-720 has been found to inhibit long-chain fatty acid incorporation into triglycerides in the liver and plasma. CI-720 does not prevent the incorporation of acetate into either sterols or triglycerides.

2. General

A. Agents used in an attempt to lower plasma cholesterol

Due to the general presumption of cholesterol as either a predisposing or direct cause of atherosclerosis, several drugs (which should include foodstuffs when used as such) have been used clinically or in experiments with animals in an attempt to lower plasma cholesterol. Obviously, research is conducted in these areas for other reasons; for example, to delineate the causes and cures of various diseases of lipid metabolism such as xanthamatosis.

Table 1 lists a number of compounds or conditions which alter the plasma cholesterol levels in various species.

B. Factors which may alter plasma cholesterol, or atherosclerosis

Table 2 lists some factors which are known to have an effect on plasma cholesterol or atherosclerosis. In general, age, stress, and immediate effect of exercise, increase plasma cholesterol; while weight stability and continued daily exercise, tend to maintain or lower blood cholesterol, and at times, lower the severity of atherosclerosis. Some researchers have found the Caucasian race to have higher plasma cholesterol than the Negroid (Pinckney and Pinckney, 1973), and familial differences in plasma cholesterol level are evident in animals and humans.

Table 1. Compounds reported to alter various cholesterol levels in different species.

Item	Species	Age	Sex	Parameter	Effect	Reference
Three different classes of hypocholesterolemic compounds (1)	MONKEY & RAT	MAT	---	B	DEC	Rodney et al. (1965)
Aureomycin	RAT	IM	M	L	INC	Guggenheim et al. (1960)
Vitamin D_2	"	"	M,F	L	INC*	
				B	DEC*	Jurgens et al. (1971)
Vitamin A	"	"	M	B,L	DEC*	Bring et al. (1965)
Nicotinic acid	"	"	M	B	NC	
	CHICKEN	"	M,F	B	DEC	Gaylor et al. (1960)
Nicarbazin® (Merck)	"	MAT	M	B	INC	Weiss (1960); Konlande and Fisher (1969)
Lithocholic acid	"	"	F	B,Y	INC	
Cholic acid	"	"	"	B	NC	Edwards et al. (1962)

*if with dietary cholesterol--throughout table

CODE: IM = immature, MAT = mature, M = male, F = female, B = blood, L = liver, A = aorta, Y = yolk, FS = fecal sterol, DEC = decrease, INC = increase, NC = no change, AHCE = antihyperlipemic effect

(1)--see footnote page (Table 1 of Appendix)

Table 1 (cont'd.).

Item	Species	Age	Sex	Parameter	Effect	Reference
Niacin	CHICKEN	MAT	F	Y	NC	Weiss et al. (1965)
	"	IM	---	A,B	DEC	Treat et al. (1960)
Methionine, betaine, or choline	"	MAT	F	B	DEC AHCE*	
				Y	NC*	Kurnick et al. (1958)
Choline chloride (.25% of diet)	"	IM	---	B	DEC*	
	"	"	---	B	NC*	Svacha et al. (1958)
	"	"	---	A,B	DEC	Treat et al. (1960)
DEAE Sephadex (5% of diet)	"	MAT	F	Y	INC	Turk and Barnett (1972)
20,25-diazocholesterol ® (2)	"	"	F	B	INC	
				Y	INC[1]	Singh and Naber (1970)
Vitamin A	"	"	F	Y	NC	Weiss et al. (1967)
	"	"	F	B,Y	NC	Dua et al. (1967)
	"	"	F	L,Y	INC[2]	Shewsbury et al. (1967)
	"	"	M,F	B	INC[3]	
	"	IM	M,F	B	AHCE	Wood et al. (1961)

1=varied with dose, 2=if high vit. A plus cholesterol in diet, 3=vit. A in dogfish liver oil

Table 1 (cont'd.).

Item	Species	Age	Sex	Parameter	Effect	Reference
SC-12937® or SC-10644® (footnotes 2 and 3)	CHICKEN	MAT	M	B	AHCE	Reynolds et al. (1971)
Benzemalecene (I.V.)	"	"	M	B	DEC	
Benzemalecene (diet)	"	"	M	B	NC	Tennent et al. (1961)
Unsaturated fatty acids	"	"	F	B,Y	NC[4]	Marion et al. (1960)
	"	"	M,F	B	INC	Wood et al. (1961)
	"	IM	M	B	INC[5]	
	"	"	M	B,L,A	INC*	Leveille and Sauberlich (1963)
Citrus Pectin	"	"	---	B	DEC	
				FS	INC	Griminger and Fisher (1967)
Pectin (2% of diet)	"	MAT	F	Y	DEC	Turk and Barnett (1972)
Vitamin B_6	"	IM	M	B	INC[6]	Daghir and Porooshani (1968)
	"	"	M	B	INC[7]	
				A	INC[7]	Daghir and Balloun (1962)

4=but found a sig. inverse relation between B and Y cholesterol levels, 5=no difference between fats, 6=if low vitamin B_6 in the diet, 7=not significant

Table 1 (cont'd.).

Item	Species	Age	Sex	Parameter	Effect	Reference
Aflatoxins	CHICKEN	IM	---	L B	INC DEC	Tung et al. (1971)
Candicidin[8]	"	"	M	B FS	AHCE INC	Fisher and Kwon (1972)
Diethylstilbestrol	" TURKEY	" MAT	M M	B B	INC INC	Reynolds et al. (1971) Simpson and Harms (1965)
X-rays (continuous)	CHICKEN	"	M	B	DEC	Estep et al. (1968)
Vanadium	"	---	M	L	NC	Dudley et al. (1961)
Inositol	"	---	---	B A	INC DEC	Treat et al. (1960)
D-thyroxine (I.V. or diet)	"	MAT	F	B Y	DEC INC	Weiss et al. (1967)
Vitamin A	HUMAN CHICKEN	" IM	--- M	B B	DEC[9] AHCE[10]	Kinley and Krause (1959) Wood (1960)
SKF -525-A (footnote 4)	DOG	MAT	M,F	B,A	DEC	Dick et al. (1960)

8=an antifungual antibiotic, 9=only if the person was atherosclerotic, 10=vitamin A in lingcod liver oil or crystalline vitamin A in the diet

Table 2. Factors which may influence plasma cholesterol or atherosclerosis.

Item	Reference
Exercise	Ratcliffe and Cronin (1958)
	Fisher and Leveille (1957)
Weight stability	Pinckney and Pinckney (1973)
	Palmer (1975)
Stress	Kaunitz (1975)
	Thornberry (1970)
Increase in age	Weiss (1957)
	Roberts and Straus (1965)
Being male or female	Wood *et al.* (1961)
	Bartov *et al.* (1970)
Genetics	Miller and Denton (1962)
	Stare (1974)
Tissue *versus* blood cholesterol level	Jurgens *et al.* (1971)

3. Specific Drugs and Other Compounds Used in Plasma and Tissue Cholesterol Level Manipulations

A. Cholestyramine

When Tennent *et al.* (1961) used cholestyramine (MK-135) to lower plasma cholesterol in the normocholesterolemic dog, they found an additive effect of this drug with either of the hepatic cholesterol synthesis inhibitors, MER-29 or benzemalecene. In the normocholesterolemic cockerel, these workers found that either MK-135 or benzemalecene decreased plasma cholesterol; and when used together, these compounds had an additive effect in lowering plasma cholesterol.

When Jones (1969) used cholestyramine, he found a highly significant decrease in the plasma cholesterol of the hen, but no change in the yolk cholesterol. This is in agreement with the earlier work of McGinnis and Ringer (1963), who in addition, found no change in body weight and an increase in the number of light colored yolks when they used this quaternary ammonium anion exchange resin.

Epley (1971) explained the decrease in plasma cholesterol of cholestyramine-treated cockerels by way of a decrease in cholesterol esters.

Epley and Balloun (1970) reported that when diets were supplemented with cholesterol, MK-135 caused a reduction in total plasma cholesterol and cholesterol esters of cockerels. If there were no cholesterol supplementation, there was still a decrease in the total cholesterol of the cockerel's blood. The free cholesterol was not changed significantly by this

drug. In another trial by Epley and Balloun (1970), when MK-135 was administered with the control diet, the serum cholesterol was not significantly lowered. These latter results are in disagreement with the other research, but are supported by the findings of Reynolds et al. (1971). Reynolds and co-workers fed four hypocholesterolemic compounds (triparanol, cholestyramine, SC-12937, or SC-10644), separately, to diethylstilbestrol-treated (DESB) cockerels. With the DESB treatment, all four compounds caused a decrease in plasma cholesterol toward normal. Without DESB, each drug caused only slight decreases in the plasma cholesterol, which were dose dependent.

Tennent et al. (1960) made a comparison of MK-135 with another bile acid binding, polymeric organic base, MK-325. They found that the effect of each compound was to inhibit what they considered to be cholesterol-induced hypercholesterolemia and aortic plaque formation in cockerels. They also noted a decrease in plasma cholesterol of normocholesterolemic cockerels and dogs that had been treated with either of these drugs in another trial. As evidence of the antiabsorbing action of these drugs on cholesterol, Tennent et al. (1960) also observed an increase in fecal and biliary acid sterol levels in both species. Tennent et al. (1959) also reported the antihypercholesterolemic action of this drug in cockerels.

B. Clofibrate

When Herman et al. (1970) fed ethyl-p-chlorophenoxy-isobutarate (clofibrate) to humans, they found some subjects to have a lowered triglyceride fraction of their plasma lipids. The mechanism of action of clofibrate was reportedly not known. Because of an excess of lipids present in their blood samples, especially triglycerides, the blood of some individuals is excessively turbid. This extreme blood turbidity was not corrected in all individuals by using clofibrate. Although some people have been successful in using a low carbohydrate type of diet to correct this hyperlipemic condition, this type of treatment does not work for all individuals.

Using rats, Thorp and Waring (1962) found clofibrate to be the most active of the aryloxyisobutyric acids in decreasing total lipid and cholesterol in blood and liver. They also found opposite effects within or between different species when this drug was used. Clofibrate enhanced the estradiol or testosterone reduction of plasma cholesterol in rats. In the monkey and chicken, these same investigators discovered that the increase in serum cholesterol due to an atherogenic diet (which has been defined as one low in protein and containing cholesterol) was exacerbated by clofibrate. In rats, clofibrate plus androsterone (which may function more as a metabolic regulator than as an androgen) was effective in decreasing plasma and liver

lipids, whereas clofibrate or androsterone alone caused no effect on the plasma or liver lipids.

According to Thorp and Waring (1962), clofibrate caused no inhibition of the acute hyperlipemia or hypercholesterolemia in rats which were intravenously given the surface active polymer, Triton WR-1339.

C. Triparanol

After feeding 1-(p-(beta-diethylaminoethoxy)-phenyl)-1-(p-tolyl)-2-(p-chlorophenyl) ethanol (also called triparanol or MER-29, patented to the Wm. S. Merrell Co.) to 24-week-old hens, Burgess et al. (1961) reported that triparanol induced an 85% decrease in yolk cholesterol, and the replacement of this cholesterol with desmosterol. Also observed after feeding the large quantity of MER-29 was a decrease in egg weight, but yolk weight was not altered. The ovaries of hens on the triparanol treatment declined from a normal weight of 69 g to 3 g per ovary.

Wong et al. (1963) reported an increase in total serum sterols, and the desmosterol component of the blood sterols, in both cockerels and laying pullets given triparanol via the feed. Desmosterol represented 76% of the total blood sterols of triparanol-treated cockerels, while none was detected in the blood of control cockerels. Desmosterol was also present (67% of sterols) in treated birds aortas, and not in control cockerels. The laying pullet's response to triparanol was the same as the cockerels, but was more

pronounced. Triparanol stopped egg production in all drug treated laying pullets. The degree and incidence of atherosclerosis of the chicken aorta was increased by triparanol.

Nelson et al. (1962) fed laying hens triparanol in the diet. The findings included: no alteration of Ca++ level in the plasma; a decrease in egg size, production rate, and yolk size; and an increase in plasma cholesterol by 90%. Following withdrawal of this drug, the plasma cholesterol level returned to normal. Perhaps the discrepancy with the other findings can be explained as a difference in dosage, as observed in the rat by Blohm et al. (1959), or to a mechanism of action for the increase in plasma cholesterol similar to nicarbazin (Weiss, 1960).

Nichols et al. (1961) fed two-week-old chicks a diet including triparanol. They initially observed a decrease in plasma cholesterol, then the cholesterol levels increased about as often as they decreased, and showed no consistent pattern. Growth was decreased with increasing amounts of triparanol, and toxic levels were reached. The egg weight of layers fed MER-29 decreased to five ounces/dozen. At higher levels, Nichols and co-workers found egg production ceased, with no change in serum or yolk cholesterol.

Blohm and MacKenzie (1959) found MER-29 decreased plasma cholesterol by decreasing the incorporation of acetate-1-^{14}C into cholesterol in the liver and intestine of rats. Blohm et al. (1959) reported this inhibition in plasma cholesterol

was specific for cholesterol, and occurred at a late stage in the cholesterol synthesis pathway, after formation of a steroid nucleus had taken place. Blohm et al. (1959) found the plasma cholesterol of rats and monkeys to be decreased, the rat having several tissues with lowered cholesterol.

Avigan et al. (1960) administered MER-29 to rats, and discovered a decrease in serum sterols. There was replacement of 27-79% of serum and tissue sterols with 24-dehydro-cholesterol.

4. Hormones

The site of carbohydrate and lipid metabolism in the chicken differs markedly from that in mammals. Work by Leveille et al. (1975) supported the view that the liver is the primary site of lipid biosynthesis in the chicken, and that adipose tissue is relatively unimportant here. In addition, insulin was reported to increase free fatty acids in the blood of fowl, which is opposite to most species. Insulin may stimulate glucagon release, with the glucagon being lipolytic in the chicken. Glucagon may be one of the most important endocrine secretions controlling avian metabolism.

According to Grande (1968), birds (geese, roosters, ducks, and turkeys) given crystalline glucagon intravenously showed prompt plasma increases in free fatty acids and sugar. He also concluded that because catecholamines have not changed plasma free fatty acids of the domestic fowl, the

effect by glucagon probably is not mediated through catecholamines (epinephrene) in birds. He referenced several papers to report that glucagon produces a decrease in plasma free fatty acids in the dog and man.

Goodridge (1968) reported that in pigeon adipose tissue the lipase was stimulated by both epinephrine and glucagon; but in the chicken, lipolysis was stimulated by glucagon and not by epinephrine. He found no change in lipolysis (free fatty acid and glycerol release to the bloodstream) in the adipose tissue when insulin was administered to embryos, 7-8-day-old chicks, or to 28-day-old chicks. In these 7-8-day-old chicks, insulin increased adipose tissue response to the lipolytic action of glucagon was about ten-fold, but did not alter the response of 28-day-old chicks. When treated with glucagon, the chick embryo tissue showed only a slight increase in lipolysis, but the other age treatments showed marked acceleration of lipolysis.

Goodridge and Ball (1966) also reported that the synthesis of fatty acids from glucose or pyruvate by pigeon adipose tissue (in vitro) proceeded at a low rate, and was unaffected by insulin. They concluded that the adipose tissue of pigeons may serve largely as a depositary for fat synthesized elsewhere, probably in the liver.

Caldwell and Suydam (1960) found that exogenous estrogen induced more rapid plaque formation in the blood vessels of cockerels than did dietary cholesterol, and that

the degree of hypercholesterolemia did not determine the extent of atherosclerosis.

Stamler et al. (1954) suggested the increased resistance to cholesterol-induced atherogenesis of mature egg producing hens (intact or after oviduct ligation) was due to their endogenous estrogen secretion. Pinckney and Pinckney (1973) reported that this same Stamler advised male human patients to treat their high blood cholesterol (and therefore supposedly arteriosclerosis) with estrogens. These authors did not state which the men found more disconcerting, the possibility of arteriosclerotic plaques, or the changes that occurred in their voices and chests.

Thorp and Waring (1962) found that subcutaneous implantation of estradiol or testosterone in the intact or gonadectomized rat, caused a decrease in blood cholesterol.

Hardy et al. (1962) found a statistical difference between the serum cholesterol of high- or low-cholesterol strains of three-week-old chicks, but that the response to treatment with different hormones was similar. In one experiment, the treatments were: control (safflower oil carrier alone, was injected), cortisone, diethylstilbestrol, or testosterone. There were no line by treatment interactions. In a second experiment, estriol benzoate had a significant line by treatment interaction in addition to increasing serum cholesterol. Cortisone and thyroxine also increased the serum cholesterol in the second experiment.

Weiss et al. (1965) used D-thyroxine, which is about 1/5 as active as the L- form, to lower plasma and increase egg yolk cholesterol. Weiss et al. (1967) also reported these results, and in addition they found that after a change of about 30% in the plasma or yolk cholesterol, the level of cholesterol of these parameters plateaued, regardless of the amount of thyroxine administered.

In the thyroidectomized chick, both D and L forms of exogenous thyroxine are capable of stimulating cholesterol biosynthesis, release, and turnover in a manner comparable to that in a normal chick (Lepp et al., 1964). These researchers also found that thyroidectomy of chickens without iodoamino acid treatment would increase these chicks serum cholesterol; and that iodoamino acid treatment with or without thyroidectomy of the chicks did not change liver cholesterol or liver weight versus euthyroid chicks. Lepp and his co-workers reported the accepted mode of action of thyroxine, and it's analogs, in cholesterol metabolism is at the biliary excretion and/or degradation stage.

When Chung et al. (1967) added diethylstilbestrol, cholesterol, and/or various fats to the feed of cockerels, they detected changes in the types of fatty acids which were esterified to plasma cholesterol.

Sturkie (1965) mentioned that the method of diethylstilbestrol administration was important in its effect of increasing blood lipids. When injected or implanted,

diethylstilbestrol has the best action, but is relatively ineffective when given orally.

5. Diet Effect on Blood Lipids

A. General observations

Lorenz et al. (1938) reported that in laying hens maintained on very low fat diets, enormous concentrations of lipids may appear in their blood, and that the total fatty acids and free cholesterol varied with the dietary fat level. In the immature female bird, dietary fat level did not change the various plasma lipid levels. The male chicken showed an increase of cholesterol ester when fed a high fat diet; but phospholipid, neutral fat or free cholesterol were not changed by dietary fat level. Lorenz and his associates showed that the laying hen experienced a decrease in variability in neutral fat and free cholesterol when given a high fat diet (by replacing carbohydrate), and reported that an interaction existed between dietary fat and ovarian activity.

Leveille et al. (1975) reported a decrease in hepatic lipogenesis of chicks fed a diet high in fat or protein (at the expense of carbohydrate).

Price et al. (1957) found that 0-14% poultry oil in the diet of caged or floor raised layers did not change the hen's plasma cholesterol level.

Dietary supplementation with menhaden oil, when fed to laying hens, caused a cessation of their egg production

(Weiss et al., 1967). They also found that safflower oil at 7.5% or 15% of the diet had little effect on egg cholesterol concentration, but at 30%, there was an increase in the egg cholesterol.

B. Egg in the diet

Corn oil, corn oil low-melting margarine (which would be high in unsaturated fatty acids), butter, beef tallow, or pork lard fed individually to rats at 10% of the basal diet, all increased serum cholesterol. Males were affected by these treatments more than females. Wachholz (1972) also found that there was no difference between the effects of fat sources or levels of supplemental cholesterol on the rat's serum cholesterol. Cholesterol was supplied either as 2% of the diet in the crystalline form, or as 10% of the diet as whole egg.

Pair-fed cockerels given 10% of the diet as dried whole egg had lower plasma cholesterol-ester and plasma total cholesterol, and gained more weight than those given crystalline cholesterol plus soy oil dietary supplementation (Epley and Balloun, 1970). These workers found no difference in the severity of atherosclerosis which resulted from the dietary supplementation of either coconut or soy oil.

El-Maguid and Quisenberry (1968) fed dried whole egg to laying hens, the amount in the diet was equivalent to 1, 2, 4, or 10 eggs/man/day. The hens had lowered plasma

cholesterol at the high egg level, and only slightly increased plasma cholesterol at the lower levels. Yolk cholesterol did not change. Two caloric levels for the diets were used, and with the high egg supplementation, the hen's performance was improved at each caloric level, versus the other egg treatments. Performance was measured by increased egg production, increased feed efficiency, and decreased mortality. El-Maguid and Quisenberry (1968) did not report the fecal sterol level, but perhaps the reason for the lowered blood cholesterol level at the higher dosage of egg equivalents was that: the excess cholesterol was in such a large amount that a portion was not absorbed (Heftman, 1970); and/or the amount of cholesterol that was absorbed from the high egg equivalent diet was sufficient to completely shut down the liver cholesterol production at the beta-hydroxy-beta-methyl glutaryl-Co A step of cholesterol synthesis. The sum of blood cholesterol from exogenous and endogenous sources then was not enough to maintain the previous level of the hens treated with the high number of egg equivalents. The cholesterol provided by the lower levels of dietary egg equivalents could have been enough to inhibit a portion of the cholesterol synthesis by the liver, the overall sum of plasma cholesterol was a biological rather than a pharmacological type of increase over the previous level. Additional results from the previously mentioned work of Epley and Balloun (1970) support the findings of El-Maguid and Quisenberry.

C. Crystalline cholesterol supplementation to the diet

Table 3 summarizes results of several researchers who added crystalline cholesterol to various feeding regimes.

6. Sitosterol and Other Plant Sterols

Weiss et al. (1967) cited a personal communication with Dr. T. A. Miettinen to report that campesterol, stigmosterol, and beta-sitosterol totaled 1.2% of the sterols in a batch of commercial eggs.

Clarenburg et al. (1971) found layers to have lower intestinal absorption of sitosterol than did non-layers (60% versus 85% absorption, respectively), and discovered a 35% decrease in egg yolk cholesterol content as a result of dietary sitosterol supplementation. This loss of yolk sterol was replaced by beta-sitosterol. These workers also reported that plasma sterols were decreased with sitosterol in the diet.

Bartov et al. (1971) concluded that the response difference of laying hens cholesterol levels to dietary soy oil, coconut oil, and safflower oil was not due to the different oil's sterol content. Dietary coconut or safflower oil significantly increased the yolk cholesterol, but the soy oil had no effect. In another series of trials, only coconut oil increased the laying hen's plasma cholesterol. These researchers stated that "it appears that plant sterols do not interfere with the cholesterol metabolism in the laying hen". Their results support the

Table 3. Effect of dietary cholesterol supplementation on various cholesterol levels in the chicken.

Item	Age	Sex	Parameter	Effect	Reference
Diet + 1% CH	MATURE	F	B Y	INC NC	Kurnick et al. (1958)
Diet + 2% CH	"	F	B Y	INC INC	Dua et al. (1966)
Diet + 1% CH	IMMATURE	M	B,L	INC	Daghir and Porooshani (1962)
Low protein diet and/or dietary cholesterol	"	M	B,A	INC	Feizenbaum et al. (1961)
Diet + 0.5% CH Diet + 1.0% CH	" "	--- ---	B B	NC INC	Morris and Hinners (1968)
Diet with or without cholesterol	---	---	B,L,A	---	Roberts and Straus (1965)[1]

CODE: M = male, F = female, B = blood, Y = yolk, L = liver, A = aorta, INC = increase, NC = no change, CH = cholesterol

1=a book devoted to atherosclerosis

assumption that the anticholesterolemic effect of plant sterols is based mainly on the inhibition of cholesterol absorption rather than the metabolism of cholesterol.

Diller et al. (1960) found that dietary beta-sitosterol fully returned the cholesterol-induced hyperlipemia of chickens to normal. Also, at 4% of the diet, beta-sitosterol prevented or reversed an increase in lipid and cholesterol concentration in the liver and aorta of these chickens.

Weiss et al. (1965) found that 1% beta-sitosterol in the diet decreased hens blood cholesterol when given a cholesterol-containing diet.

Weiss et al. (1967) reported that in normal laying hens, 1% dietary beta-sitosterol had little effect on blood or egg sterol concentrations. They again reported that 1% beta-sitosterol in the diet would decrease egg and blood cholesterol if the hen was given a cholesterol-containing diet. The egg cholesterol was increased 60% when the hen was fed cholesterol at 1% of the diet. They found no detectable beta-sitosterol in the egg of hens fed 1% beta-sitosterol and 5% lecithin, or safflower oil as 30% of the diet, but attributed this absence to technical error.

Boorman and Fisher (1966) reported that with no dietary sterols, all sterols present in the egg were cholesterol. Also, when hens were fed a diet having a mixed sterol content, cholesterol was preferentially absorbed from the gut versus phytosterols; and that more campesterol was absorbed

than beta-sitosterol. The extent to which absorption of plant sterols occurs is in part dependent on the fat content of the diet, plus phytosterols and cholesterol competitively inhibit the absorption of the other.

Konlande and Fisher (1969) showed evidence for a non-absorptive antihypercholesterolemic action of phytosterols in the chicken. Campesterol appeared as the major active component of soy and wheat sterols in relation to the antihypercholesterolemic effect of these sterols.

7. Turnover of Cholesterol

In his review, Kaunitz (1975) stated that the cholesterol turnover in the body of man was 1000-2000mg/day. The plasma turnover amounts to about 1000 mg/day. The usual American diet supplies 300-800 mg cholesterol/day, of which only about 150-300 mg are absorbed. About 2% of the tissue cholesterol is used in steroid hormone synthesis in humans.

According to Andrew et al. (1968), the laying fowl has two main excretory pathways for the elimination of cholesterol. These pathways are the egg formation mechanism and the feces. They showed that there was a plasma "isotopic steady state" in hens that were orally administered labelled cholesterol. They used the steady state data to conclude there is little ovarian cholesterol synthesis. Andrew and co-workers calculated the half-life of

cholesterol in the laying hen to be about 36 hours, and stated the cholesterol half-life in humans and dogs was 8-12 days.

8. Summaries of Cholesterol or Atherosclerosis

Kaunitz (1975) has reviewed atherosclerosis etiology, and has presented alternate theories to those currently being presented.

An atherosclerosis monograph guest edited by Stare (1974) and provided by Best Foods, Inc., a division of CPC (Corn Products Corporation) International, dealt mostly with lipid metabolism in adult humans, and was obviously biased toward an unsaturated fatty acid cure-all for atherosclerosis.

Palmer (1975) also discussed dietary aspects of human atherosclerosis. She attempted to link intake of cholesterol and saturated fatty acids with the morbidity and mortality from coronary disease.

A book by Roberts and Straus (1965) categorized atherosclerosis according to animal types and humans, giving differences between them in many aspects of this disease.

Page (1954) summarized investigational and clinical trends of the research concerning atherosclerosis.

Kritchevsky, as well as Cook, has written a book (both in 1958) which dealt with cholesterol history and various aspects of cholesterol metabolism.

Pinckney and Pinckney (1973) have written a book which is understandable by the lay-man concerning the role of

cholesterol and saturated fatty acids in atherosclerosis. They expose fallacies of the theory that "normal" amounts of cholesterol or saturated fatty acids are detrimental to ones health. They also detail some of the politics behind this cholesterol controversy.

Recently Brown and Goldstein (1976) offered their explanation for cholesterol metabolism and control.

The World Health Organization (1973) issued a helpful memorandum which classified types of hyperlipidemias and hyperlipoproteinemias.

Atherosclerosis

1. Effect of Hormones

Stamler et al. (1954a) reported that in the male chicken, cortisone caused a small increase in blood pressure and an intensification of atherosclerosis, but was relatively inactive as a glycocorticoid. They also found that hydrocortisone caused no increase in atherosclerosis (aortic or coronary artery) or hypertension of cockerels with steroid-induced diabetes and hyperadrenalism, which was associated with an enhancement of hypercholesterolemic hyperlipemia. ACTH (corticotropin) action was the same as that of hydrocortisone.

2. Plaques

A writer for the monograph guest edited by Stare (1974) stated that the first step in the atherosclerotic process is

the accumulation of lipid deposits in smooth muscle cells that have begun to proliferate in the intima.

Fox (1938) reported that the simplest atherosclerotic change is intimal hyperplasia. Some animals with the worst cases of atherosclerosis had lived the longest and did not die from atherosclerosis. Furthermore, he found differences between birds and mammals in the type of arteriopathy they exhibited, and that the atherosclerotic type vascular changes occurred in all animals with increasing age.

Palmer (1975) suggested that plaque lipids are derived chiefly from serum lipids and accumulate and stimulate an initial change in smooth muscle proliferation.

Palmer (1975) and Fox (1938) both mentioned that the primary atheroma sites are those where blood flow is turbulent and damage to epithelium is mechanically more probable. The theory of Kaunitz (1975) that cholesterol increase in an area is part of the body's response to injury, would seem applicable here. Fox (1938) stated that in the bird, the area just above the heart is the point in the vascular system stressed most, and perhaps more in the chicken than in any other animal.

Kaunitz (1975) indicated that cholesterol may be present in huge amounts in some pathological conditions, as in scars, fibroids, and granuloma, and that cholesterol is usually associated with Ca++ in atherosclerotic plaques.

Feizenbaum et al. (1961) stated that day-old-male chicks on a coconut oil plus cholesterol supplemented diet,

"showed a clear trend toward greater atherosclerosis" than those fed corn oil plus cholesterol type diet. The atherosclerosis observed in the chicks was most prevalent in the abdominal section of the aorta, versus the thoracic segment. These researchers also reported that a low protein diet and/or dietary cholesterol caused an increase in plasma and aortic cholesterol.

Thorp and Waring (1962) cited a personal communication with R. S. F. Campbell et al. (1960) to report that in the monkey, aortic atheroma increased in proportion to the rise in serum cholesterol; but in the chicken, aortic or coronary atherosclerosis was not changed by an increase in serum cholesterol.

Ratcliffe and Cronin (1958) have proposed a hypothesis of atherosclerosis etiology which states that social pressure may create a stress, and thereby an imbalance of adrenal secretions. These secretions would then cause the plaque formation. They reported that atherosclerosis presence or absence was independent of age or diet, but was associated with an increased population density of various animal species in a zoo. Their hypothesis was based on conclusions they drew from data of 55 years of autopsy records of these animals and birds.

In Table 4 are listed factors that may or may not alter an organism's susceptibility to circulatory pathologies.

Table 4. Factors which may or may not alter different species chances of circulatory disease.

Item	Reference
Ascorbic acid; water hardness; diet fiber	Palmer (1975)
High calorie diets	El-Maguid and Quisenberry (1968)
Egg consumption	Epley and Balloun (1970) Fisher et al. (1963)
Alcohol (drinking)	Kritchevsky (1958)
Oil type in diet	Epley and Balloun (1970) Swell et al. (1960) Chung et al. (1965) Banerjee et al. (1965) Fisher et al. (1963) Kaunitz (1975)
Blood pressure	Krista et al. (1970) Miller and Balloun (1968)
Cholesterol biosynthesis in the aorta	Eisley and Pritham (1955) Azarnoff (1958)
Vitamin A	Bayer et al. (1972)
Diet with or without cholesterol	Fisher et al. (1961) Caldwell and Suydam (1960)

OBJECTIVES

The purpose of this study was to discern the effect of CI-720 (an antilipemic agent in other species) on the following parameters of the Single Comb White Leghorn laying hen:

1. Total and free plasma cholesterol
2. Plasma triglycerides
3. Total yolk cholesterol
4. Body weight
5. Percent egg production
6. Total egg weight; plus component weights and their per cent of total egg weight
7. Feed consumption
8. Excreta cholesterol

MATERIALS AND METHODS

Experiment 1

1. General Comments

Twenty-four Single Comb White Leghorn (SCWL) hens in their first year of lay were brought from the Poultry Science Research and Teaching Center to a controlled-environmental cage room maintained at about 22°C. The hens were allowed to acclimate in 40 x 20 x 40 cm individual wire cages before being randomly assigned to treatments. The hens were housed in these same type cages during the experiment. Light was provided 17½ hours each day (0630 to 2400 hours).

Feed and water were available ad libitum. Care was taken, by the treatment space allocation, to avoid cross contamination of feed. Individual daily feed intake was recorded, usually before 0800 hours.

The diet used was MSU Ration 72-10 (Table 5), which was blended in a Mix Mill model 911 A2 nutri blender.

For the drug feed treatment, CI-720 was blended upward at 2600 ppm with a portion of the control diet. A tumble type mixer was used for the final blending.

During the pre-treatment period, all placebo capsules were partially filled with corn starch and collectively

Table 5. MSU Breeder Ration 72-10

Ingredient	Parts/1000
Corn, #2 yellow	576.2
Soybean meal, 48%	200.0
Meat and bone meal	30.0
Alfalfa, 17%	40.0
Tallow, stabl.	55.0
Methionine hydroxy analog	0.8
Dicalcium phosphate	18.0
Limestone	69.0
Salt, iodized	3.0
Choline chloride, 50%	2.0
Vitamin mix	3.0
Mineral mix	3.0
	1000.0

Vitamin mix (supplies per kg diet): Vitamin A--10,000 I.U.; Vitamin D--1,000 I.C.U.; Vitamin E--10 I.U.; Vitamin K--4.0 mg; Thiamine--3.0 mg; Riboflavin--10.0 mg; Pantothenic acid--15.0 mg; Niacin--100.0 mg; Pyridoxine--6.0 mg; Biotin--150.0 mcg; Folacin--3.0 mg; Vitamin B_{12}--15.0 mcg; Ethoxyquin--125.0 mg; Dist. dr. solubles* to 5.0 g.

Mineral mix (supplies per kg diet): Manganese--55 mg; Magnesium--500 mg; Iron--80 mg; Copper--11 mg; Zinc--80 mg; Dist. dr. solubles* to 5.0 g.

*with 4% corn oil.

Specifications: Ca--%, 3.5; P--%, 0.59; Fat--%, 8.3; Fiber--%, 2.8; Meth.--% of protein, 2.0; Prot./E., 5.83; Protein--%, 16.9; 3,000 kcal M.E./kg diet.

stored in a clean, dry screw-cap bottle. Drug treatment capsules were packed with CI-720 at 150 mg CI-720/kg body weight. During the first week of drug treatment capsules were filled with drug, based on the weight recorded for respective birds, for the first day of pre-treatment. Successive weekly individual body weight determinations were used to calculate the drug dosage for that hen in respective treatment weeks. Drug capsules for a treatment week were stored in containers, individually labelled for each hen.

All capsules used were size 1, made of gelatin, and produced by the Eli Lilly Company.

The new drug level in the capsules started the day of body weight determination, at the end of each treatment week.

The daily capsule was administered to the appropriate hen by pushing it into the esophagus, aided by water as a lubricant.

Egg production was recorded at approximately 1600 hours daily. All eggs were marked with the corresponding date and cage number and stored at 4°C until processed for total yolk cholesterol, total egg weight, and yolk, albumen, and shell weights. Evans et al. (1967) found no consistent change in lipid distribution of eggs after storage for six and twelve months, versus fresh eggs.

Twelve hens with egg production over 75% were selected and randomly distributed to treatments, namely: control

feed; drug feed (CI-720 @ 2600 ppm); control *per os* capsules containing starch); drug *per os* (CI-720 in capsules at 150 mg/kg body weight). Three hens were in each treatment.

Five ml heparinized blood samples were withdrawn from the brachial vein of each hen, between 0800-1000 hr. on 15 May (day zero of treatment with drug), and days 4, 7, 14, and 21 of treatment, and on day 7 of the post-treatment period. Body weight was also determined at these times ($\pm$ 1 g).

After all blood samples were withdrawn, they were immediately centrifuged at 2000 rpm (using an International Centrifuge model SBV size 1) for thirty minutes.

An aliquot of plasma was aspirated to a screw cap tube and diluted one to ten with acetone:ethanol (1:1), according to the method of Searcy and Bergquist (1960), for each sample. The diluent was then stored in a refrigerator at 5°C until processed for total and free cholesterol. Two dilutions were processed for each hen's plasma sample at each data collection date.

Plasma samples for triglycerides were processed following the method of Technicon Instruments (no date noted), at the same time as the total and free plasma cholesterol determinations were made.

In order to determine the total cholesterol content, total excreta was collected on days 15 through 21 of drug treatment.

Class A Mohr pipettes plus Propipet® (Spectronics Corp.) were used for most volume transfers, except the ferrous sulfate-acetic acid reagent and the concentrated sulfuric acid of the Searcy and Bergquist procedure color reaction. For these latter volume transfers, a Repipet® (Lab-Industries), or a glass syringe plus needle was used for the injection of reagent into the test tubes, respectively.

2. Total Plasma Cholesterol

Flow Chart 1 outlines the Searcy and Bergquist (1960) procedure used to determine the total and free plasma cholesterol.

3. Free Plasma Cholesterol

See Flow Chart 1 for this procedure.

4. Plasma Triglycerides

After processing the plasma to a stop point, the samples were stored in refrigerators at 18°C or 1°C. The modified version of the Technicon Auto-analyzer method (no date noted) that was followed is outlined in Flow Chart 2. Due to analytical difficulties, most sample data were discarded. Meaningful statistical analysis of the remaining samples was not possible. The values determined for the remaining data are included in the appendix for those interested.

Flow Chart 1. Total and free plasma cholesterol determination by the Searcy and Bergquist (1960) procedure.

.3 ml plasma + 2.7 ml acetone:ethanol (1:1)

thoroughly mix

centrifuge 10 minutes at 2000 rpm

aspirate supernatant

discard precipitate

store excess at 5°C

Total Cholesterol

0.4 ml to test tube

add 6.0 mo $FeSO_4$-acetic acid reagent

add 2.0 ml concentrated H_2SO_4

wait at least 10 minutes for color development

read absorbance of unknowns against a 0.4 ml acetone: ethanol (1:1) + color reagent blank*

determine sample mg/dl cholesterol from the regression line of standard increments read**

Free Cholesterol

1.0 ml to a conical centrifuge tube

add 1.0 ml digitonin sln.

thoroughly blend on a vortex type mixer

wait at least one hour for precipitate formation

centrifuge 10 minutes at 1700 rpm

aspirate supernatant

resuspend precipitate in ca. 2.0 ml acetone:ethanol (v/v)

centrifuge 5 minutes at 1700 rpm

aspirate, discard supernatant

Flow Chart 1 (cont'd.).

Free Cholesterol (cont'd.)

:

dissolve precipitate in 6.0 ml $FeSO_4$-acetic acid reagent

:

add 2.0 ml concentrated H_2SO_4

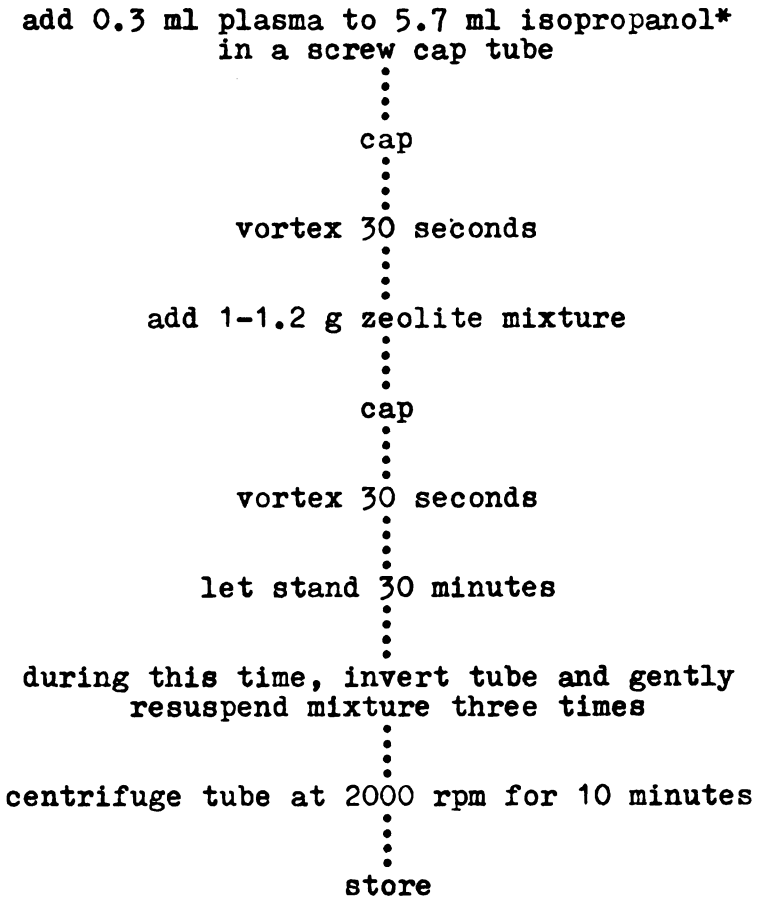
:

determine optical density as in total plasma cholesterol determination

*All total or free cholesterol determinations of either experiment were made on a Hitachi Perkin-Elmer Model 139 UV-VIS Spectrophotomer, the wavelength was set at 490 nm

**Standards were prepared by adding known amounts of crystalline cholesterol to known volumes of acetone: ethanol (1:1)

Flow Chart 2. Sample preparation for plasma triglyceride analysis on the Technicon Autoanalyzer.



*redistilled at 82°C to 85°C

5. Egg Parameters

Eggs were removed from storage and segregated by hen, for the entire experiment. The procedure used for selecting eggs to be processed was: the egg laid on the day of blood sample collection was used when possible. If no egg was laid on that date, the egg from the following day was used. Again, if there was no egg from that date, the egg laid the day preceeding blood withdrawal was used. If none of these conditions could be met, the egg closest to the blood sampling date was chosen (randomly if there were two eggs the same distance from the data collection date).

On the rare occasion the yolk broke during its separation from the albumin, a pasteur pipette with the tip filed off at the base was used in conjunction with a Propipet® to salvage enough uncontaminated yolk for a total yolk cholesterol determination.

A single procedure for the determination of total yolk cholesterol was not used. Instead, the modified Zlatkis method of Weiss et al. (1964) was utilized through the filtering and dilution phase, then the extraction and saponification of cholesterol was as reported by Abell et al. (1952). The color reaction and total cholesterol determination of Searcy and Bergquist, as previously discussed, was followed for the final steps. There was also limited input by other researchers. For instance, Brown (as cited by Weiss et al., 1964), stated that "a wide range of conditions may be used for saponification in a constant volume without

necessarily altering the accuracy of the determination of blood cholesterol" and suggested "saponification at 65°C for one hour with the same concentration of alcoholic potassium hydroxide used by Abell et al. (1952), in order to be certain that all the cholesteryl esters present were saponified". Also, Fisher and Leveille (1957) did not wait between the addition of chloroform:methanol (at 2:1) to the yolk, and the subsequent filtration. Weiss et al. (1964), however, waited several hours. For these experiments, the yolk/solvent mixture stood about one hour before being filtered. The recovery for this combination of procedures was 102%.

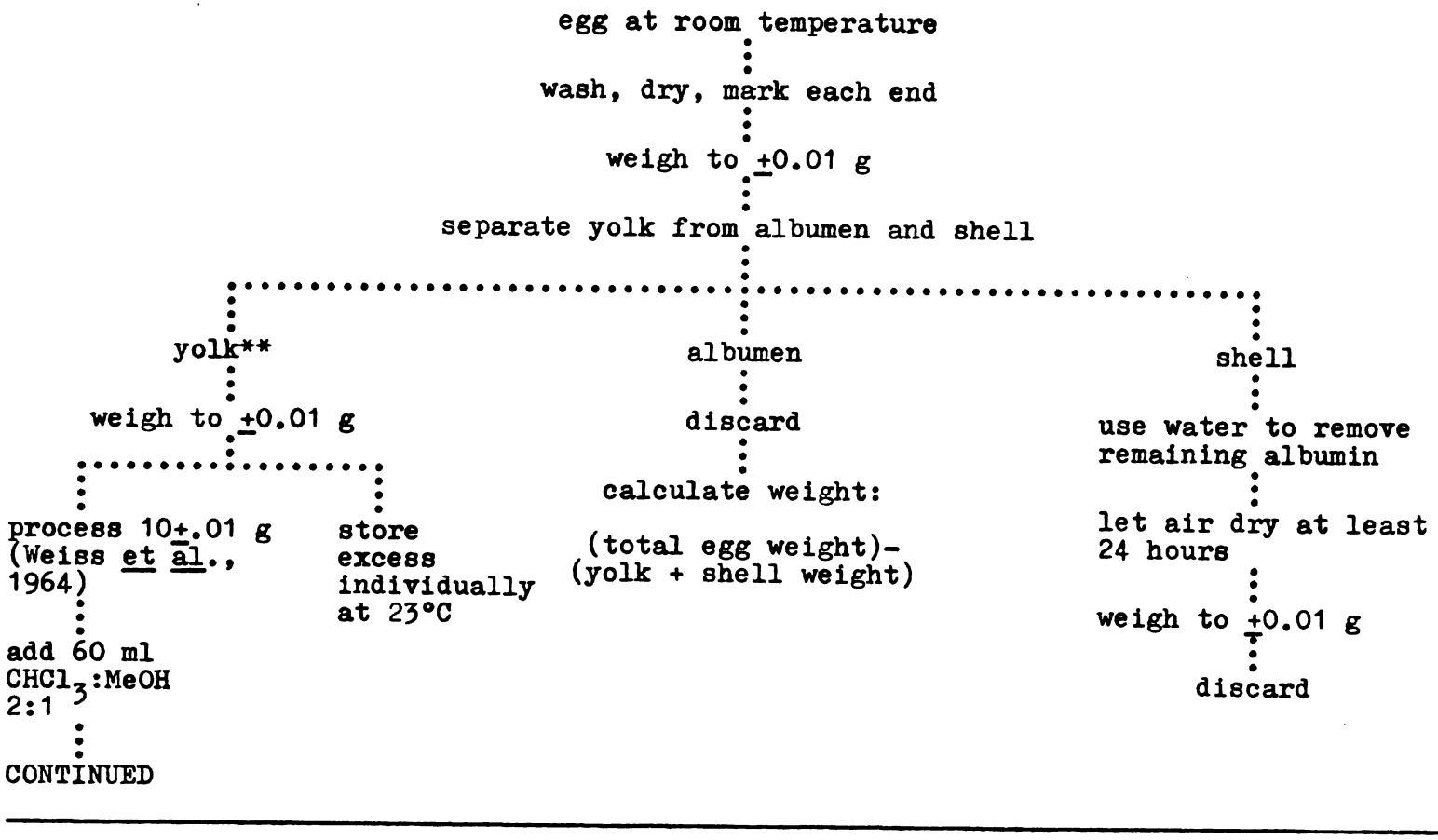
The column used in the filtration of denatured protein from the solvent was a 22 x 450 mm chromatography column, having a tapered tip and sintered glass filter immediately above the taper. The tip was fitted with a cork, which was firmly inserted into a 125 ml microfiltering flask with tubulation. The apparatus was held in place by a Thomas support stand and utility clamps. A vacuum was used to facilitate the filtering process.

Flow Chart 3 shows the method used to determine total yolk cholesterol, plus egg and component weights.

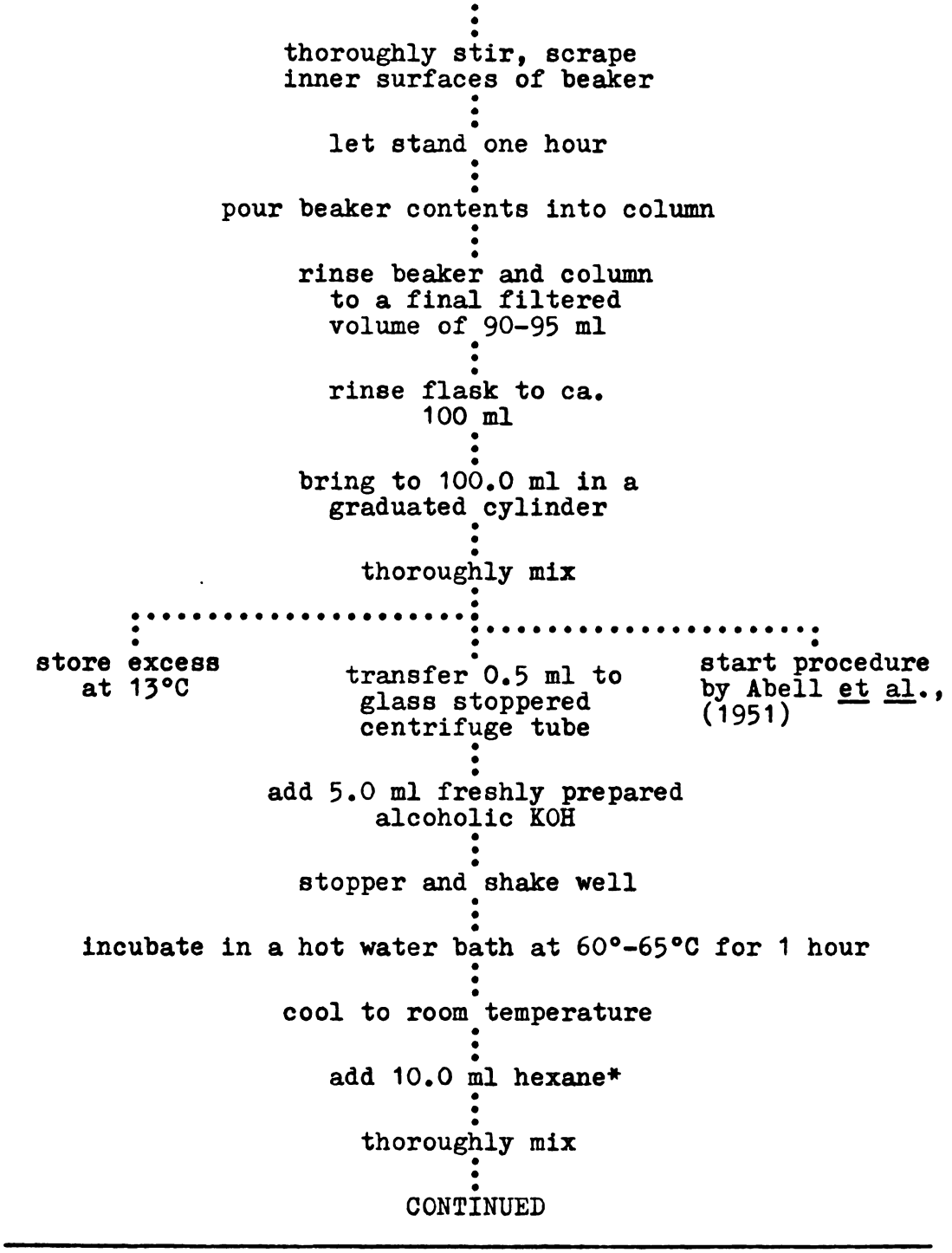
6. Excreta Cholesterol

The excreta from individual hens was air dried about 16 weeks. Then the feathers and other extraneous material were separated from the excreta and discarded. The excreta was

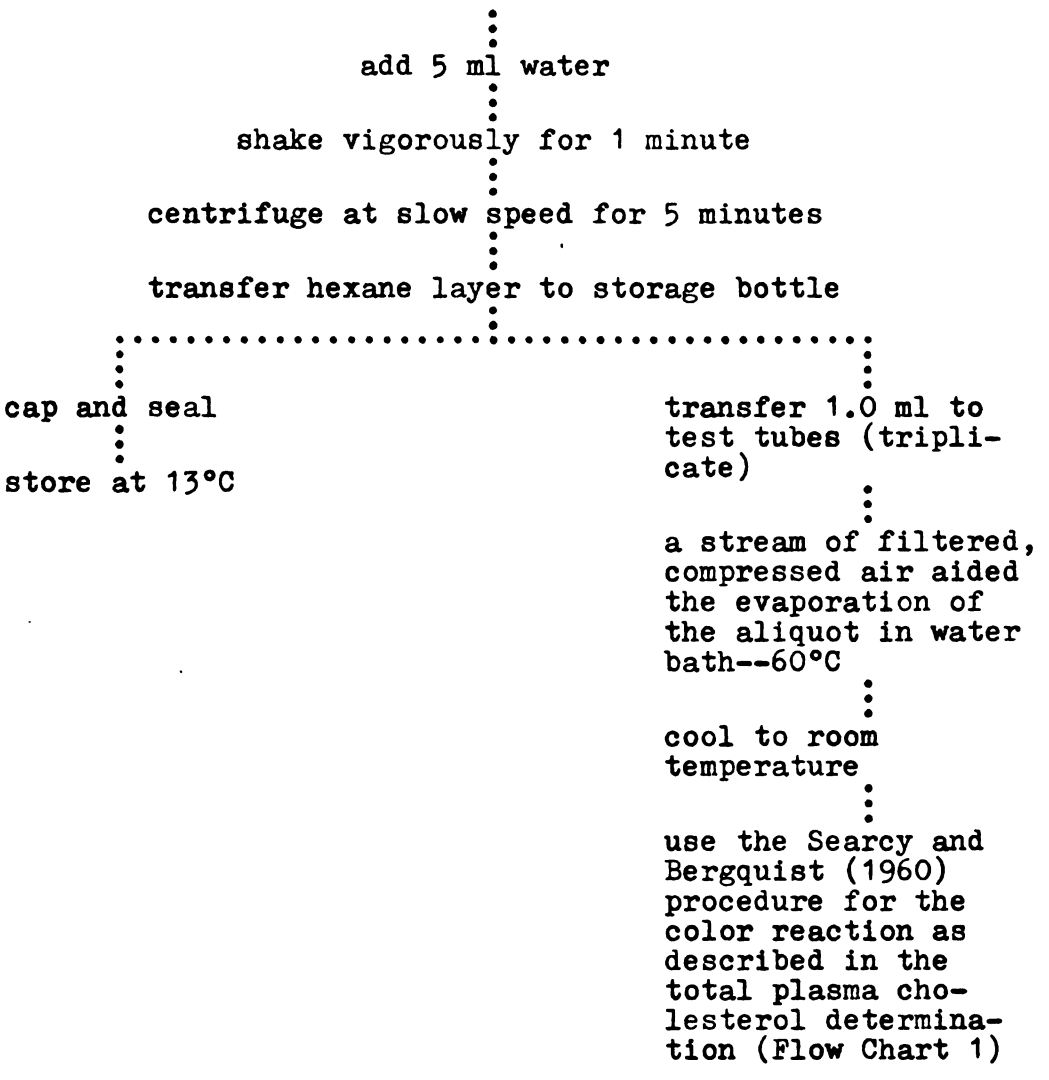
Flow Chart 3. Egg parameters.



Flow Chart 3 (cont'd.).



Flow Chart 3 (cont'd.).



*68.7 ± 1.4°C boiling point

**Recovery was 102%--see Table 2 of Appendix for all recoveries.

then weighed to ± .01 g. The total amount of excreta was ground to a fine consistancy in a hand operated meat grinder. The product of this grinding was transferred to Whirl Pak® (NASCO) bags and stored at room temperature until processed for total cholesterol. The excreta cholesterol was extracted in a manner similar to that of Fisher et al. (1961). Flow Chart 4 outlines the steps used in this process.

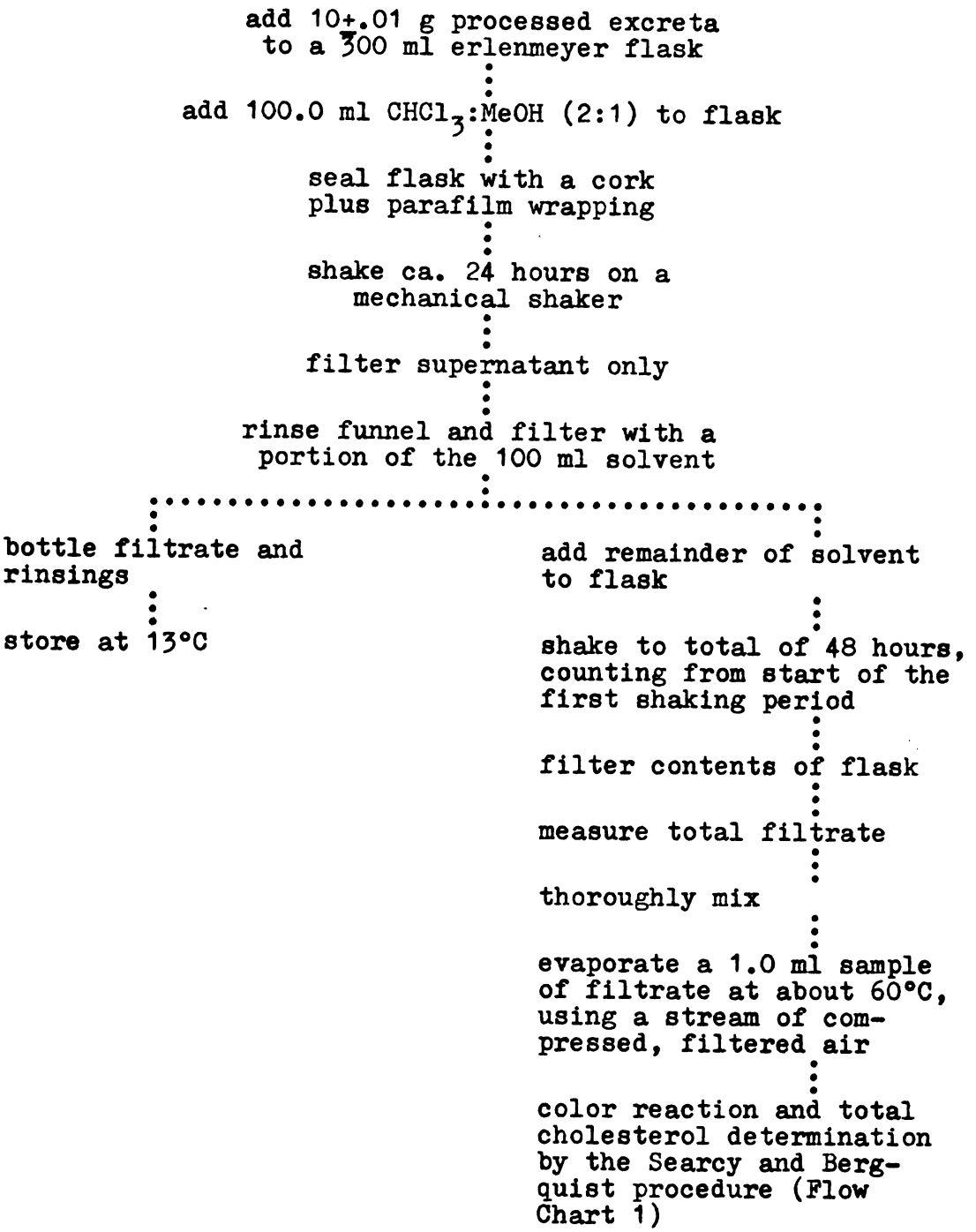
7. Statistics

A split plot method of analysis (Gill, personal communication) was used for all information with full replication. These data were processed using a computer program. As a check of the computer results, the plasma total cholesterol data were manually analyzed by this method. The appendix contains a table comparing these results.

The data put on the computer were: 1. total plasma cholesterol 2. free plasma cholesterol 3. percent free of total plasma cholesterol 4. change in total plasma cholesterol using the pre-treatment value as 100% 5. same as 4, but for free plasma cholesterol 6. body weight 7. percent change in body weight by week 8. percent egg production 9. change in percent egg production.

The rest of the data were highly unbalanced and were analyzed statistically using a one-way ANOVA for each time period, in an attempt to segregate time trends.

Flow Chart 4. Excreta processing.



When an f-value indicated possible differences in treatment means at a date, the f-max test (Gill, personal communication) for homogenous variance was used; if variances were equal, the Tukey HSD test was used, and if unequal, the Dunnett test of means was a better one to use. For unbalanced data within a time period, a modified Tukey HSD test of means was followed. All statistical tests were from personal communication with Gill, and are summarized in the appendix, as is the split plot model and sums of squares formulas.

Experiment 2

1. General Comments

The preparation and physical environment of the SCWL laying hens studied were as in experiment 1. The animals were in their first year of production, and from two different strains. Hens known to be in laying condition were selected and placed on three dietary treatments, according to the previous 13 days egg production. Hens from each level of production were placed in each treatment, ensuring that each group started with hens of similar production. Eight hens were used per treatment.

The control diet was the same as in experiment 1. The low drug treatment was the control diet plus CI-720 added at 2600 ppm. The drug was blended upward with control diet, and mixed with an amount of control diet sufficient for the entire experiment in the nutri blender described in part one.

The high drug treatment level had CI-720 at twice the concentration (5200 ppm) as the low drug treatment feed, and was prepared in the same manner.

Using values of total plasma cholesterol, egg production, and other factors, the end of the third week of drug treatment was determined to probably be the best time to sample the blood of hens for this one-time plasma cholesterol measurement.

Only a weekly mean treatment value for feed consumption was recorded; at this time, the body weight was also recorded ($\pm$ 1 g). The birds were generally fed before 0800 hr. daily, with egg collection at about 1600 hr. each day. Eggs of the collection week (days 15 through 21 of drug treatment) were marked with the date and cage number and stored with the excess eggs from experiment 1. All other eggs from the drug treated groups were destroyed.

Between 0800-1000 hr. on day 21 of drug treatment, 10 ml heparinized blood samples were taken either from the brachial vein or by heart puncture from all hens of this experiment. The blood was centrifuged directly after all samples were taken, as in experiment 1, and the plasma frozen in individual screw-cap tubes.

2. Total and Free Plasma Cholesterol

Total and free plasma cholesterol were determined as in experiment 1.

Procedural changes from experiment 1 were: 1. all volume transfers 1 ml and under were made using Micro-pettors® (Scientific Manf. Industries), in place of pipettes, whenever possible; 2. a Repipet® was substituted for the syringe and needle, with beaker reservoir, used for the concentrated sulfuric acid of the color reaction.

3. Total Yolk Cholesterol

Total yolk cholesterol was determined as in experiment 1.

4. Plasma Triglycerides

Plasma triglycerides were analyzed by the method reported by Mendez et al. (1975), which is diagrammed in Flow Chart 5.

Rather than the tri-olein standard Mendez and his co-workers recommended, corn oil in isopropanol was used. Corn oil is about 97% compounds which have a glycerol base. The glycerol moiety is the part reacted and determined in this procedure, so the decision was made that this degree of purity was satisfactory.

5. Egg Weight; Shell, Albumen, and Yolk Weights

These parameters were all determined as in experiment 1.

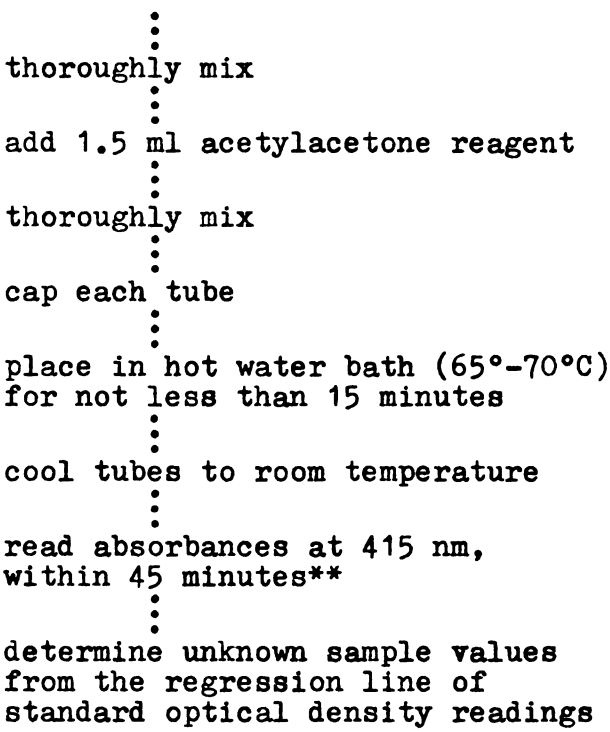
6. Statistics

Because low egg producing hens were thought to be affected more by the drug for some traits than were high producers, the blocking of hens on egg production does not constitute valid blocking. The reason is that a basic

Flow Chart 5. Triglyceride analysis by the Mendez *et al.* (1975) procedure.

blank*	standards*	unknowns
add 0.5 ml water	add 0.5 ml of each standard to the respective tube	add 0.5 ml unknown plasma
	add 0.5 ml water	
	add 2.0 ml heptane	
add 3.5 ml isopropanol	add 3.0 ml isopropanol	add 3.5 ml isopropanol
	add 1.0 ml dilute H_2SO_4	
	mix 30 seconds on a vortex type mixer	
	let phases separate without centrifugation	
	transfer 0.2 ml heptane layer to a clean dry screw cap tube	
	add 2.0 ml isopropanol	
	add 0.6 ml saponification reagent	
	thoroughly mix	
	let stand at room temperature not less than 5 minutes	
	add 1.5 ml sodium metaperiodate reagent	
	CONTINUED	

Flow Chart 5 (cont'd.).



*15 ml screw cap tubes were used for all observations

**used the previously described Spectrophotometer
(Flow Chart 1)

premise of blocking has been violated; that a nuisance variable not interact with treatments. All data were analyzed as a one-way analysis of variance (anova). If the f-statistic was significant, the f-max test for homogenous variance was applied. For equal variances, the Tukey HSD test of means was employed; if unequal, Dunnett's test of means was used. Unless noted to the contrary, the Tukey HSD test was the statistical analysis used.

For all the egg data, there was not balanced replication, so a modified Tukey HSD test of means was used, as in the first experiment. In addition, the values for the only egg from the high drug treated group of hens was in the midst of those from the low drug level. Therefore, the values from drug treated hens were combined, and the statistical analysis done as a comparison of the control group of hens <u>versus</u> the hens in the combined drug treated groups.

RESULTS AND DISCUSSION

Total Plasma Cholesterol

1. Experiment 1

According to the split plot statistical analysis of the data, the presence of CI-720 significantly decreased ($P < .046$) the hen's total plasma cholesterol, from 133 to 114 mg/dl plasma $\pm$ 5.6 as the experiment standard error of the mean.

Figure 1 shows a comparison of the plasma cholesterol treatment means from hens on sample dates of experiment 1. From this graph, one can see a possible reason for the significance of a drug effect. The three hens on the drug *per os* treatment started out with low total plasma cholesterol values, and did not change much. This would cause the final mean for the drug treated hens to be predictably lower than the final mean for those hens not given the drug. When these drug treatment means did change over time, their respective control values generally changed in a similar manner, so there was no interaction over time between the treatments.

When the hen was used as her own control (the cholesterol levels expressed as a percent of their respective

Figure 1. Experiment 1. Total plasma cholesterol in mg/dl. Values represent the mean of three hens per treatment. The standard error for the experiment was 5.7.

A = last day of pre-treatment

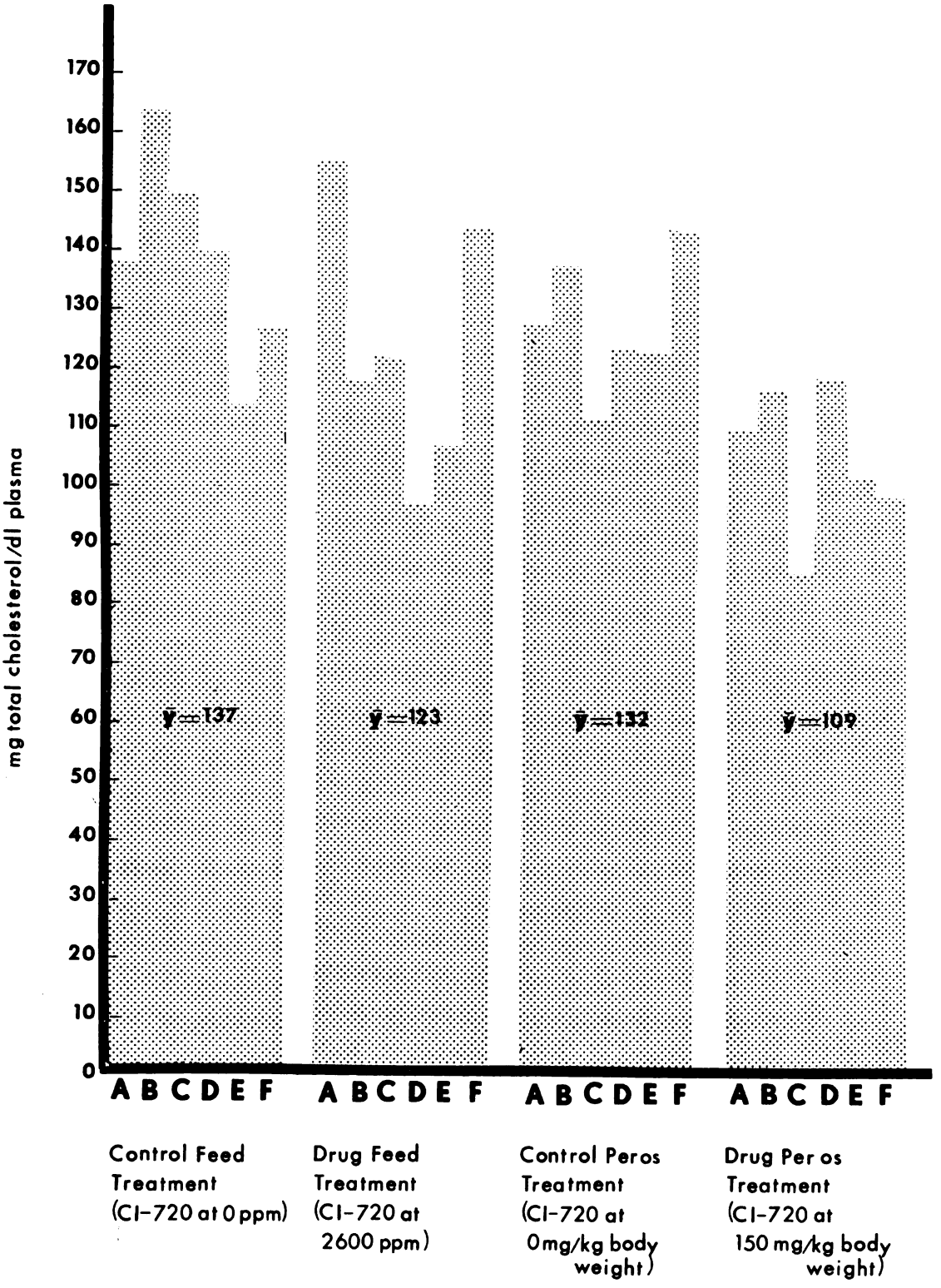
B = day 4 of drug treatment

C = day 7 of drug treatment

D = day 14 of drug treatment

E = day 21 of drug treatment

F = day 7 of post-treatment



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pre-treatment level) no difference could be detected between any of the four treatments, for any split plot factor.

The values for total plasma cholesterol of hens in all treatments were examples of considerable biological variation.

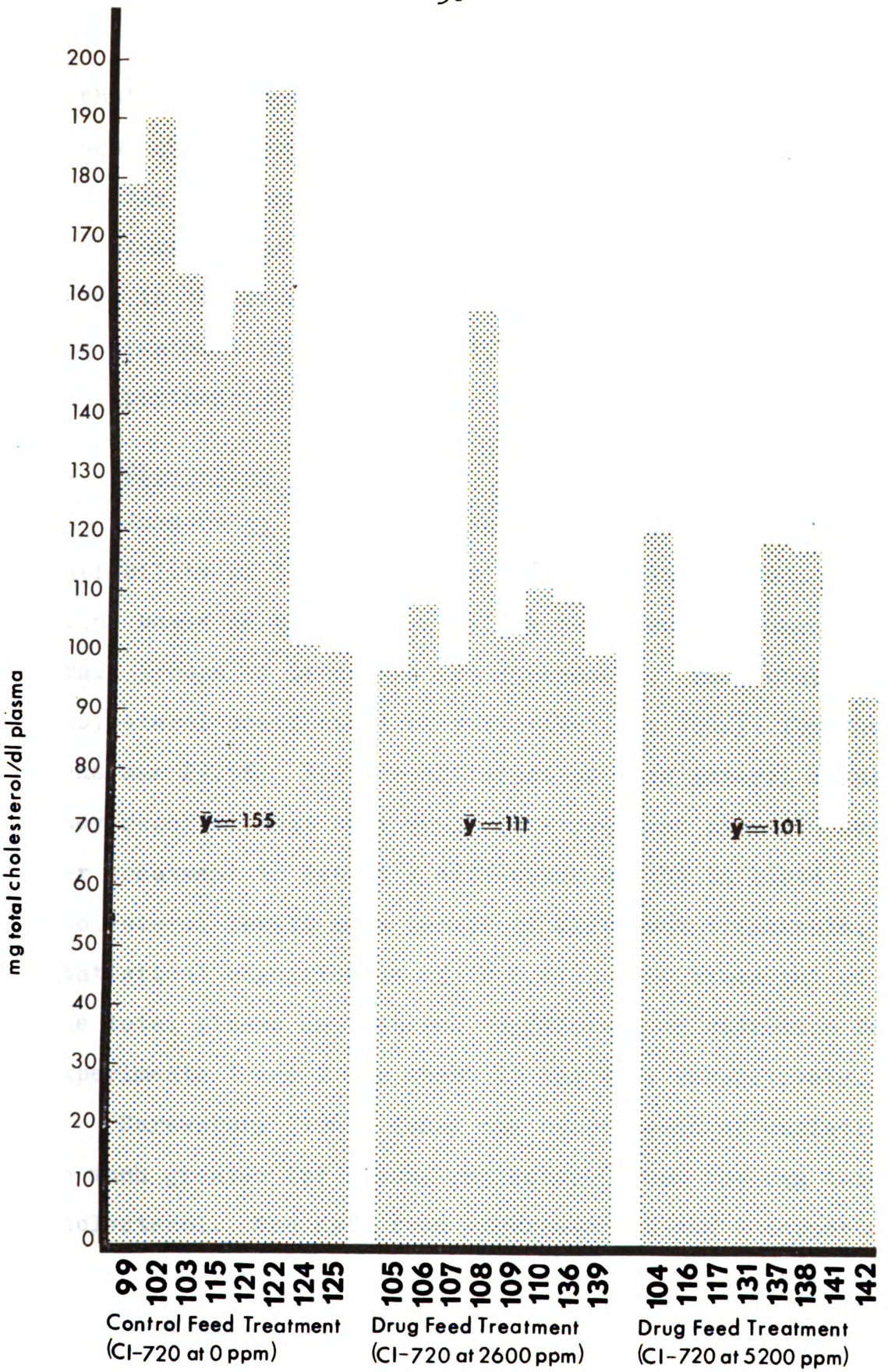
2. Experiment 2

Statistical analysis of treatment means by the Tukey HSD test of means indicated that hens of the control group had higher total plasma cholesterol than hens of either of the drug treated groups of hens ($P < .01$).

In Figure 2 are shown the total plasma cholesterol level from hens in each treatment. In general, there was little difference between the total plasma cholesterol level of hens in either of the drug treatments. Two hens of the control treatment are shown to have total plasma cholesterol values close to the means of the drug treated groups.

Total plasma cholesterol values for individual hens were, in general, higher in the second than in the first experiment. For example, the mean of all the control hen's values were 137 and 155 mg/dl plasma for experiments 1 and 2, respectively, for total plasma cholesterol. Possible explanations are, that mixed strains were used in the second experiment, and all hens were of the same strain and age in experiment 1. Also, these values may have been evidence of seasonal variation, as Weiss (1957) suggested. He additionally listed reproductive state, diet and analytical

Figure 2. Experiment 2. Total plasma cholesterol in mg/dl. These are the individual hen levels on the 21st day of drug treatment. The standard error for the experiment was 5.32



Numbers represent the hen number

technique as factors in the variability of chicken total plasma cholesterol. Another consideration is that there was only one observation over time in experiment 2, while the overall mean was calculated from hen levels at six dates in the first experiment. Also, three hens were used per treatment in experiment 1, and eight hens per treatment in experiment 2.

3. Discussion

Johnson et al. (1959) suggested that due to the great variability of laying chicken's plasma cholesterol, conclusions based on the average values obtained from unreplicated small groups of birds are not dependable. The total plasma cholesterol coefficient of variation ($s \times 100/\bar{y}$) for these hens was 29.5%. For a coefficient of this general magnitude, they reported that they would have required about 70 hens per treatment to detect a difference of 15% in blood cholesterol levels as a result of different treatments, with statistical significance of ($P < .01$), nine times out of ten. The total plasma cholesterol coefficient of variation for experiments 1 and 2 was about 23 and 21%, respectively.

Shrewsbury (1967) found the coefficient of variation to be much greater for plasma cholesterol than for egg total cholesterol. The data from experiments 1 and 2 supported the findings of Shewsbury (see Table 3 of the appendix).

In experiment 1, the range of all values of total plasma cholesterol was 75-209 mg/dl, with a mean of

123 mg/dl. In experiment 2, hens had a total plasma cholesterol range of 71-195 mg/dl, with a mean of 122 mg/dl. Table 6 gives values for total plasma cholesterol others have found.

In experiment 2, many of the hens on drug treatment were "off feed" and out of production. This was most evident in the group of hens given CI-720 via the feed at 5200 ppm, whose feed intake was about half the maintenance level of about 70 g feed intake/hen/day for SCWL (Miller and Denton, 1962), during the week of sample collection. In experiment 1, the feed consumption average dropped for hens given either drug treatment, but few were below the maintenance level. With insufficient food for the body functions, lipids would be used for energy so the blood levels of these lipids would be expected to decline. Acetate then would not be available for cholesterol synthesis, and other lipids, and one would expect those hens with below maintenance feed intake to have lesser plasma cholesterol, triglycerides, and other blood lipids. Also, the inability of the hens to provide adequate nutrients for the egg would cause the egg production to cease.

Lorenz *et al.* (1938) found the total plasma cholesterol changed less than any other lipid during the laying period.

Bartov *et al.* (1971) observed that plasma cholesterol concentration exhibited a pronounced rhythmic change associated with the egg formation cycle, and that these fluctuations were not reflected in the yolk cholesterol

Table 6. Chicken total plasma cholesterol values taken from the literature.

Type	Cholesterol Range or Mean in mg/dl blood	Reference
SCWL hens	54-422, $\bar{y}$=211	
SCWL hens +5% of diet as dried egg yolk	140-220, $\bar{y}$=184	
Barred Plymouth Rock hen	88-238, $\bar{y}$=137	
Barred Plymouth Rock hen+ 5% of diet as dried egg yolk	138-210, $\bar{y}$=165	Miller and Denton (1962)
SCWL floor layers	248	
SCWL floor non-layers	272	
SCWL floor layers 17% protein; 937-1050 Cal./lb. diet	255-340	
18% protein; same energy	285-294	
SCWL non-layers; 17% protein; same energy	368-470	
18% protein; same energy	518-645	Price *et al.* (1957)
14-day-old SCWL males	86±34	Peterson (1951)
4-week-old to 28-day-old SCWL males	ca. 55-95	Peterson *et al.* (1952)
SCWL laying hen	178-285	Wagh and Hinners (1961)
SCWL 2-week-old males	141±12	Banerjee *et al.* (1965)

Table 6 (cont'd.).

Type	Cholesterol Range or Mean in mg/dl blood	Reference
Laying hen--meat pop.	169±59	
Laying hen--mixed pop.	239±63	Washburn and Nix (1974)
Laying hen	76-156	Walker et al. (1951)
Range layer New Hampshire	116-288, $\bar{y}$=205	
New Hampshire cage layer	110-450, $\bar{y}$=217	Johnson et al. (1959)
Laying hen	88	Dua et al. (1966)
Laying hen low fat diet	73-215	
Laying hen high fat diet	79-242	
Immature nonlaying hen	92-155 $\bar{y}$=121±3	
Male chicken low fat diet	102-147	
Male chicken high fat diet	103-187	Lorenz et al. (1938)

concentration. There appeared a definite increase in plasma cholesterol when no egg was laid, even though one was present in the shell gland. Bartov and co-workers also noted a marked decline in blood cholesterol after repeated daily (six) blood withdrawal.

By comparing layers versus non-layers, Price et al. (1957) found that layers have a lower plasma cholesterol level than non-layers; and for these latter birds in cages, they reported a 50-250% increase in total plasma cholesterol over non-layers on litter. In the present study, the values for the control hen's total plasma cholesterol did not reflect the inverse relationship Price and associates reported. The reason could be that none of the hens were completely out of production; the only hens of these experiments to have ceased production were hens given the CI-720. The wide difference between the control hens total plasma cholesterol values would then be explained by biological variation.

The CI-720 administration appears to have results similar to those observed following the administration of cholestyramine, regarding total plasma cholesterol and total yolk cholesterol, i.e., to lower the plasma cholesterol, and have no effect on yolk cholesterol. The fact that hens in one drug treatment from the first experiment did produce eggs with significantly higher yolk cholesterol than the eggs produced by hens of a control treatment at one sample date, should be mentioned to qualify this comparison

with cholestryamine. The treatment means for yolk cholesterol were nearly identical in experiment 2.

Weiss et al. (1967) have reported results which indicate that clofibrate may decrease plasma total cholesterol of hens, but they did not report statistical analyses for the data because of the intense individual variability of the subject's plasma cholesterol.

Free Plasma Cholesterol

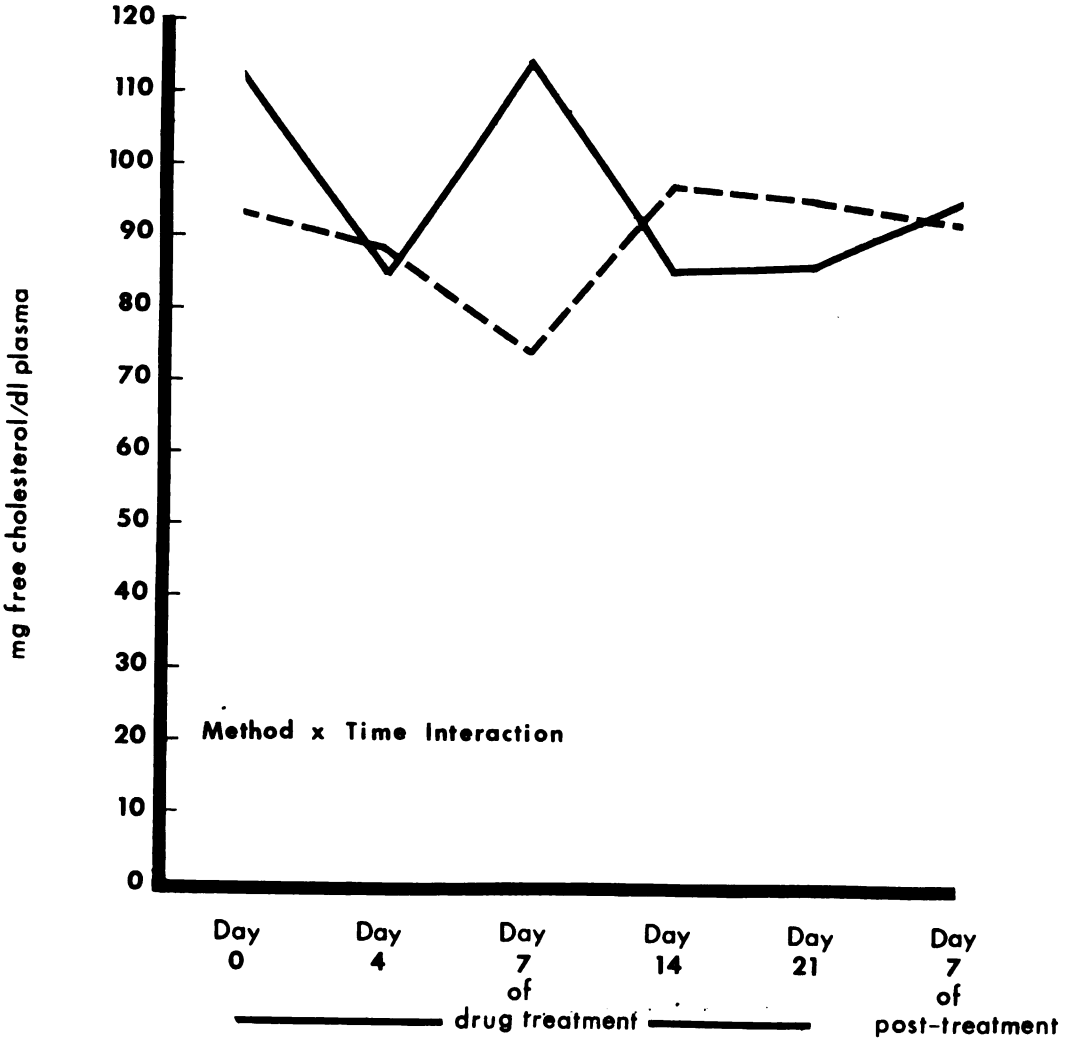
1. Experiment 1

Statistical analysis using the split plot model with a computer, showed a highly significant ($P < .009$) lowering of the hen's free plasma cholesterol by administration of CI-720, from 107 to 79 (± 5.7 SEM) mg/dl.

The method of drug administration over time was significant ($P < .044$). The drug given via the feed seemed to be more variable in effect than when the capsule was used to administer the drug. Free plasma cholesterol values for all six data collection dates are reported in Figure 3. The response to method of drug administration over time is about the same except at the end of week one of treatment. This time has no particular significance as far as any treatment change is concerned, and was probably due to chance. This one out-lying value was undoubtedly influential in causing the level of significance to be present.

The only other item of interest for this parameter is the three-way interaction of method x time x drug ($P < .014$).

Figure 3. Experiment 1. Free plasma cholesterol. Plot of method of drug administration x time interaction; according to the split plot statistical analysis. The means plotted represent the data from six hens, as the control and drug feed treatments were compared against respective per os (capsule) treatments.

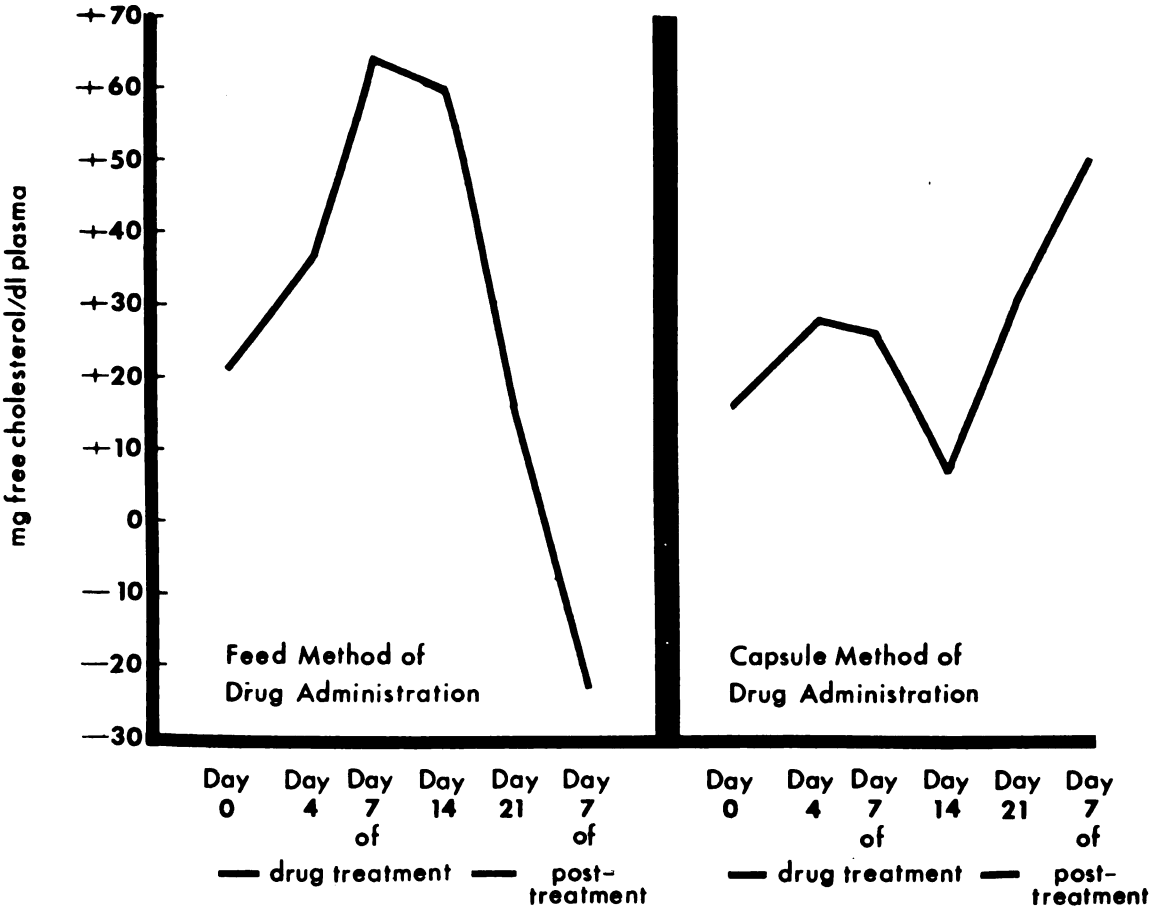

120
110
100
90
80
70
60
50
40
30
20
10
0
mg free cholesterol/dl plasma
Method x Time Interaction
Day 0
Day 4
Day 7 of drug treatment
Day 14
Day 21
Day 7 of post-treatment
= Feed Treatments, combined
= Capsule Treatments, combined

Plotting the difference in response between presence and absence of the drug over time (Figure 4), one can see that a difference does exist between feed or capsule treated groups of hens, and that they also do not react in the same way with time.

Figure 5 illustrates the antilipemic effect of CI-720 by using the hen means of free plasma cholesterol at each treatment date. The antilipemic effect of CI-720 was apparent for the hens of the drug feed group, which also showed a rebound to the pre-treatment level when the drug was removed; this rebound effect was not evident for hens in the drug per os treatment. Hens of the drug per os treatment had levels of free plasma cholesterol during the treatment periods which were higher than the pre-treatment reading, as was true for the total plasma cholesterol levels. This drug per os group of hens had started out with low total and free plasma cholesterol, and remained that way. These drug per os treated hens were probably primarily responsible for the overall indication of lowered free plasma cholesterol, as a result of administering CI-720.

When the hen was used as her own control, (all subsequent free plasma cholesterol levels taken as the percent of the respective hen's pre-treatment value) no difference could be found in comparisons between treatments, using the split plot statistical analysis.

Figure 4. Experiment 1. Free plasma cholesterol. Three-way interaction of method of drug administration x presence or absence of drug x time.



VALUES REPRESENT THE DIFFERENCE IN RESPONSE (NO DRUG MINUS DRUG TREATED HEN VALUES), AS SEGREGATED BY METHOD OF DRUG ADMINISTRATION

Figure 5. Experiment 1. Free plasma cholesterol in mg/dl. Values represent the mean of three hens per treatment. The standard error for the experiment was 11.6.

A = day 6 of pre-treatment

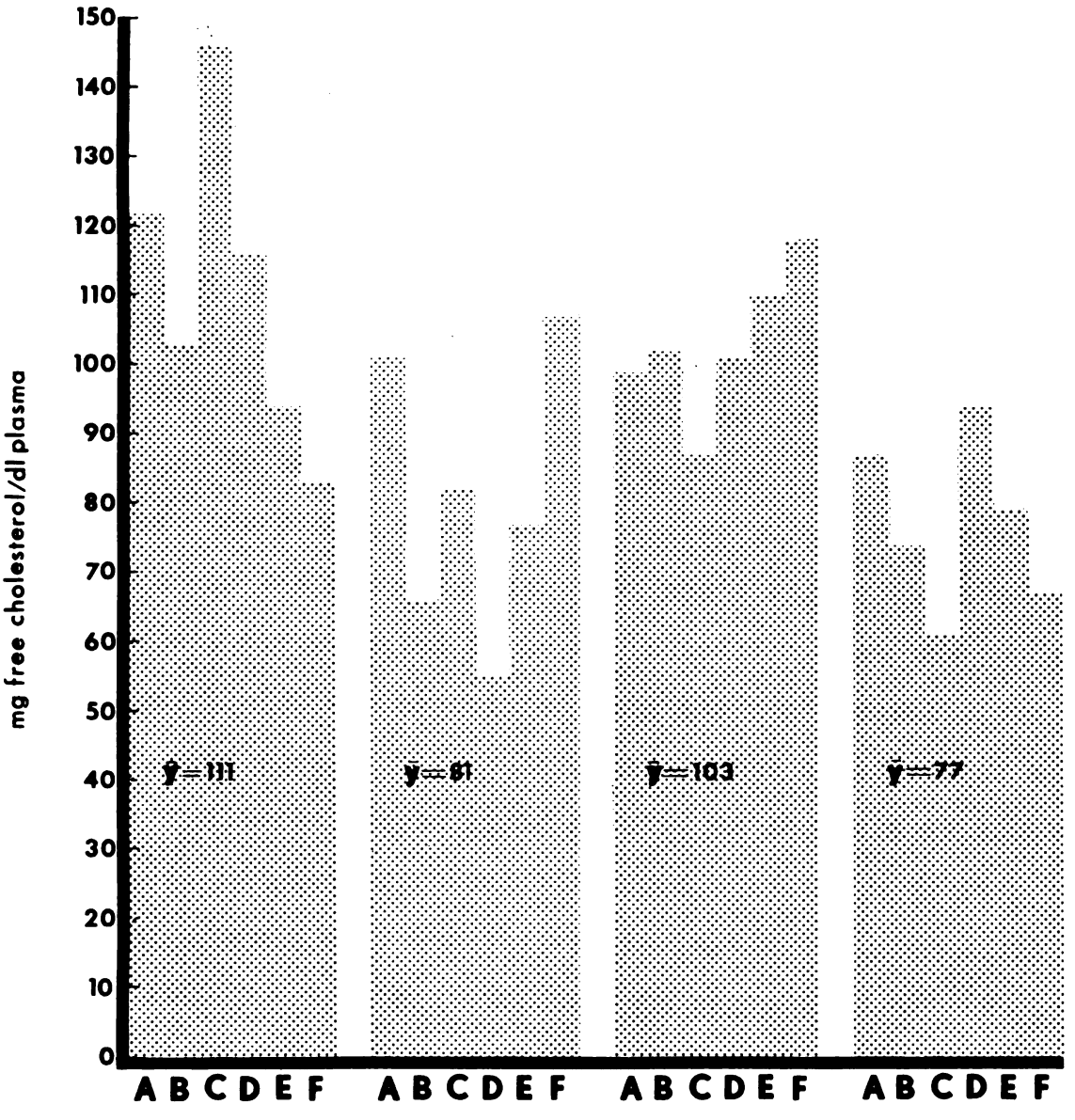
B = day 4 of drug treatment

C = day 7 of drug treatment

D = day 14 of drug treatment

E = day 21 of drug treatment

F = day 7 of post-treatment



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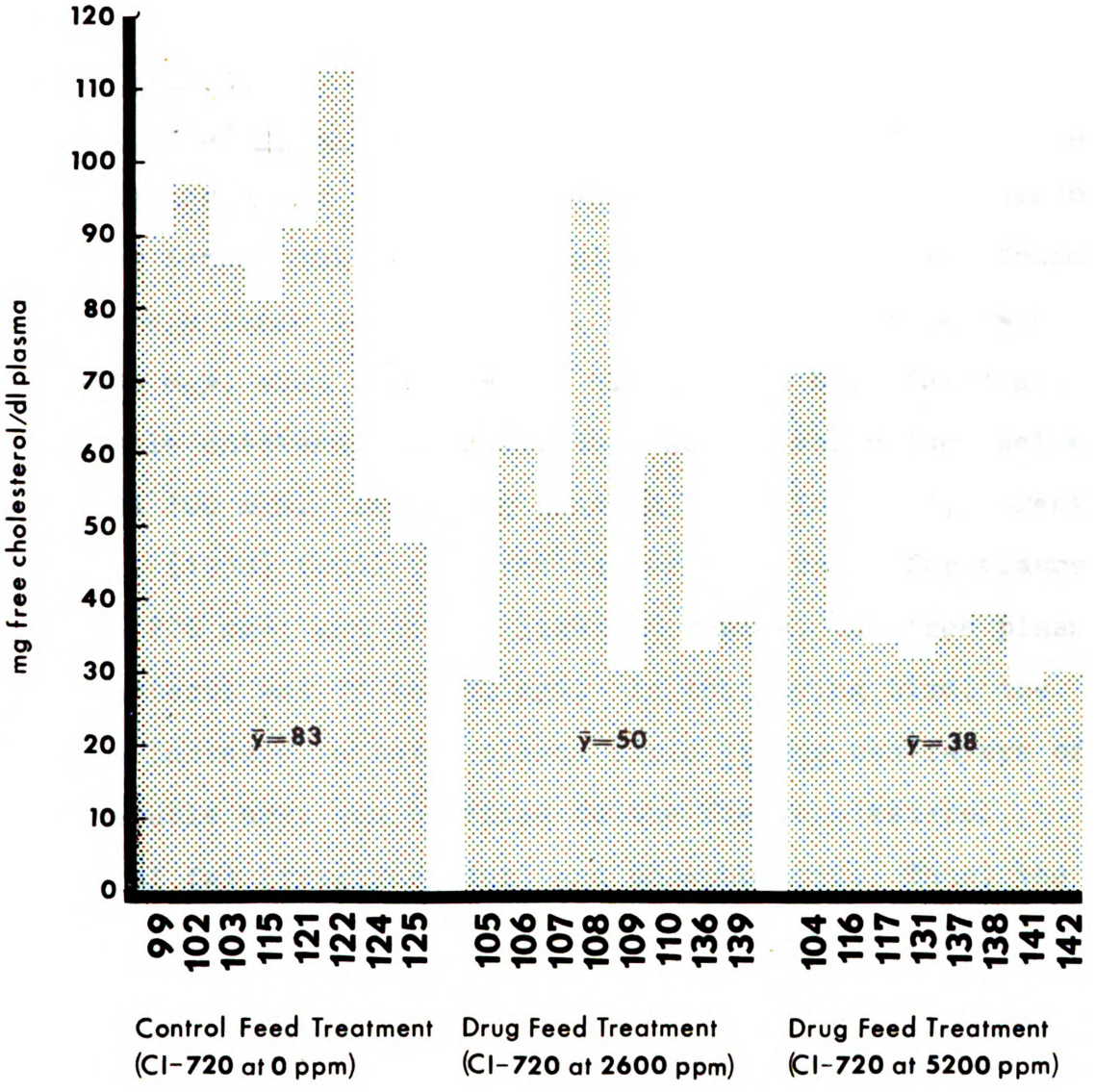
2. Experiment 2

As in the first experiment, the statistical analysis indicated that the drug lowered the free plasma cholesterol from the control hen's level, this time at a 99% confidence level. The values for treatments of 0, 2600, or 5200 ppm CI-720 were 83, 50, and 38 mg free cholesterol/dl plasma, respectively. The two treated groups of hens were also tested statistically by the Tukey HSD test of means, and were found to be similar.

When the single observation of free plasma cholesterol for each hen was compared with that hen's egg production (Figure 6), there seemed to be an indication that the effect of CI-720 was associated with the hen's capability of producing eggs. This would logically be an effect of a drug that altered plasma lipid levels, as the yolk is about 32% lipid, and the egg about 11% lipid. In the high drug level treatment, only hen 104 was in laying condition, and in the 2600 ppm treatment the hens in cages 106, 107, 108, and 110 were the only ones in production. These hens had free plasma cholesterol levels approximately 1.5 to 3 times that of the remainder of the hens given the drug. These drug treated hens, with the slightly depressed from control levels of free plasma cholesterol, could have had a higher tolerance toward the drug. Those hens with low plasma cholesterol and low egg production, also were not eating at their normal level. Therefore, the change in diet

Figure 6. Experiment 2. Free plasma cholesterol in mg/dl plasma. Plotted are the individual hen values for the eight hens per treatment on day 21 of drug treatment. The standard error for the experiment was 4.02.



Numbers represent the hen number

could explain the decline in egg production and plasma cholesterol, at least in part.

3. Discussion

Lorenz et al. (1938) found considerable variation in the laying hen's free plasma cholesterol. The immature non-laying female chicken's free plasma cholesterol was found to have a range of 72-106 mg%. The range for males was 68-106 mg%, when on a low fat diet. On a high fat diet, the male chickens had free blood cholesterol values between 74 and 100 mg%. In laying hens on a low fat diet, Lorenz et al. (1938) found the free cholesterol range for plasma was 67 to 206 mg%; and on the high fat regime, the free plasma cholesterol was 73 to 190 mg%. In the laying bird, the large increase in total plasma lipid versus non-layers of comparable age, was confined to the free cholesterol, neutral fat (triglycerides) and phospholipid. Thus, the free percent of total plasma cholesterol would increase with the onset of egg production. These changes associated with maturity were not uniform, even for birds on the same diet.

Caldwell and Suydam (1960) found free plasma cholesterol in cockerels to be about 24 to 31 mg%.

The values in either of the current experiments are in the range of free plasma cholesterol levels reported by other researchers for both layers and non-layers.

Because of the low free plasma cholesterol values and low free percent of total plasma cholesterol, the data of the second experiment appeared to be in error, therefore, the plasma diluent of each hen was re-evaluated using freshly prepared reagents. In table 2 of the appendix is given a comparison of these results; this table also gives an indication of the high degree of precision of this procedure.

Free Cholesterol as a Percentage of Total Plasma Cholesterol

1. Experiment 1

This parameter showed responses which were similar to changes in free plasma cholesterol. The split plot statistical analysis indicated a highly significant decrease in the percent free of total plasma cholesterol due to CI-720 administration ($P<.013$), and the three-way interaction had a significance level of .029. The time effect was also highly significant ($P<.003$).

Figure 7 illustrates the variability of the hen's percent free of total plasma cholesterol values over time. Because the pattern of each control group was similar in appearance to it's drug counterpart (but not in magnitude), the statistical significance of a drug effect may not have been entirely accurate. Also, the hens given the drug in the feed started with and maintained low values for this parameter throughout the experiment; and the drug treated groups of hens had values on treatment dates greater than

Figure 7. Experiment 1. Percent free of total plasma cholesterol. There were three hens per treatment.

A = day 6 of pre-treatment

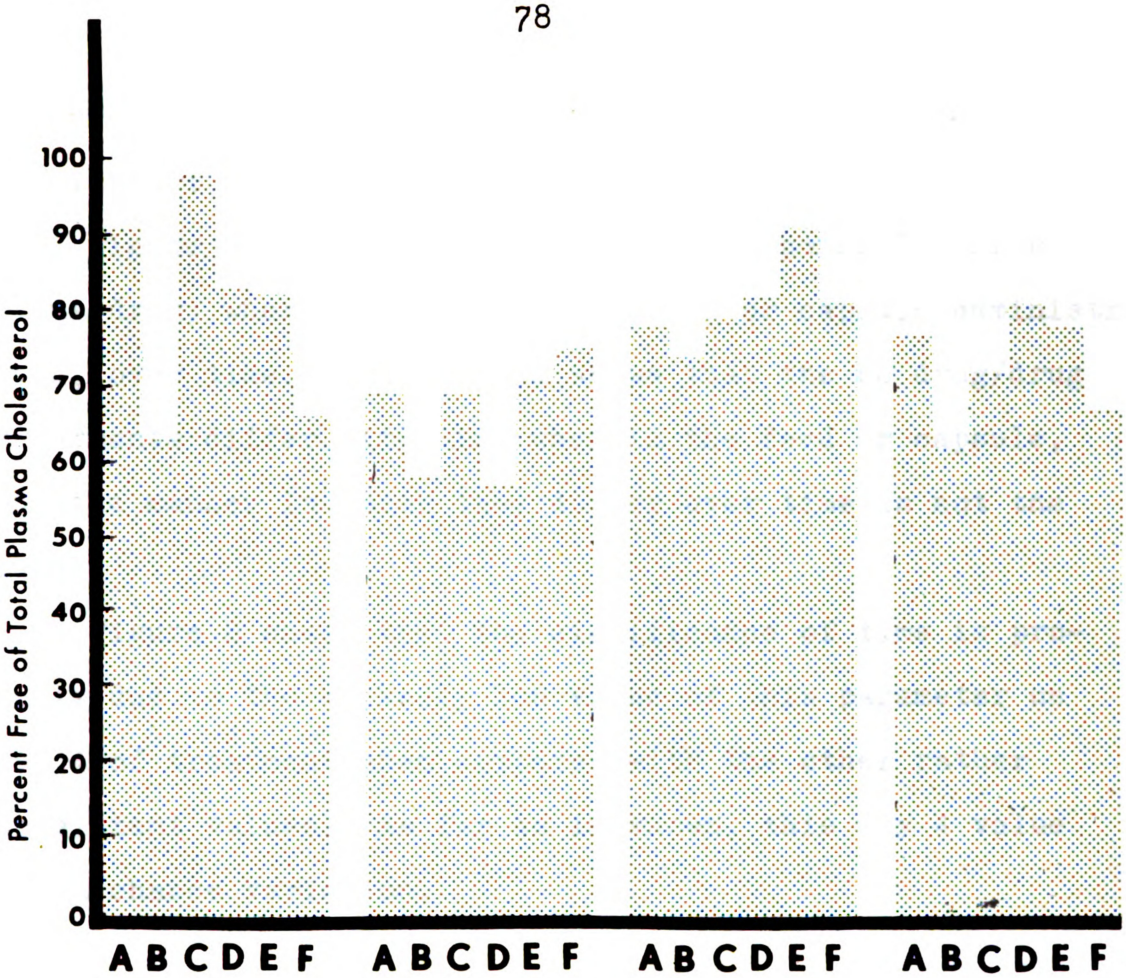
B = day 4 of drug treatment

C = day 7 of drug treatment

D = day 14 of drug treatment

E = day 21 of drug treatment

F = day 7 of post-treatment



Control Feed Treatment (CI 720 at 0 ppm)

Drug Feed Treatment (CI-720 at 2600 ppm)

Control Per os Treatment (CI-720 at 0 mg/kg body weight)

Drug Per os Treatment (CI-720 at 150 mg/kg body weight)

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the respective pre-treatment level. Table 7 is the counterpart of Figure 7.

Figure 8 shows the difference in response (no drug minus drug) when interacting with feed or capsule administration, over time. This figure shows that the no drug/drug treatments differ with time when in the feed or capsule, and the manner in which they differ over time is not the same.

Figure 8 shows that the significance of time is probably due to the markedly low value of this parameter on day 4 of treatment when compared with the other rather consistent values. As far as is known, this day 4 value was a chance variation.

2. Experiment 2

Because of non-homogenous variance, as indicated by the f-max test on the three treatment variances, the Dunnett method of testing means was used. The control hen's values versus the low drug level treated hens, and the latter versus the hens given the high CI-720 dosage were not significantly different from each other ($P > .05$). But the control hens mean for the percent free of total plasma cholesterol was greater than the mean of the hens given the high drug level ($P < .01$).

Table 8 shows the percent free of total plasma cholesterol for experiment 2, and also contains the values for total and free plasma cholesterol for each hen. Figure 9

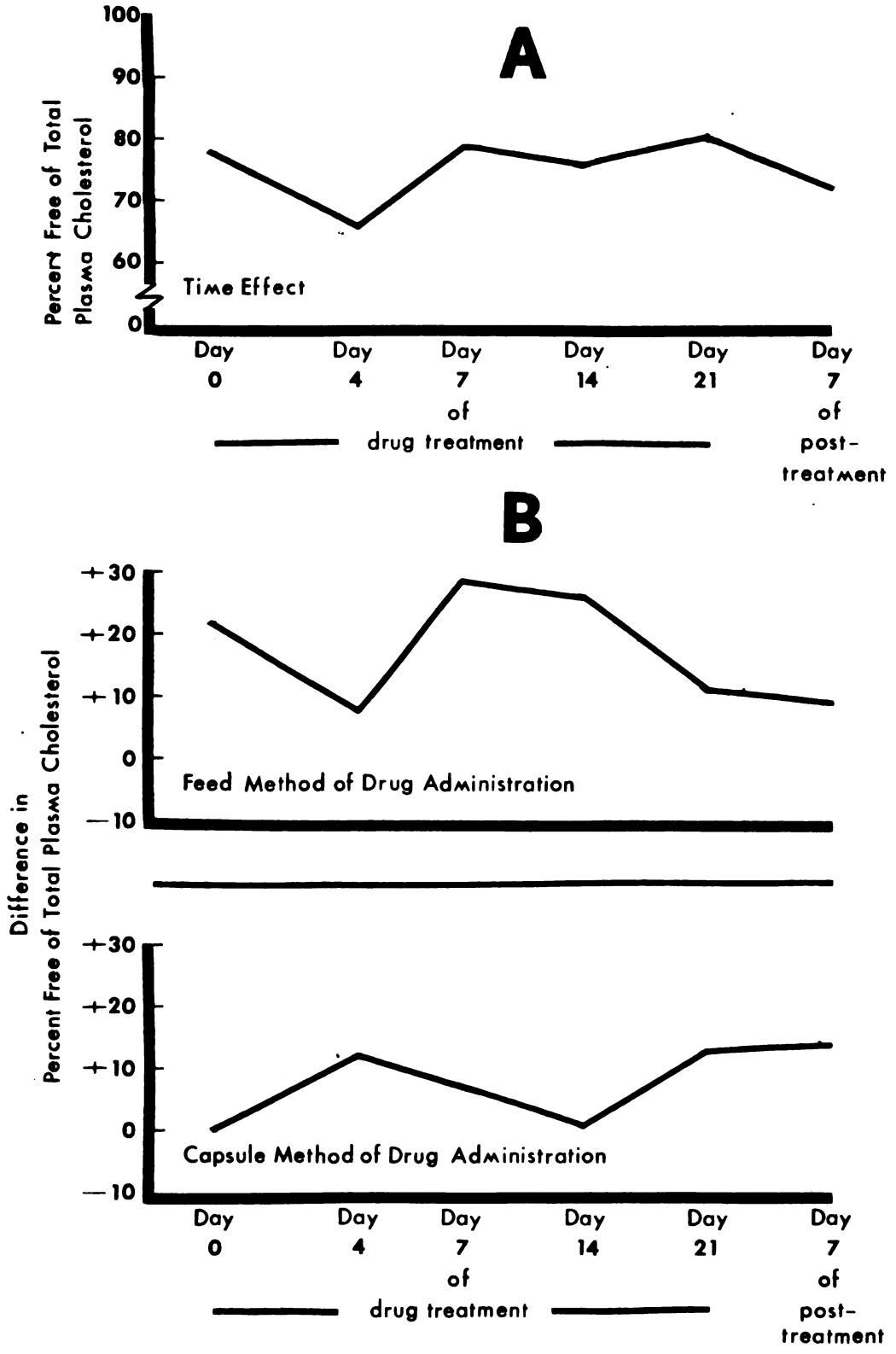
Table 7. Experiment 1. Percent free of total plasma cholesterol.

Hen #	Day 0	Day 4	Day 7	Day 14	Day 21	Day 7	$\bar{y}$
Control Feed Treatment (CI-720 @ 0 ppm)							
1	100	62	95	85	81	60	82
2	86	57	102	72	86	71	79
3	86	71	97	92	80	68	82
mean	91	63	98	83	82	66	81
Drug Feed Treatment (CI-720 @ 2600 ppm)							
6	96	60	75	49	80	74	72
7	57	46	63	51	52	78	58
8	54	67	68	72	80	72	69
mean	69	58	69	57	71	75	66
Control *per os* Treatment (CI-720 @ 0 mg/kg body weight							
9	82	82	76	90	96	87	86
10	71	73	85	89	89	68	79
11	80	68	76	67	87	87	78
mean	78	74	79	82	91	81	81
Drug *per os* Treatment (CI-720 @ 150 mg/kg body weight)							
14	80	48	71	74	78	70	70
15	58	54	78	81	70	56	66
16	94	84	67	88	86	76	83
mean	77	62	72	81	78	67	73

Figure 8. Experiment 1. Percent free of total plasma cholesterol. Results of the split plot statistical analysis.

A = the effect of time on the percent free of total plasma cholesterol

B = the three-way interaction of method of drug administration x presence or absence of drug x time



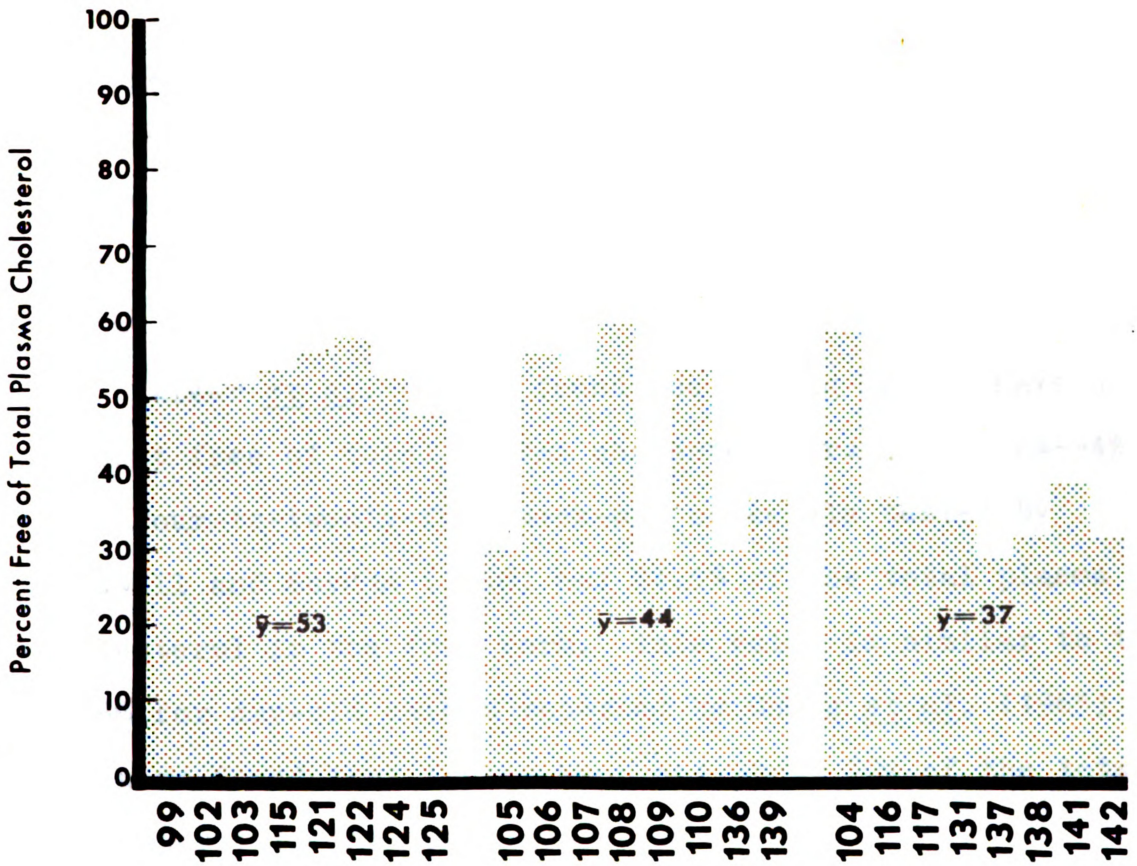
B = VALUES REPRESENT THE DIFFERENCE IN RESPONSE (NO DRUG MINUS DRUG TREATED HEN VALUES), AS SEGREGATED BY METHOD OF DRUG ADMINISTRATION

Table 8. Experiment 2. Percent free of total plasma cholesterol.*

Control Feed Treatment (CI-720 @ 0 ppm)								
Hen Number	99	102	103	115	121	122	124	125
Total Plasma Cholesterol	179	190	164	151	161	195	101	100
Free Plasma Cholesterol	90	97	86	81	91	113	54	48
Percent Free of Total	50	51	52	54	56	58	53	48
Low Drug Level Treatment (CI-720 @ 2600 ppm)								
Hen Number	105	106	107	108	109	110	136	139
Total Plasma Cholesterol	97	108	98	158	103	111	109	100
Free Plasma Cholesterol	29	61	52	95	30	60	33	37
Percent Free of Total	30	56	53	60	29	54	30	37
High Drug Level Treatment (CI-720 @ 5200 ppm)								
Hen Number	104	116	117	131	137	138	141	142
Total Plasma Cholesterol	121	97	97	95	119	118	71	93
Free Plasma Cholesterol	71	36	34	32	35	38	28	30
Percent Free of Total	59	37	35	34	29	32	39	32

*Included are the individual values for the total and free plasma cholesterol.

Figure 9. Experiment 2. Percent free of total plasma cholesterol. Values are calculated as the difference between the total and free plasma cholesterol levels on day 21 of drug treatment. Experiment standard error was 1.96.



Control Feed Treatment (CI-720 at 0 ppm)

Drug Feed Treatment (CI-720 at 2600 ppm)

Drug Feed Treatment (CI-720 at 5200 ppm)

Numbers represent the hen number

is a graph of only the percent free of total plasma cholesterol for the individual hens.

3. Discussion

Lorenz et al. (1938) observed levels of 57-99 percent free of total plasma cholesterol when they fed a high fat diet to laying hens; and on a low fat diet, the range was 63% to 105%.

Walker et al. (1950) found the laying hen to have a percent free of total plasma cholesterol range of 66%-94%.

Four-to-twenty-five-week-old cockerels tested by Caldwell and Suydam (1960) had 24.5% of the total plasma cholesterol in the free form. This value is similar to the 26.6% for day-old chicks reported by Chung et al. (1965) who also found that laying hens had about 72% of the total cholesterol in the free form.

Kaishio (1933) has reported that the plasma ratio of free cholesterol to total cholesterol in non-laying hens was within the limits of variation of the male birds he used. His range for esterified cholesterol for hens was 11.8% to 70.2%, with a mean of 45.7%. Therefore, the percent free of total plasma cholesterol range was about 29 to 88%.

Human circulating cholesterol is about 2/3 esterified, and 1/3 in the free form, according to information in the monograph which was guest edited by Stare (1974). This is

comparable to non-laying hens and immature chickens, but is the inverse of laying hens.

In experiment 2, the reason is not know why the percent free of total plasma cholesterol values did not exceed 60% even for the control hens. This is about the lower limit of the range observed by others for laying hens.

In the first experiment, the hens out of production only had values of 40%-50% free of total plasma cholesterol. The second experiment had non-laying hens with a range of 29%-39% free of total plasma cholesterol. Although the magnitude is different, the trend of hens out of production is the same in both experiments.

Plasma Triglycerides

1. Experiment 1

Due to technical difficulties, most sample results were discarded. Because some values fell within the range as reported by others, the data from this experiment are included in Table 4 of the Appendix, but final conclusions are left to the reader.

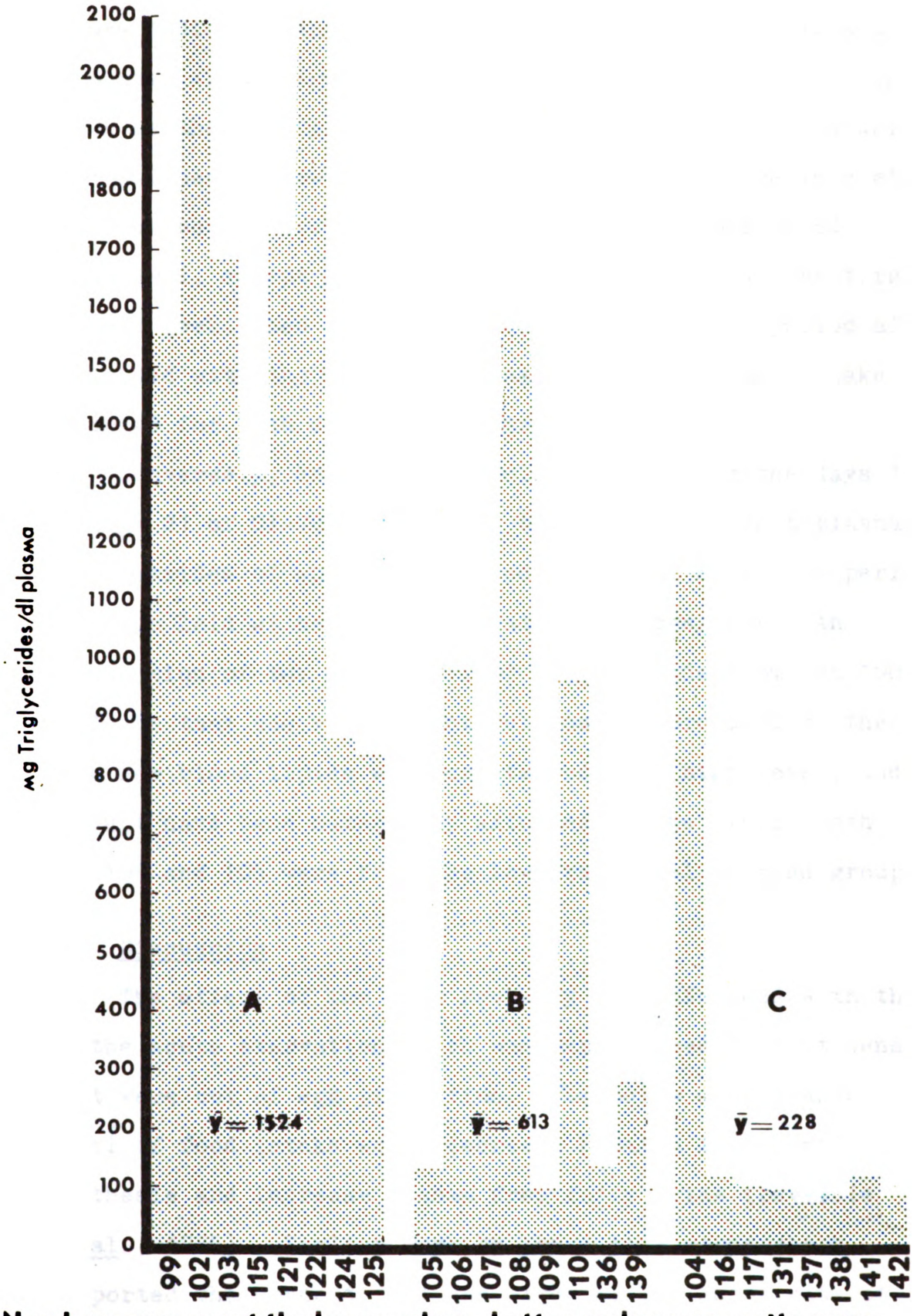
2. Experiment 2

The plasma triglycerides statistical analysis (Tukey HSD), indicated the mean for the control group of hens was higher than the mean for either group of hens on drug treatment ($P < .01$).

Figure 10 is a plot of the individual hen triglyceride values obtained for this one-time blood withdrawal. This

Figure 10. Experiment 2. Plasma triglycerides in mg/dl. Plotted are the individual hen values at day 21 of drug treatment. There were eight hens per treatment. The standard error for the experiment was about 96 mg/dl.

A = control feed treatment (CI-720 @ 0 ppm)
B = drug feed treatment (CI-720 @ 2600 ppm), also referred to as the low drug level treatment
C = drug feed treatment (CI-720 @ 5200 ppm), also referred to as the high drug level treatment



Numbers represent the hen number; Letter code on opposite page

figure indicates there is considerable individual variation of plasma triglyceride levels among the control hens. Also shown is the biological variation in response to the drug.

All hens below 300 mg triglycerides/dl plasma were at zero production for at least a week prior to the blood withdrawal, except hen 105, which laid one egg at the first of that week, then ceased to produce. This time period also coincided with the below maintenance level of feed intake recorded for both drug treated groups of hens.

Conversely, hen 108 laid only two eggs for the days 14 through 21 of CI-720 treatment, but had the highest plasma triglycerides of any drug treated hen. The next time period this hen laid at her previous rate of 4 eggs/week. An explanation of the high level of blood lipids from hen 108 could be that she was not entirely out of production, therefore her blood lipids were maintained at a high level, and may not have been materially affected by the drug. Both hen 108 and 105 were from the low drug level treated group.

3. Discussion

The effect of the drug probably is confounded with that of the acute starvation which was experienced by most hens that were out of egg production. The below maintenance level of food intake would decrease hepatic fatty acid synthesis and increase plasma free fatty acids (Leveille et al., 1975). Earlier work by these same researchers supported the concept that the liver is the major site of

avian lipid biosynthesis (Leveille et al., 1968). These changes would mean that plasma triglycerides should decrease by fasting. Fasting also inhibits liver cholesterol synthesis at the beta-hydroxy-beta-methylglutaryl-Co A step. This fasting effect is apparent when comparing blood lipid levels with that of feed intake.

Herman et al. (1970) found that dietary carbohydrate removal caused a decrease in some human serum triglycerides.

Muruiri et al. (1975) reported that when meal fed chicks are fed, their plasma triglycerides were increased from 38±4 mg% toward the level of the ad lib. group of chicks, which was 117±16 mg%.

Walker et al. (1950) reported the total lipid of the laying hen ranged from 652 to 2308 mg%. Of the total lipid, neutral fats (triglycerides) were about 62% (404 to 1431 mg%), phospholipids were about 30%, and total cholesterol about 7% (46 to 162 mg%).

Lorenz et al. (1938) reported hens to have higher total lipids than the hens Walker and his associates used. The former researchers found that the lipid levels in the blood of laying hens were variable, as evidenced by the range of a single hen, over about a three month period of time, which was recorded to be from 1030 to 4129 mg% total lipid. Lorenz et al. (1938) also reported that by using analysis of covariance, there was no relationship between duration or intensity of production and the plasma lipid

level, provided the hen was in production at the time of the observation.

Total Yolk Cholesterol

1. Experiment 1

Although Figure 11 shows that the hens given the drug, on the average, had higher yolk cholesterol than hens used as their control, only the comparisons of control *per os* versus the drug feed treatment groups of hens, or the control *per os* versus the drug *per os* treatment for hens on day 21 of drug treatment were statistically significant ($P < .05$). The Tukey HSD test of means was used to analyse the data.

Figure 12 shows the values for total egg yolk cholesterol which was determined at the end of each treatment week and post-treatment week as a percent of the respective hens pre-treatment level of yolk cholesterol. Because of the intense individual variation of the percent of pre-treatment values, statistics were not used to evaluate these results. In Figures 11 and 12 it can be seen that the probable reason for the significance of the above comparisons was the below 100% of pre-treatment yolk cholesterol values of hens in the control *per os* treatment, and the much greater than pre-treatment level of several of the drug treated hens. Also, according to these figures, the drug may have been more effective in altering the yolk cholesterol levels when the capsule was used for CI-720 administration, than via the feed.

Figure 11. Experiment 1. Egg yolk total cholesterol in mg/g yolk. Plotted are the means of three hens/treatment.

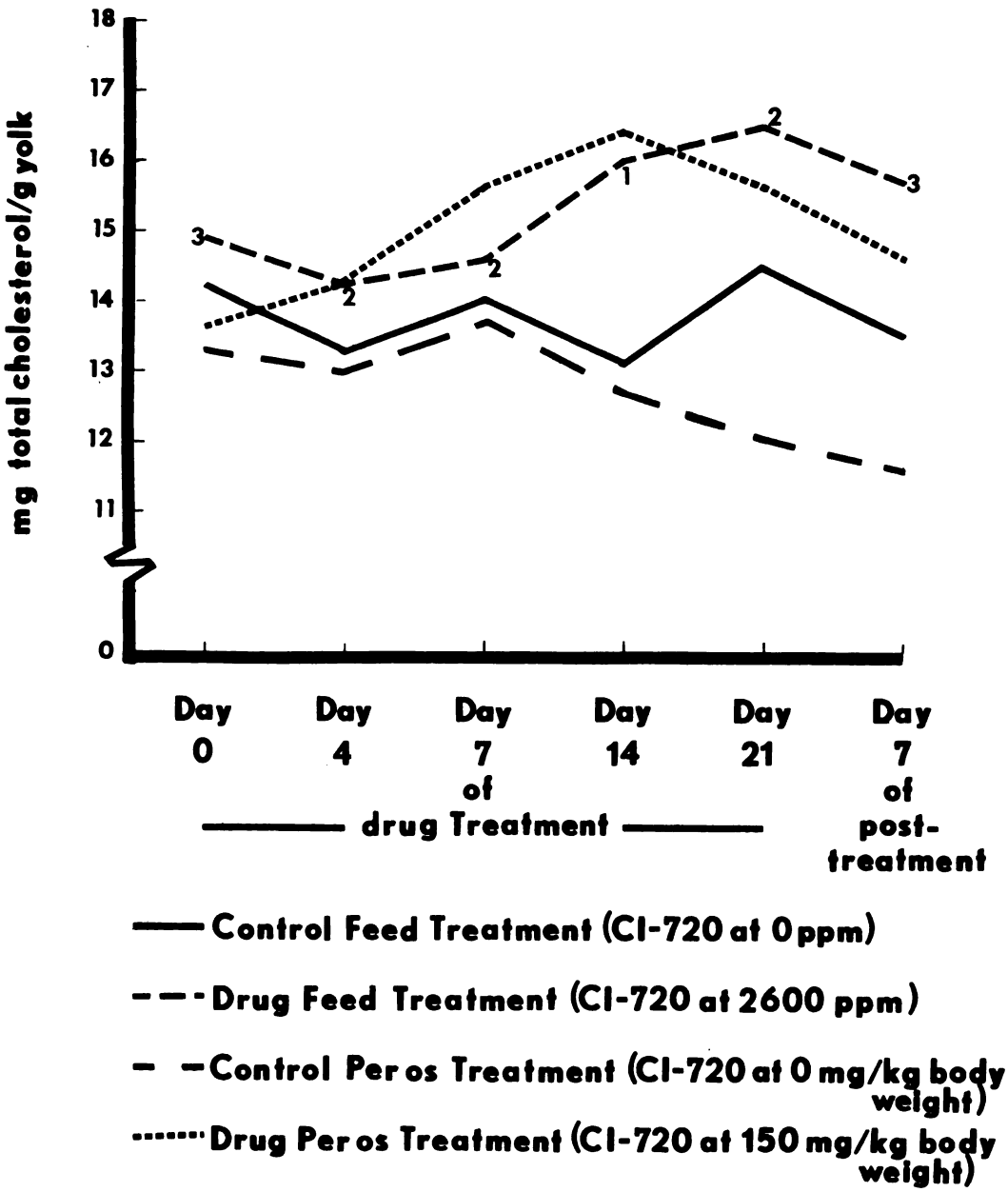

mg total cholesterol/g yolk
18
17
16
15
14
13
12
11
0
Day 0
Day 4
Day 7 of drug Treatment
Day 14
Day 21
Day 7 of post-treatment
Control Feed Treatment (CI-720 at 0 ppm)
Drug Feed Treatment (CI-720 at 2600 ppm)
Control Per os Treatment (CI-720 at 0 mg/kg body weight)
Drug Per os Treatment (CI-720 at 150 mg/kg body weight)

Figure 12. Experiment 1. Egg yolk total cholesterol percent of the individual hen's pre-treatment level.

A = day 4 of drug treatment

B = day 7 of drug treatment

C = day 14 of drug treatment

D = day 21 of drug treatment

E = day 7 of post-treatment

(1) Control Feed Treatment (CI-720 @ 0 ppm)

——— = Hen # 1

····· = Hen # 2

— — — = Hen # 3

(2) Drug Feed Treatment (CI-720 @ 2600 ppm)

——— = Hen # 6

····· = Hen # 7

— — — = Hen # 8

(3) Control Per os Treatment (CI-720 @ 0 mg/kg body weight)

——— = Hen # 9

····· = Hen # 10

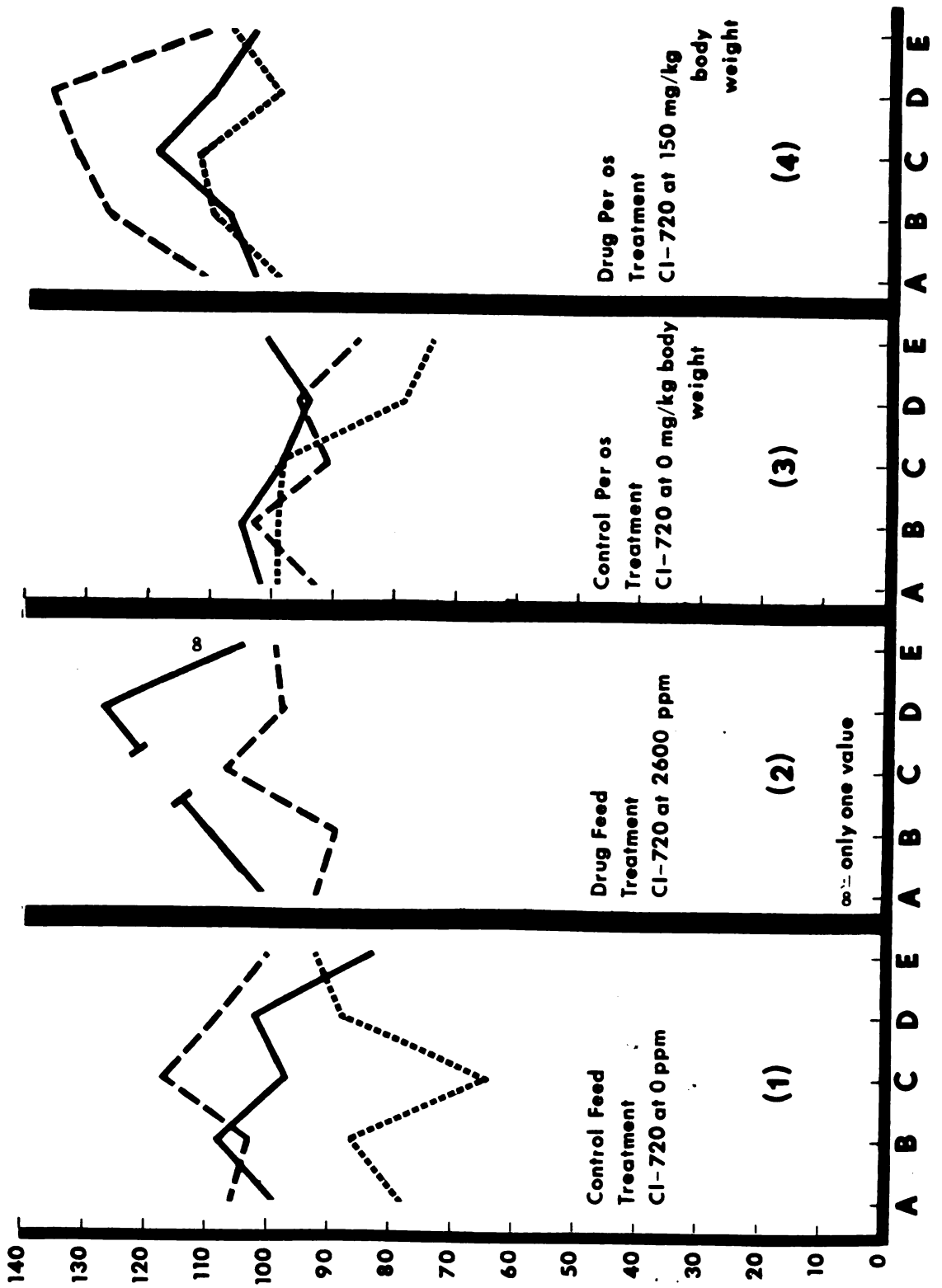
— — — = Hen # 11

(4) Drug Per os Treatment (CI-720 @ 150 mg/kg body weight)

——— = Hen # 14

····· = Hen # 15

— — — = Hen # 16



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Other than biological variation, the reason why hens of the control feed group showed so much change from the pre-treatment level of yolk cholesterol over time, as well as the downward trend of yolk cholesterol levels of hens in the control per os treatment, is not known.

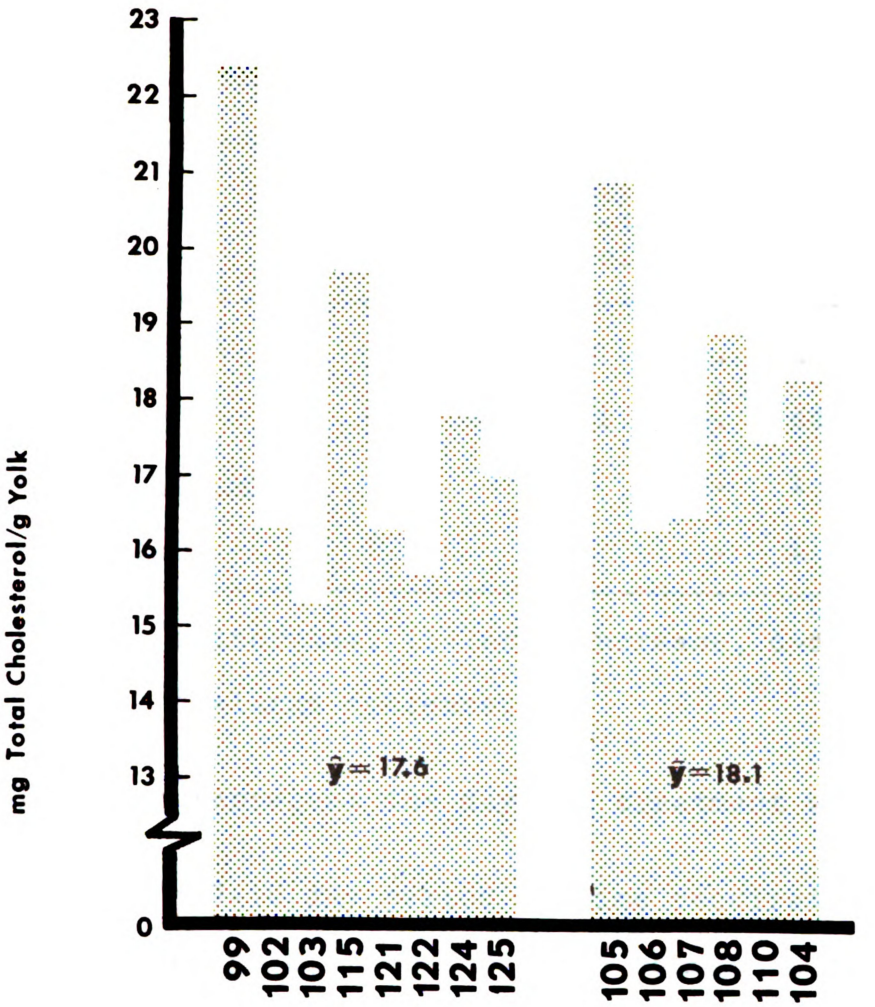
2. Experiment 2

No change was noted in the yolk cholesterol of hens of any treatment. The final treatment mean of the drug treated hens had only slightly more cholesterol/g yolk than did the control hens (Figure 13). In the high drug level treatment, the only hen to lay an egg had about an average amount of cholesterol for these drug treated hens. Therefore, the drug may have a maximum effect on the amount of cholesterol placed into the yolk, if there is any effect at all.

3. Discussion

If the drug had caused the hen to excrete cholesterol through the egg, those hens given the CI-720 would have been expected to have higher levels of cholesterol in the yolk than the control hens. This appeared to be the case only in experiment 1. For hens of any treatment in experiment 1, the change in the amount of cholesterol deposited in the yolk during the following week's drug treatment was usually opposite in direction to the change in total plasma cholesterol (see Table 9). This inverse relationship cannot be evaluated for experiment 2, because this was a one-time

Figure 13. Experiment 2. Individual hen values of total cholesterol/g yolk, from an egg laid on or about the 21st day of drug treatment. Because only hen 104 yielded information of this parameter, and the value was close to the mean of the hens given the low drug treatment, all drug treated hens yolk cholesterol values were combined. The standard error of the experiment was 0.57.



Numbers represent the hen number

Table 9. Relationship between plasma and yolk total cholesterol.

Treatment	Day* 0-7 Plasma	Day 7-14 Yolk	Day 7-14 Plasma	Day 14-21 Yolk
Control Feed	i	d	d	i
Drug Feed	dd	ii	dd	i
Control per os	d	d	i	d
Drug per os	d	i	i	d

Legend: i=increase, ii=large increase, d=decrease, dd=large decrease.

*The days represent the days of treatment with CI-720.

There is a 7-10 day period of time required for the formation of the yolk, therefore, the change in the blood cholesterol would not be reflected in that day's egg, but in the egg laid sometime during the following week. For example, the change in blood cholesterol from day 0 to day 7 of drug treatment may show an increase; and because the blood lipids of the hen during this week are forming a future yolk, which is not completed until the following week, then this level of blood cholesterol would not be reflected in the yolk cholesterol until the following week. This time period for the yolk would be from day 7 to 14 of drug treatment.

blood withdrawal and egg sample. The experiment 1 control *per os* treatment is the only group of hens in this experiment not to follow this trend of an inverse relationship between blood and yolk cholesterol. Marion *et al.* (1960) found a significant inverse relationship between serum cholesterol and the cholesterol present in the yolk (-.288 correlation), when dietary fats and fatty acids were manipulated.

Others have noted that if the blood is overloaded with cholesterol, as in the addition of cholesterol to the diet, this cholesterol supplementation will cause an increase in the yolk cholesterol content. For example, Weiss *et al.* (1967) concluded that egg cholesterol concentration did not parallel the blood levels when there were different types of fat in the diet except in those cases where hypercholesterolemia was produced by feeding diets that contained cholesterol.

Singh and Naber (1970) fed a hypocholesterolemic drug (20,25-diazacholesterol, also called SC-12937) and reported that at .1 to 5 ppm, blood sterols were increased and egg sterols were decreased, but long term feeding of 5 ppm caused an increase in egg sterols above the control hen's sterol level.

Bartov *et al.* (1971) found that yolks from hens of about equal rates of production vary significantly in their total yolk cholesterol concentration, but the yolk cholesterol from the same hen was quite uniform. They reported that the

yolk cholesterol values were independent of egg production, but that the group of low-producing hens contained more hens yielding high yolk cholesterol values than did the group of high-producing hens. These researchers indicated that these "good" layers excreted more cholesterol per week through the egg than did those hens laying less eggs.

Jones (1969) noted that there was a slight difference in the yolk cholesterol content between strains of birds, but variation from egg to egg, even between birds of the same strain was enough to prevent any statistical differences. Turk and Barnett (1971) also reported considerable variation among individual hens from the same flock, regardless of age, feed, management, strain of layer, or geographic location, and therefore no difference statistically between these. Clarenburg et al. (1971) also made the observation of lack of uniformity of hen's yolk cholesterol.

In the present studies, the hens producing at lower rates tended to have more cholesterol/g yolk than the better producers. Fluctuations in production tended to create opposite changes in the total yolk cholesterol concentration. These data are in quasi-agreement with Bartov et al. regarding the egg production:yolk cholesterol relationship, but the eggs produced by control hens was less uniform than that reported by Bartov et al. and other authors. Table 10 gives the means for each hen in both experiments.

Table 10. Total egg yolk cholesterol in mg cholesterol/g yolk.

Hen #	day zero	day 4	day 7	day 14	day 21	post-trt.	mean
Experiment 1:							
Control Feed Treatment (CI-720 @ 0 ppm)							
1	14.2*	14.1	15.4	13.9	14.5	11.8	14.0
2	14.4	11.2	12.4	9.2	14.1	14.7	12.7
3	13.9	14.7	14.3	16.2	15.0	13.9	14.7
mean	14.2	13.3	14.0	13.1	14.5	13.5	13.8
SEM	0.15	1.08	0.88	2.06	0.26	0.86	
Drug Feed Treatment (CI-720 @ 2600 ppm)							
6	14.3	14.5	15.7	n/e	18.2	14.8	15.5
7	15.5	n/e	n/e	n/e	n/e	17.4	16.5
8	15.0	13.8	13.4	16.0	14.7	14.8	14.6
mean	14.9	14.2	14.6	16.1	16.5	15.7	15.5
SEM	0.35	0.35	1.15	0.00	1.75	0.87	
Control Per os Treatment (CI-720 @ 0 mg/kg body weight)							
9	12.7	13.0	13.4	12.5	12.0	12.8	12.7
10	12.4	12.3	12.3	12.1	9.8	9.2	11.4
11	14.8	13.8	15.3	13.4	14.2	12.7	14.0
mean	13.3	13.0	13.7	12.7	12.0	11.6	12.7
SEM	0.75	0.43	0.88	0.38	1.56	1.18	

*all are means of triplicate readings

n/e = no egg

Table 10 (cont'd.).

Hen #	day zero	day 4	day 7	day 14	day 21	post-trt.	mean
Drug Per os Treatment (CI-720 @ 150 mg/kg body weight)							
14	13.4	13.8	14.3	15.9	14.8	13.8	14.3
15	14.8	14.6	16.3	16.6	14.7	15.8	15.5
16	12.7	14.1	16.1	16.7	17.3	14.1	15.2
mean	13.6	14.2	15.6	16.4	15.6	14.6	15.0
SEM	0.62	0.23	0.64	0.25	0.85	0.62	

Experiment 2:

Hen #	99	102	103	115	121	122	124	125
mean	22.4	16.3	15.3	19.6	16.3	15.7	17.8	17.0
group mean ± SEM	17.55±0.85							
	105	106	107	108	110		104	
mean	20.9	16.3	16.5	18.9	17.5		18.3	
group mean ± SEM	18.1±0.70							

Harris and Wilcox (1963b) found a seasonal difference in yolk cholesterol, which increased from August to February, then decreased in April and June. They also calculated a negative correlation between yolk size and its cholesterol concentration, regardless of the season.

In this work, the first experiment was conducted in May and June, and the second experiment occurred in October and November. The information reported by Harris and Wilcox may explain in part the larger values for yolk cholesterol observed in the second experiment. In a companion paper, Harris and Wilcox (1963a) reported that environmental influences contribute a greater effect on yolk cholesterol than does genetics. Others who found a seasonal difference in yolk cholesterol levels were Jones (1969); and Whiteside and Fluckinger (1965) for total yolk sac cholesterol.

Edwards et al. (1959) found a positive correlation of .44 between weight of the hen and the egg cholesterol level, and a value of .38 for the correlation between yolk cholesterol and the yolk iodine number.

Weiss et al. (1963) reported that the Zlatkis method "as adopted to the determination of cholesterol in eggs gave abnormally high values when compared to the method of Abell." The values of the present experiments tend to support this statement, when a comparison is made between these values and those reported in the literature when the

Zlatkis method was used; this is especially true in the first experiment.

The values of total yolk cholesterol obtained in the present experiments are in the range of values found by others. The yolk cholesterol of the first experiment was lower and the range larger than in the second experiment. In neither experiment were the values of the range marked by dramatic differences between adjacent values over time.

Comparing experiment 1 egg yolk cholesterol values with those of experiment 2, there is some difficulty in making a definite statement of the effect of CI-720 on yolk cholesterol levels. The effect of CI-720 (assuming it's existence) is comparable to the drug used by Singh and Naber (to decrease the blood cholesterol and move it to the yolk) in only the first of two experiments. There did not appear to be any drug effect in experiment 2. Egg production of most hens treated with CI-720 in experiment 2, was stopped by the drug; this could have been due to a lack of feed intake or to altered lipid metabolism so the yolk may not have been formed. The first yolks produced after the egg formation mechanism was stopped, tended to be higher in yolk cholesterol (see hen #6 of experiment 1 in Table 9).

Table 11 lists yolk cholesterol concentrations reported in the literature.

Table 11. Total yolk cholesterol values reported in the literature.

Description	mg Cholesterol per gram yolk	Reference
Experiment 1:		
Laying hen	11.7-14.8	
Experiment 2:		
Good layer	11.3-14.1 mean=12.5	
Poor layer	11.3-14.7 mean=13.2	Bartov et al. (1971)
SCWL	15.6±0.5--S.D.	
BPR	17.3±3.2--S.D.	Miller and Denton (1962)
Laying hen	29.4-30.1	Combs and Helbacka (1960)
Laying hen	11.2-13.7	Weiss et al. (1967)
Laying hen	14-14.5	Clarenburg et al. (1971)
Laying hen, mixed strain (Athens-Canadian)	19-27 family range mean=22.8±2.8 S.D.	
mixed strain (American breeds)	17.5-21.5 family range mean=19.2±1.2 S.D.	Washburn and Nix (1974)
Laying hen	17.8	Dua et al. (1966)
SCWL	21.8±0.22 (1957)	
	25.2±0.19 (1958)	Harris and Wilcox (1963a)

Body Weight (in grams)

1. Experiment 1

The split plot statistical analysis showed no f-value to be even close to being significant. The hens of the drug treatments did decrease in body weight, possibly because of lowered feed consumption. The hens given CI-720 showed a return toward the pre-treatment level the week after drug withdrawal. Body weights of individual hens are listed in Table 12. The split plot model was used for the observed values only.

2. Experiment 2

Figure 14 shows hen treatment means of body weight at each data collection date, the individual hen values used to calculate these means are in Table 12.

In both experiments, the CI-720 seemed to cause a decrease in body weight, which then either plateaued, or the hen partially recovered the weight before the experiment ended. The body weight changes paralleled feed consumption patterns.

The body weight of hens in the control group was reasonably uniform, and in most cases these hens gained weight throughout the experiment.

For the last two weeks of drug treatment, the control hens were significantly heavier than the hens given the high dosage of CI-720 ($P < .02$), using the Tukey HSD test of means.

Table 12. Body weight (±1 g) of hens at the end of each treatment week.

Hen #	Day 0 Expt.	Day 0 Trt.	Day 7 Trt.	Day 14 Trt.	Day 21 Trt.	Day 7 Post-trt.
Experiment 1:						
Control Feed Treatment (CI-720 @ 0 ppm)						
1	2544	2570	2524	2502	2243	2236
2	1707	1728	1784	1880	1904	1920
3	2174	2184	2200	2210	2161	2177
Drug Feed Treatment (CI-720 @ 2600 ppm)						
6	2069	2046	2087	1926	2005	2057
7	2274	2191	2163	2176	2197	2224
8	1868	1841	1762	1802	1818	1817
Control Per os Treatment (CI-720 @ 0 mg/kg body weight)						
9	1764	1744	1739	1694	1699	1738
10	1826	1777	1791	1787	1816	1843
11	2114	2158	2243	2198	2267	2267
Drug Per os Treatment (CI-720 @ 150 mg/kg body weight)						
14	1920	1871	1794	1858	1847	1866
15	2177	2093	2130	2107	2098	2027
16	2216	2198	2140	2098	2162	2238

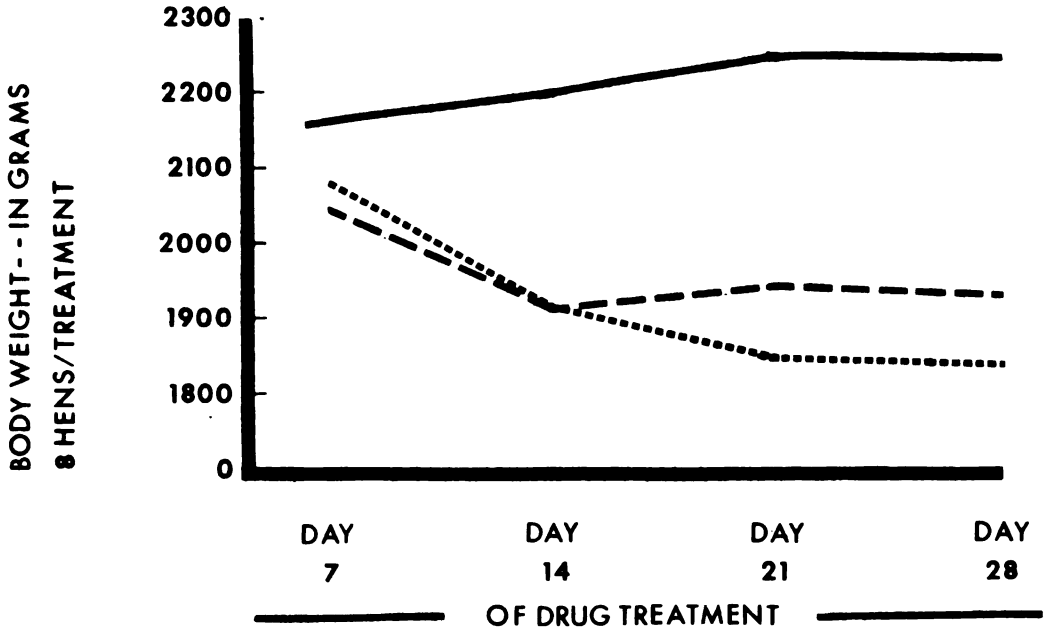
Table 12 (cont'd).

Hen #	Day 0 Expt.	Day 0 Trt.	Day 7 Trt.	Day 14 Trt.	Day 21 Trt.	Day 28 Trt.
Experiment 2:						
Control Feed Treatment (CI-720 @ 0 ppm)						
99			2537	2563	2682	2714
102			1771	1871	1881	1868
103			1874	1880	1917	1884
115			2210	2251	2226	2257
121			2275	2267	2373	2358
122			2286	2315	2411	2399
124			1869	1968	2010	1965
125			2467	2489	2489	2526
Drug Feed Treatment (CI-720 @ 2600 ppm)						
105			2355	2045	2043	2054
106			1845	1868	1887	1822
107			2109	2221	2161	2170
108			2312	2171	2299	2266
109			2212	2081	2018	1926
110			1934	1854	1885	1838
136			1823	1548	1527	1566
139			1800	1546	1725	1805

Table 12 (cont'd.).

Hen #	Day 0 Expt.	Day 0 Trt.	Day 7 Trt.	Day 14 Trt.	Day 21 Trt.	Day 28 Trt.
Experiment 2 (cont'd.):						
Drug Feed Treatment (CI-720 @ 5200 ppm)						
104			2309	2303	2206	2182
116			2190	1949	1857	1923
117			2009	1836	1676	1723
131			2082	1930	1836	1850
137			1800	1645	1557	1446
138			1934	1535	1597	1627
141			2155	1935	2054	1889
142			2163	2108	2007	2071

Figure 14. Experiment 2. Body weight in grams ($\pm$1).



INITIAL BODY WEIGHT WAS NOT RECORDED

——— = CONTROL FEED TREATMENT (CI-720 at 0 ppm)

- — - = DRUG FEED TREATMENT (CI-720 at 2600 ppm)

····· = DRUG FEED TREATMENT (CI-720 at 5200 ppm)

The body weight of hens in the control treatment was not greater than that of hens of the low drug treatment ($P > .05$), for all dates--see Figure 14. The Tukey test was used here also. The hens on drug treatment started with body weights that were slightly less than the control hens, which would cause over confidence in the decrease in body weight caused by the drug, but the trend seems to be clear from Figure 14.

During the second and third weeks of drug treatment, the feed consumption of hens given the high drug level (5200 ppm) was about half the maintenance level. Obviously, if feed is not available to the hen's body tissues, the energy stored as adipose tissue will be used to maintain life. There would thus be a reduction in the hen's body weight.

Percent Change in Body Weight

1. Experiment 1

The absolute value (mathematically) of the percent change in body weight of individual hens was calculated on a weekly basis, throughout the experiment. Considerable variation was evident in all treatments. Nothing in the computer analysis of this variable, using the split plot procedure, was close to having a significant f-ratio.

Using the hen as her own control, and calculating the percent of pre-treatment body weight value at each date, for each hen, no difference could be detected in the means

of any treatment given the hens. The Tukey HSD test of means was also used for the evaluation of the data. Control groups were combined and compared against the combined drug group, due to the split plot model characteristics, for means in this analysis.

When the change in body weight was evaluated as the initial minus final body weight, there was obviously no difference between the treatment group of hen's means because the ranges overlapped, if the hen #1 values were included; and there probably was a difference between treatments if values of hen #1 were excluded. Hen # 1 was injured on day 14 of drug treatment, and so lost body weight even more than the drug treated hens. Although some hens given this drug gained weight, they usually lost at the rate of 3-4%. The control hens fluctuated about the pre-treatment level, and their maximum loss was about 4% of their body weight during a week. There was a 2.0 vs. a 2.4% change in body weight for control and drug treated hens, respectively; SEM = 0.259.

2. Experiment 2

Comparing the overall hen mean of the absolute change in body weight from the preceeding week, for each treatment hens on the control treatment were found to be significantly less changed than those on either level of drug treatment ($P < .05$) for the days 8-14 of drug treatment period. Hens

on the drug treatments were not different from each other in any comparisons.

Because of non-homogenous variance, the Dunnett test of means was used for the statistical analysis of the data from the second week of treatment; the Tukey HSD test of means was used for all other comparisons. In this second experiment, there was no weight recorded at the start of the experiment, so the first change in body weight is from the day 8 through 14 time period.

3. Discussion

During experiment 1, the body weight fluctuations (on a weekly basis) were very similar for hens on all four treatments. Considering the overall percent change for the combined control treatments, three hens increased, one did not change, and two declined in body weight. One of the drug treated hens of this experiment gained weight, four lost body weight, and one did not change in body weight.

Hens in experiment 2 showed similar responses in the weekly change for the 0 ppm and 2600 ppm drug treatments, but the 5200 ppm drug treated hens usually declined in body weight. Results of the overall weight change for hens of this second experiment showed the control hens all gained weight, the 2600 ppm treated hens all lost body weight except for one hen that did not change, and one that gained weight. The 5200 ppm drug treated hens all lost body weight, some as much as 300 grams.

Weekly Percent Egg Production

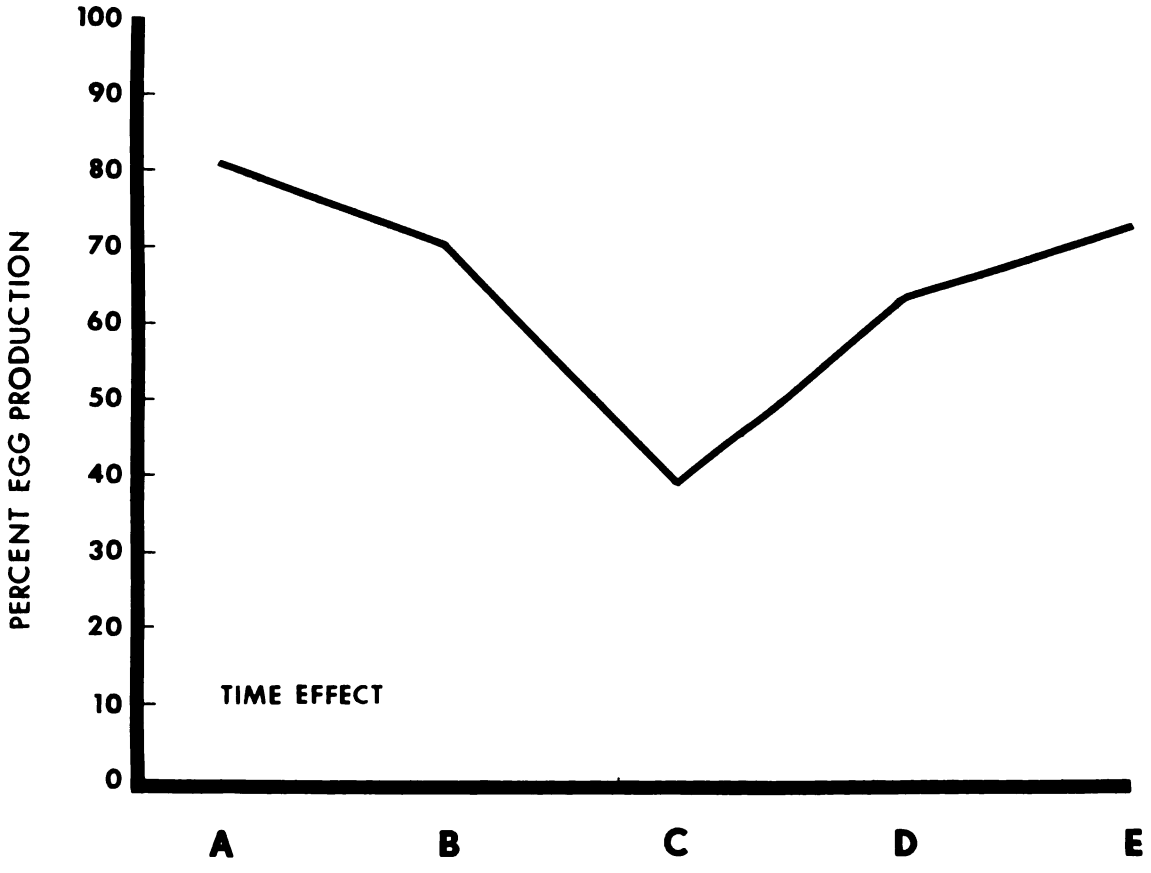
1. Experiment 1

According to the split plot statistical analysis, the effect of time and of CI-720 presence was significant ($P < .0005$; and $P < .019$, respectively), for this parameter.

For the time effect, the second week of drug treatment was undoubtedly the point that caused such a high level of confidence (see Figure 15). During this second week of drug treatment, two of the three hens in the drug feed treatment were out of production, and the other hen laid only one egg. This treatment had hens which were adversely affected by the drug, but the other drug treatment did not have hens which showed such a dramatic effect of CI-720. Table 13 shows the number of eggs produced by each hen during each treatment week, for both experiments.

The mean of the three hens/treatment at each sampling date is plotted in Figure 16. The capsule drug treated hens did not seem to be affected by CI-720 except at day 14 of treatment (also see Table 14) but on this treatment date, all groups of hens also experienced a decrease in percent egg production, with the treated hens being affected more than the control hens. The hens given the drug feed treatment produced eggs at a rate (zero then rebounded) which would suggest that there was a definite lowering effect of CI-720 on egg production. The rebound of egg production after the withdrawal of CI-720 commenced too quickly for the effect of CI-720 to be totally mediated through the lowered

Figure 15. Experiment 1. Effect of time on percent egg production, according to the split plot statistical analysis. All hen values were combined and segregated according to time for this parameter. n=12 hens at 6 times; SEM for the experiment was 10.5.



A - DAY 0 OF DRUG TREATMENT

B - " 7 " " "

C - " 14 " " "

D - " 21 " " "

E - " 7 " POST TREATMENT

Table 13. Egg production record in total eggs per week.

Hen #	pre-trt.	days 0-7*	days 8-14	days 15-21	post-trt.
Experiment 1:					
Control Feed Treatment (CI-720 @ 0 ppm)					
1	5	5	5	5	6
2	7	7	4	7	8
3	6	5	2	4	5
Drug Feed Treatment (CI-720 @ 2600 ppm)					
6	6	5	0	0	5
7	3	0	0	0	0
8	6	4	1	5	5
Control *Per os* Treatment (CI-720 @ 0 mg/kg body weight)					
9	7	5	5	6	6
10	6	6	4	6	6
11	6	6	4	6	6
Drug *Per os* Treatment (CI-720 @ 150 mg/kg body weight)					
14	6	6	2	6	6
15	5	6	3	5	4
16	5	4	3	3	4

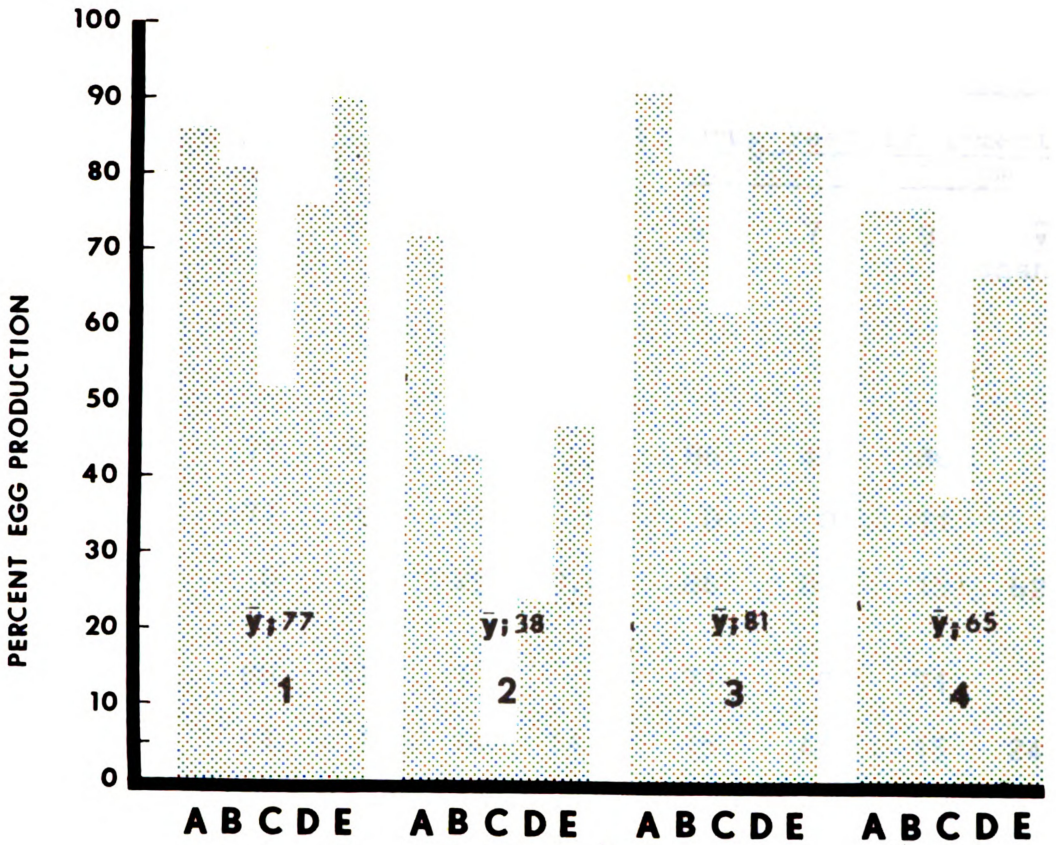
*days of drug administration

Table 13 (cont'd.).

Hen #	days 0-7*	days 8-14	days 15-21	days 22-28
Experiment 2:				
Control Feed Treatment (CI-720 @ 0 ppm)				
99	5	3	3	5
102	6	4	6	6
103	4	5	5	5
115	1	2	3	5
121	6	6	7	6
122	7	6	7	7
124	6	6	4	5
125	6	6	6	6
Drug Feed Treatment (CI-720 @ 2600 ppm)				
105	6	4	1	0
106	7	6	6	7
107	5	4	6	4
108	4	6	2	4
109	5	1	0	0
110	3	5	5	6
136	4	5	0	0
139	5	1	0	0
Drug Feed Treatment (CI-720 @ 5200 ppm)				
104	6	5	6	5
116	4	0	0	0
117	2	0	0	0
131	5	0	0	0
137	4	1	0	0
138	5	4	0	0
141	6	1	0	0
142	2	0	0	0

*days of drug administration

Figure 16. Experiment 1. Percent egg production average for the three hens/treatment, at each week of the experiment. SEM for the experiment was 10.5.



A- PRE-TREATMENT WEEK

B- DAY 0 through 7 OF DRUG TREATMENT

C- " 8 " 14 " " "

D- " 15 " 21 " " "

E- POST-TREATMENT WEEK

1- CONTROL FEED TREATMENT; CI-720 at 0 ppn

2- DRUG FEED TREATMENT; CI-720 at 2600 ppm

3- CONTROL PER OS TREATMENT; CI-720 at 0 mg/kg body weight

4- DRUG PER OS TREATMENT; CI- 720 at 150 mg/kg body weight

Three hens per treatment

Table 14. Experiment 1. Change in percent egg production from the pre-treatment level.

	Control Feed Treatment CI-720 @ 0 ppm				Drug Feed Treatment CI-720 @ 2600 ppm			
Hen #	1	2	3	$\bar{y}$ change	6	7	8	$\bar{y}$ change
Time Period								
end pre-trt.*	71	100	86		86	43	86	
end week 3	71	100	57		0	0	71	
change	0	0	29	9.6	86	86	15	62
end post-trt.	86	114	71		71	0	71	
change	14	14	14	14	15	43	15	24

	Control Per os Treatment CI-720 @ 0 mg/kg B.W.				Drug Per os Treatment CI-720 @ 150 mg/kg B.W.			
Hen #	9	10	11	$\bar{y}$	14	15	16	$\bar{y}$
Time Period								
end pre-trt.	100	86	86		86	71	71	
end week 3	86	86	86		86	71	43	
change	14	0	0	4.7	0	0	28	9.3
end post-trt.	86	86	86		86	57	57	
change	14	0	0	4.7	0	14	14	9.3

*percent egg production

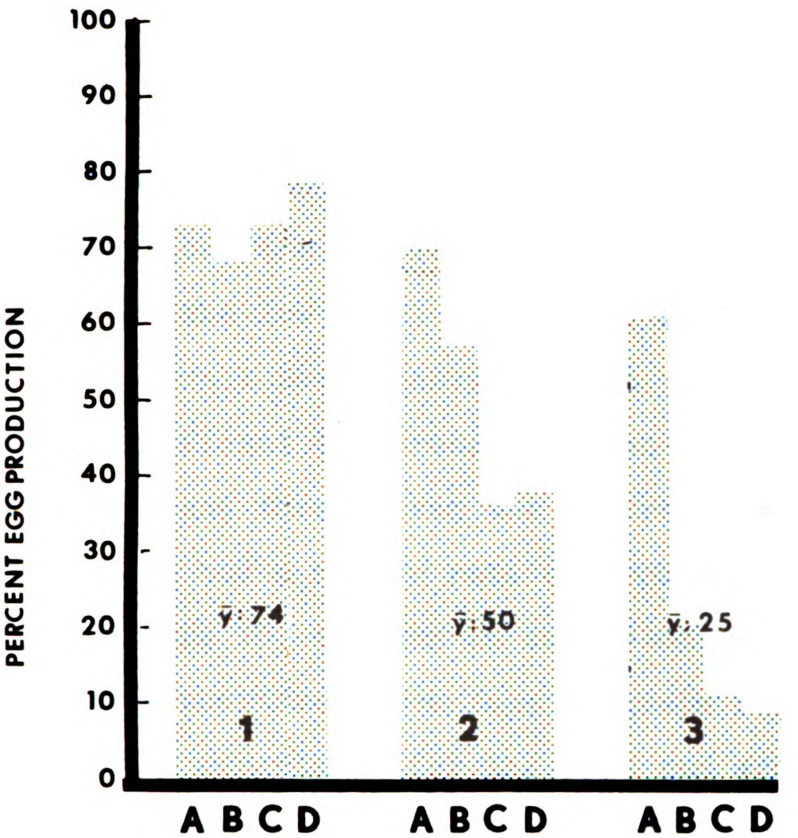
feed intake or any resorption of egg yolk, or probably even an alteration in the lipids produced for the yolk.

2. Experiment 2

The percent production mean of the eight hens fed each of three treatments is shown in Figure 17, apparently there are significant differences between the treatments. Tukey's HSD test of treatment means showed no difference between any of the treatments during the first week. But in the second week of treatment, egg production of the control hens was significantly ($P < .01$) greater than that of the high drug level treated group of hens. This is the same period of time after drug administration that the hens of the first experiment were initially affected. In experiment 2, when a hen given a drug treatment went out of production, she did not return. Possibly the post-treatment occurred at such a time to allow the hen to recover from low production in experiment 1; but in experiment 2 there was no post-treatment. Half of the hens on each drug treatment molted in experiment 2. This could have been due to a direct effect of the drug, or to the stress CI-720 may have produced, or to lowered feed intake. In general, the egg production of the poorer layers seemed to be affected more than the "good" layers egg production for experiment 2.

For this second week of drug administration, the hens given the high level of CI-720 had less eggs produced than

Figure 17. Experiment 2. Percent egg production. The standard error of the mean was about 8.7 for the experiment.



A - DAY 0 through 7 OF DRUG TREATMENT

B - " 8 " 14 " " "

C - " 15 " 21 " " "

D - " 22 " 28 " " "

1 - CONTROL FEED TREATMENT (CI-720 at 0 ppm)

2 - DRUG FEED TREATMENT (CI-720 at 2600 ppm)

3 - " " " (" at 5200 ppm)

EIGHT HENS PER TREATMENT

the hens treated with the low level of CI-720 ($P < .05$). For both of the last two weeks of drug treatment, the control hens produced more eggs than the hens given CI-720 at 5200 ppm, ($P < .01$). For the last two weeks of CI-720 administration, the control hens laid more eggs than did the hens on the low level of drug ($P < .05$).

The overall mean weekly percent production showed a highly significant difference between the hens of the high drug level, and those given the control treatment. The control hens had a higher rate of production.

In Figure 15, the hen in cage #104 was the only reason there was a positive percentage to graph for the last two weeks of the experiment for the 5200 ppm drug treated hens.

The Dunnett test of means was used for the means of the last week only, the rest of the data was tested by the Tukey HSD test of means.

3. Discussion

As may be the situation with CI-720, many compounds that alter blood lipid levels (by various mechanisms), also stop egg production. Some of these compounds are: nicarbazin (Weiss, 1960; Konlande and Fisher, 1969); lithocholic acid (Edwards, 1962); DEAE Sephadex (Turk and Barnett, 1972); or 20,25-diazocholesterol (Singh and Naber, 1970).

CI-720 may act by depressing feed consumption, in possible combination with other mechanisms to cause a decrease in the egg production, such as the disruption of

lipid metabolism. Obviously, for egg production, hens must have sufficient levels of nutrients available to her body for self preservation and then the extra demands of reproduction. Walker et al. (1950) state that a laying fowl may eliminate about 5 g of fat daily; while Lorenz et al. (1938) placed the figure at about 4 g, but for a lighter bird.

Because 7-10 days are required for a yolk to mature in the ovarian follicle (Card, 1972), the effect of increased feed consumption in experiment 1 (during or after drug administration) on increasing egg production should not occur until at least this period of time had elapsed. For example, in experiment 1, hen number 8 (drug feed treatment) had returned to her previous level of egg production after a week of essentially zero egg production, all during the time the drug was being administered and the feed intake was below her normal level, but above that required for maintenance for this breed of chicken. Also hen number 6 returned immediately to her previous level of egg production and feed intake, after the CI-720 was withdrawn. Hen number 6 was at zero production the two previous weeks. In experiment 2, no return of egg production accompanied the increase in feed intake during the fourth week of drug treatment (which was still below the 70 g feed intake/hen/-day required for maintenance), at the end of which the experiment was terminated. Apparently the CI-720 has some direct effect on the egg production in some hens.

Perhaps the reason the hens given CI-720 via the capsules were not affected as much as the ones given the *per os* drug treatment, may be due to the fact that the drug given orally was in one massive dose. Therefore, the absorption time for the CI-720 was much different for hens given *per os versus* drug feed treatment. In experiment 1, the drug *per os* treated hens had CI-720 administered at a rate of about 1.5 times that of the drug feed treated hens (based on the overall averages of 309 and 202 mg CI-720/hen/-day). The difference in the amount of CI-720 administered is due to the fluctuation in feed consumption due to the presence of the drug; the hen could not control the amount of CI-720 injested when the drug was in the capsule. The egg production in the capsule treated group of hens was not affected as much as the 2600 ppm drug treated group of hens, but the hens given the latter treatment in either experiment showed similar responses for this parameter.

Whether the effect of CI-720 is direct, is mediated through the low feed consumption, alterations in the lipid metabolism, some other mechanism, or a combination of these, CI-720 is clearly at least a predisposing cause of a decline in egg production.

Change in Weekly Percent Egg Production

1. Experiment 1

No difference in the change in percent egg production (using absolute values, as in the percent change in body

weight) was detected by using the split plot statistical analysis. The absolute value (mathematically) of the change in weekly percent egg production was calculated for individual hens.

Hens in drug treatments generally declined in egg production from week to week. There was an overall decrease in egg production for four of these hens, while one hen did not change, and one increased while on drug treatment. The control hen's egg production generally did not change, or was increased, between the different weeks. There was no overall change in egg production for four of the control hens, with one decreasing and one increasing.

Probably the natural variation in egg production was sufficient to hide the fact that hens of the drug feed treatment were out of production versus their much higher previous record.

2. Experiment 2

A comparison of the average change in percent egg production (calculated as in experiment 1) for control hens for the first week of drug treatment, versus that of the 2600 ppm CI-720 treated hens, showed no statistical significance even though this low drug level treated group mean change was about twice that of the control treated hens. The control versus the high drug level treatment comparison showed the control hens changed less (the control hens had higher egg production) than the hens of this drug treatment ($P < .01$).

No other comparison was significant. The Tukey HSD test of means was used for all comparisons of these means.

All hens administered CI-720 at 5200 ppm had an overall decline (no production for 7 of 8 hens) in egg production. Five hens treated with the low level of drug decreased, two did not change, and one increased in overall egg production. One control hen decreased, two increased, and five showed no change in their overall egg production.

3. Discussion

For experiment 2, the changes in the treated groups of hen's egg production were associated with a 61% decline in feed consumption after the hens were given CI-720 @ 5200 ppm, and with a decline of about 30% in feed intake for the hens fed CI-720 @ 2600 ppm.

The normal positive and negative fluctuations in the egg production of control hens were apparently sufficient to mask the continued decline in egg production to zero of several hens. In experiment 1 only hens of the drug feed treatment were out of production for a week (plus or minus). In experiment 2, several hens of the low drug treatment, and all the high drug level treated hens (except at the first week of treatment) but one had stopped producing eggs by the third week of treatment.

Judging by the average amount of change from the pre-treatment period to either the third week of drug treatment, or to the end of the succeeding post-treatment week (see

Table 14) the CI-720 seems to have an effect only in the hen's egg production of the drug feed treatment. The other drug treatment change was almost identical to the control hen's change.

Total Egg Weight: Yolk, Albumen, and Shell Weights, Plus Their Percent of Total Egg Weight

1. Experiment 1

When computing an analysis of variance for the eggs laid on the day which ended a treatment week, no f-value was significant for any of the parameters of this section. See Table 15 for the mean values and ranges for these parameters for either experiment.

2. Experiment 2

All these parameters were statistically tested and found to have means which were not significantly different from each other, according to the f-ratio or by the Tukey HSD test of means if the f-value was significant. Only the total egg weight and yolk weight means showed any tendency toward statistical significance ($P < .2$ and $P < .1$, respectively).

Only eggs laid (by the eight hens per treatment) on the day ending the third week of drug treatment were evaluated for these parameters. Only one egg was available from the hens given the high drug treatment, and only five eggs from those given the lower drug treatment.

Table 15. Experiments 1 and 2. Means for egg weight; and the egg component weights, plus their percent of total egg weight.*

	Experiment 1		Experiment 2	
	Control	Drug	Control	Drug
Egg weight (g) mean**	55.9±0.59	55.7±0.59	62.4±1.60	57.2±2.40
range	48.1-62.3	49.9-63.0	55.9-69.9	52.5-67.5
Yolk weight (g) mean	17.9±0.25	17.5±0.22	19.2±0.43	17.4±0.35
range	14.9-20.1	15.8-20.5	16.8-21.1	16.5-18.6
Shell weight (g) mean	5.2±0.12	5.4±0.09	5.0±0.19	4.6±0.30
range	4.0-6.5	4.9-6.5	4.5-5.9	3.7-5.5
Albumen weight (g) mean	32.6±0.30	33.0±0.47	38.3±1.37	35.2±1.83
range	29.0-36.4	28.3-39.0	32.5-43.9	32.0-43.5
Yolk weight percent of total egg mean	32.1±0.20	31.4±0.35	30.8±0.63	30.6±0.73
range	30.1-34.4	28.0-34.5	28.4-32.8	27.6-32.9
Shell weight percent of total egg mean	9.4±0.15	9.8±0.15	8.0±0.30	8.0±0.29
range	7.9-11.3	8.9-11.3	7.0-9.4	7.0-8.9
Albumen weight percent of total egg mean	58.6±0.25	58.8±0.33	61.3±0.77	61.4±0.59
range	56.7-61.7	55.1-64.7	56.6-63.0	60.2-64.3

*Experiment 1 had three hens per treatment and six dates on which eggs were collected for evaluation. Experiment 2 used eight hens per treatment, and only one egg per hen (where available) was analyzed. There were unequal numbers in each.

**±S.E.M.

If the yolk and/or egg size were decreased by CI-720, the mechanism may be as that proposed by Burgess et al. (1962) for triparanol. Triparanol supposedly interrupted the estrogen production in the ovary, and inhibited the primary maturation of ova. The percent of the yolk of total egg weight was not changed by CI-720 administration. Probably these marginal significance levels were due to chance fluctuations in the egg and yolk sizes, as these values were very similar in the first experiment, and the experiment 2 albumen did not appear to compensate for the yolk size difference.

3. Discussion

The values of the egg and components reported for hens of both experiments are well within the ranges reported in the literature for control hens.

Romanoff and Romanoff (1949) stated that the actual and relative weights of the egg's structural elements, especially the shell, may deviate rather widely; even the weights for eggs of a "single individual hen".

Romanoff and Romanoff (1949) have cited other authors to report values that are pertinent to this discussion, they are as follows: V. S. Asmundson, for shell percent of total egg weight of 11.0, and values of 61.6 and 27.4 percent of total egg weight for albumen and yolk, respectively; N. Olsson found the shell to be 10.9 and 10.6 percent of the total egg weight, with the albumen percent being 61.1 and

59.6 percent, and the yolk 28 and 29.8 percent of total egg weight. In their table 11, which was composed of data from various sources, these authors reported that for a 58 g egg, the albumen percent of total egg weight was 55.8 (32.4 g); yolk was 31.9 percent (18.5 g); and shell was 12.3 percent (7.1 g) of the total egg weight.

The mean shell percent of total egg weight C. C. Morgan (1932) reported was 9.72, with a range of 6.76 to 12.5 percent.

Asmundson and Baker (1940) reported a range of shell percent of total egg weight of 5.28 to 9.46.

In 1961 Chung and Stadetman reported that the albumen represented 56.3 percent, and the yolk 30.4 percent of the total egg weight in the chicken. By simple arithmetic, the shell then was about 13.3 percent of the total egg weight. These authors found that the total egg weight was highly correlated with the albumen and yolk weights, as would be expected.

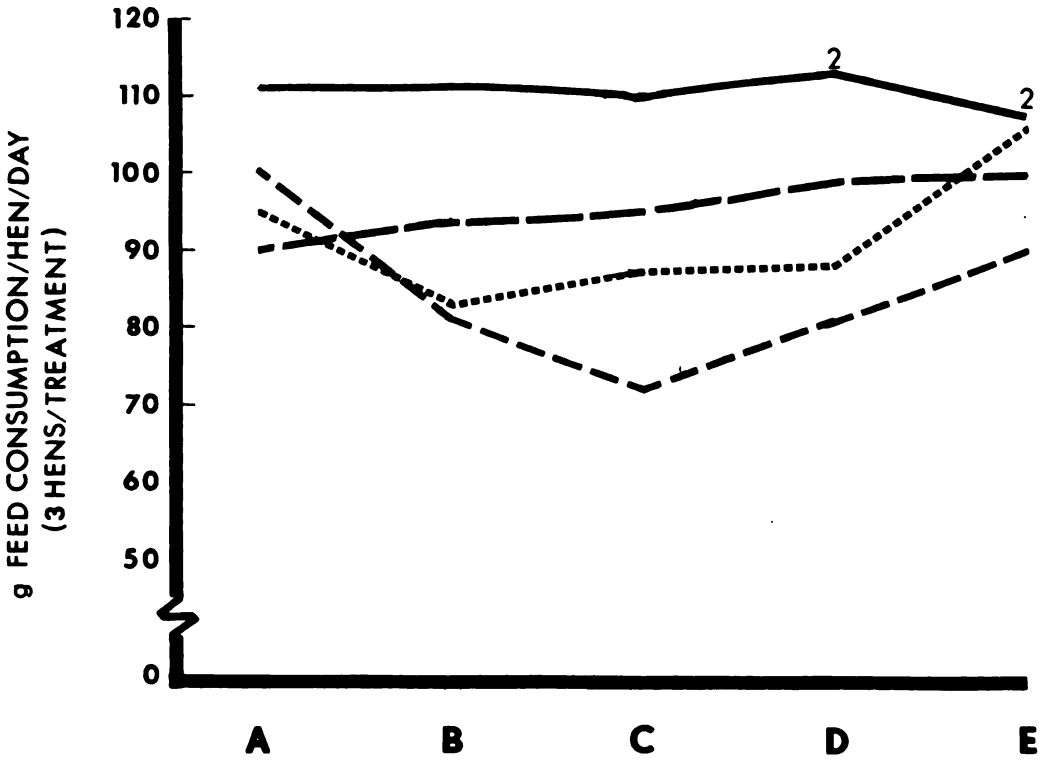
Weiss et al. (1967) believed that individual thyroid activity in a hen, with it's seasonal variation, probably influenced the level of the hen's egg production, egg yolk size, and yolk cholesterol content.

Feed Consumption

1. Experiment 1

Figure 18 deals with the feed intake of hens used in experiment 1, using corrected values to eliminate the data

Figure 18. Experiment 1. Feed consumption, average of three hens per treatment. For the last two weeks of the experiment only the values of two hens were used to calculate the mean for the control feed treated hens for this figure, as indicated by the numeral 2 on the line in the graph.



A = FEED CONSUMPTION MEAN DURING PRE-TREATMENT PERIOD

B = " " " " WEEK 1 DRUG TREATMENT

C = " " " " " 2 " "

D = " " " " " 3 " "

E = " " " " POST TREATMENT PERIOD

——— = CONTROL FEED TREATMENT (CI-720 at 0 ppm)

– – – = DRUG FEED TREATMENT (CI-720 at 2600 ppm)

——– = CONTROL PER OS TREATMENT (CI-720 at 0 mg/kg BODY WEIGHT)

····· = DRUG PER OS TREATMENT (CI-720 at 150 mg/kg BODY WEIGHT)

of hen # 1 for the last two weeks of the experiment. This hen was injured on day 14 of drug treatment and was off feed from that date until the post-treatment week. The feed intake analysis was calculated with the values of the injured hen considered as valid, and with her observations taken as the mean of the other two hens for that date. Hen # 1 had maintained this exact level of feed consumption for each of the previous weeks. By these manipulations, the reader has the option of viewing the data as it was recorded, as well as what probably would have happened. These alterations resulted in no change in the post-treatment period statistical results, but importantly altered the results from week three of drug treatment. See Table 16 for a comparison of these data.

The three control hens' feed intake was found to be significantly ($P < .05$) greater than that of the drug per os treated hens, on day 21 of drug treatment. For this same date, as well as the second week of drug treatment, the control feed treated hens consumed more feed ($P < .01$) than the hens of the drug feed group.

2. Experiment 2

There was pronounced individual variation among the birds given the drug treatments, especially those hens offered the high level of drug (5200 ppm), where hen 104 ate at nearly a normal rate, and some hens consumed essentially zero feed for several days, as indicated by the

Table 16. Experiment 1. Results of statistical analysis by the Tukey HSD test of means, comparing the feed intake of the four treatments.*

Treatment Comparison	End of Treatment Week 1	2	3	post-trt.	using expt. trt. $\bar{y}$
Control feed vs. drug feed	NS[1]	sig.[2] .01	sig.[3] .01 NS	NS[3] NS	sig. .05
Control feed vs. control per os	NS	NS	NS NS	NS NS	NS
Control feed vs. drug per os	NS	NS	sig. .05 NS	NS	NS
Drug feed vs. control per os	NS	NS	NS NS	NS NS	NS
Drug feed vs. drug per os	NS	NS	NS NS	NS NS	NS
Control per os vs. drug per os	NS	NS	NS NS	NS NS	NS

*The data tested was the mean of feed intake for the three hens of a treatment. Because hen # 1 was injured on day 14 of drug treatment, and was thereafter off feed, the data was calculated with her feed consumption as valid as well as corrected to what it had been previously.

[1]NS=not significant P>.05

[2]sig.=significant P<.05

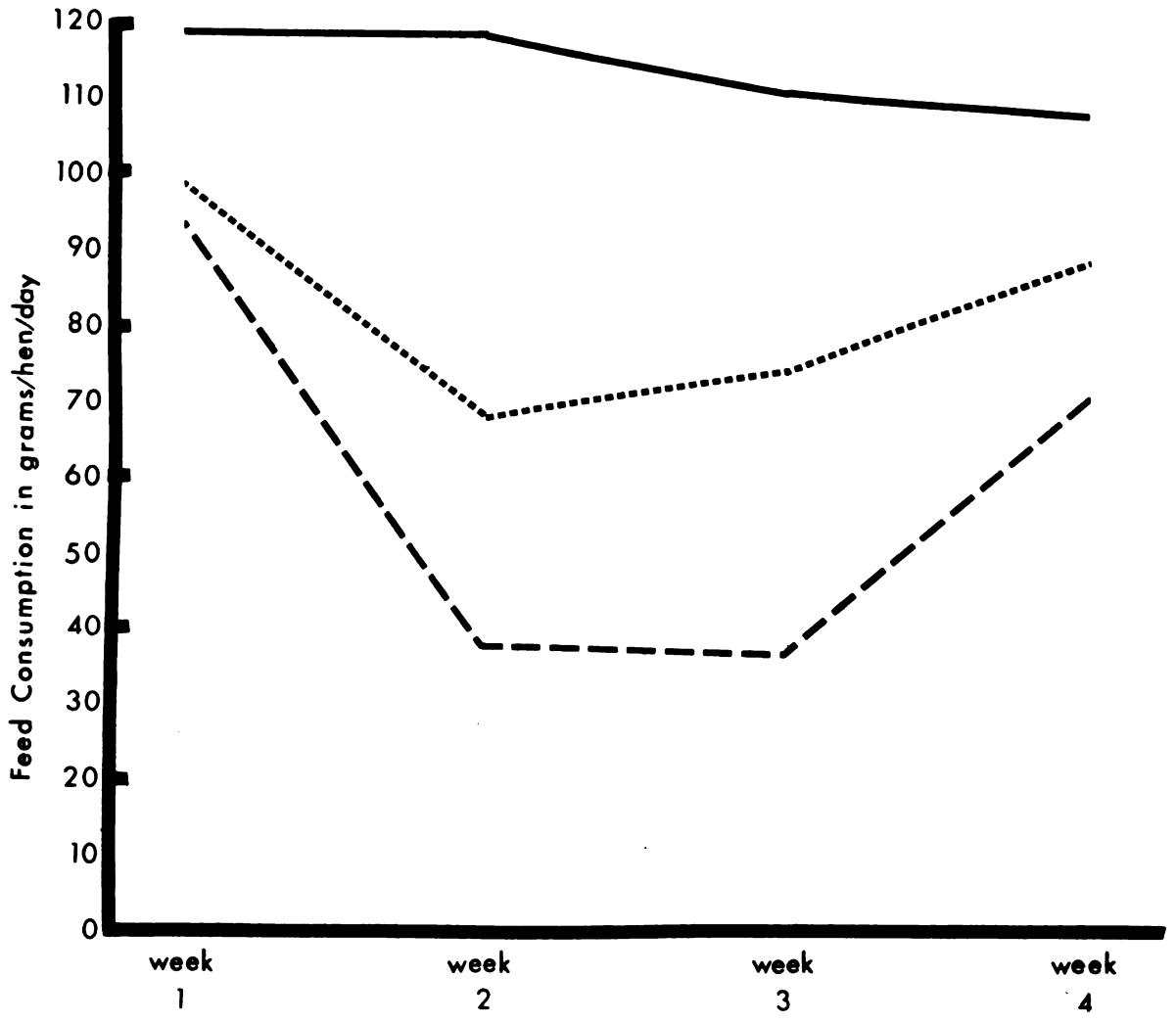
[3]statistical analysis including the hen in cage # 1 as a valid observation is below the results computed from data using the corrected values (the mean of hens 2 and 3).

amount of feed left in the container each day. Even if hen 104 ate only at the maintenance level (70 g/hen/day), that left the other seven hens with much less than the approximately 36 g/hen/day calculated as the mean for that treatment in each of the second and third weeks of drug administration.

By the fourth week of drug treatment, those hens eating feed with CI-720 at 5200 ppm (see Figure 19) appeared to bounce back from the near starvation level of feed intake of the preceeding two weeks to almost a maintenance level. This rebound effect was evident for hens given the low drug treatment, in both experiments (compare Figures 18 and 19). After a decline in feed intake of 30 g/hen/day, those hens given the 2600 ppm treatment started an upward trend to near their pre-treatment level by the time the experiment had ended.

Analysis of the final treatment means by Tukey's HSD test of means indicated the control group of hens had higher feed intake than those of either of the drug treatments ($P < .05$). There was no difference statistically between the drug treatments. Statistical analysis for means of the hen's feed intake at the end of each treatment week was not performed. Instead, Figure 19 serves to better illustrate the dramatic change in feed consumption using the mean for feed intake at the end of each treatment week. Any time the feed intake values drop below the individual hen's

Figure 19. Experiment 2. Feed consumption average of the eight hens/treatment, as calculated at the end of each week of drug treatment.


120
110
100
90
80
70
60
50
40
30
20
10
0
Feed Consumption in grams/hen/day
week 1
week 2
week 3
week 4
= Control feed treatment (CI-720 at 0 ppm)
= Drug " " (" " 2600 ppm)
= " " " (" " 5200 ppm)

requirements which allow her to be of economic value, that is significant, whether or not it is statistically.

3. Discussion

Table 17 lists the amount of CI-720 taken in by individual drug treated hens of experiment 1 as a mean for the week, plus the mean of the eight hens per drug treatment in the second experiment. Hens given the CI-720 via the gelatin capsule injested the drug in larger quantity (107 mg as the difference between treatment means) than those hens receiving CI-720 in the feed. The effect of the CI-720 bolus was not as great as when this drug was given in the feed, in lowering the feed consumption.

The rationale behind these drug levels was that at 2.6 g CI-720/kg diet, an average hen would consume about 260 mg CI-720/hen/day (depending on energy content of the diet). An average S.C.W.L. laying hen might weigh 1.8 kg, therefore at 150 mg CI-720/kg body weight, the amount of drug consumed based on these two different calculations, would be about equal (for experiment 1). The assumption was made that feed treated hens would ingest about 260 mg CI-720/hen/day; and the hens treated according to their body weight would be administered about 270 mg CI-720/hen/day.

By doubling the dosage of the first experiment for the feed method of drug administration, the second experiment in part, attempted to find an upper limit of CI-720

Table 17. Experiments 1 and 2. Intake of CI-720, as calculated from the feed consumption means in each experiment. Also, the intake of drug based on the body weight in a treatment in experiment 1.

Experiment 1[a]:

Drug Feed Treatment (CI-720 @ 2600 ppm)

Hen #	6		7		8	
	Feed[b] Intake	CI-720[c] Consumed	Feed Intake	CI-720 Consumed	Feed Intake	CI-720 Consumed
Day 1-7[d]	99[e]	257	63	164	80	208
Day 8-14	68	177	68	177	80	208
Day 15-21	85	221	71	185	87	226
Mean	84	218	67	175	82	214
Treatment Mean		202				

Drug Per os Treatment (CI-720 @ 150 mg/kg B.W.[f])

Hen #	14		15		16	
Weigh[g] date	B.W.[f]	CI-720 capsule (mg)	B.W.	CI-720 capsule (mg)	B.W.	CI-720 capsule (mg)
9 May	1.920	288	2.177	327	2.216	332
15 May	1.871	281	2.093	314	2.198	330
22 May	1.794	269	2.130	320	2.140	321
Mean	1.862	279	2.133	320	2.185	328
Treatment Mean		309				

Table 17 (cont'd.).

Experiment 2[a]:

	Mean feed intake/hen/day[b]	Mean CI-720 intake/hen/day[c]
Drug Feed Treatment (CI-720 @ 2600 ppm)		
Day 1-7[d]	98	255
Day 8-14	67	174
Day 15-21	73	190
Day 22-28	88	229
Mean	82	212
Drug Feed Treatment (CI-720 @ 5200 ppm)		
Day 1-7[d]	93	484
Day 8-14	37	192
Day 15-21	36	187
Day 22-28	71	369
Mean	59	308

a = 3 and 8 hens/treatment in experiments 1 and 2, respectively.
b = in g feed consumed/hen/day.
c = (feed intake) x (2600 g CI-720 per kg diet) = g CI-720 consumed x 1000 = mg CI-720 consumed.
d = days of drug treatment.
e = all values are rounded off.
f = hen body weight in kg+1 g.
g = the date weight used to calculate CI-720 packed into the capsule for 1-3 treatment weeks.

administration. This doubled dose of CI-720 (5200 ppm) was enough to inhibit the feed intake of all but one of the eight hens of that treatment.

The response of the 2600 ppm CI-720 treated hens in each experiment showed almost identical patterns of feed consumption (see Figures 18 and 19), even to the average amount of feed eaten over the length of the experiment. Hens in experiment 1 that were given this particular treatment averaged about 81 g feed consumed/hen/day, and the hens in experiment 2 for this treatment averaged 82 g feed consumed/hen/day.

In experiment 2, comparing the amount of drug consumed by the 2600 ppm treated hens with the amount consumed by those hens given the high level of drug for the second and third weeks of drug treatment, the values were about the same. Yet the feed intake was about completely inhibited for some hens of the high drug level treatment, and not in the lower drug level treatment. The level of feed consumption of hens of the 2600 ppm drug treatment was about twice that of hens of the 5200 ppm drug treatment. If the effect of CI-720 was to influence the appetite center in the brain, then the large amount of CI-720 ingested during the first week by the hens given the higher level of drug could have been enough to nearly stop the desire to eat (except hen 104). Then at the lower levels of drug which occurred later because of no appetite, the effect of CI-720 may have dissipated enough to allow some food consumption.

Alternatively, the hens may have come to a point of "eat or die", and the drug was not strong enough to force the latter choice.

Apparently the effect of CI-720 on feed consumption is not only on palatability, as those hens given the drug in the capsule were similarly effected as those given CI-720 in the feed, but to a lesser magnitude. Possibly an action of CI-720 was the result of an inhibition of the appetite center. Because the capsule treated hens injested about 120 mg CI-720 more than the corresponding drug feed treated hens, on a regular basis, and yet maintained a feed intake that was greater than those hens eating the contaminated feed, a safe assumption could be that the effect of CI-720 is at least partially mediated through a palatability and/or instinctive rejection of foreign substances.

Several hens molted in the drug treated groups, in both experiments but more so in the second. This could have been due to an action of the drug, but more likely to the acute starvation or below maintenance levels of feed intake of the hens. In experiment 1, hen # 7 started to molt by the end of the first week of drug treatment; during this time, she averaged about 63 g of feed intake per day. By day 17 of drug treatment, hen # 6 started to molt, the preceeding week her feed intake averaged about 68 g. Both hens were from experiment 1.

In experiment 2, records of individual feed intake were not kept, but for the 2600 ppm treatment, four hens showed evidence of molting (cessation of egg production, and dropped feathers) the week after their feed consumption had declined to 66 g feed consumed/hen/day, and several showed signs of illness (watery droppings, ruffled feathers, crouched position, eyes closed). Four hens on the 5200 ppm treatment dropped feathers the third week of treatment; this group of hens had taken in only about 36 g feed/hen/day average during the second and third weeks of treatment.

Total Excreta Cholesterol

1. Experiment 1

Excreta samples were collected during the days 15 through 21 of drug treatment; in experiment 1, only. The collection pan served as the storage container for air drying.

When the excreta cholesterol was expressed as mg cholesterol/g dry excreta, there was no difference between the different treatment means, as judged by the insignificant f-value of the analysis of variance. The mean for the three hens/treatment were: 1) control feed treatment (CI-720 at 0 ppm)--- 4.1; 2) drug feed treatment (CI-720 at 2600 ppm)---3.4; 3) control *per os* treatment (CI-720 at 0 mg/kg body weight)---3.5; 4) drug *per os* treatment (CI-720 at 150 mg/kg body weight)---3.2 mg cholesterol/g dry excreta. For an approximation of these values when based on wet excreta

weight, simply divide by four. The standard error for the experiment was 0.144.

March et al. (1964) expressed their results as g sterol per kg diet. They reported values of 4.89 and 5.86 as control hen's means. When the values of the present experiment are expressed as March and co-workers did, these values are much lower, more on the order of 1+ g cholesterol excreted/kg diet.

This may indicate a technical error in this experiment, or degredation of the cholesterol as Clarenburg et al. (1971) have suggested.

Konlande and Fisher (1969) reported that control chicks had values of 6.2 mg cholesterol/g excreta, but did not state whether these values were based on wet or dry excreta weights.

Because of the extended period of time (about 18 weeks) between time of sample collection and processing, the cholesterol content could have been reduced due to bacterial degredation (Clarenburg et al., 1971). Also worms were very abundant in the excreta collection trays, and they probably consumed part of the excreta, thus making total excreta erroneous.

SUMMARY

1. During two experiments, laying hens were administered an antilipemic compound (CI-720) for the purpose of determining its effect on a number of blood, reproductive, and physical parameters. The results were as follows:
   A. Total plasma cholesterol was lowered by CI-720 from 133 to 114 mg/dl plasma in experiment 1, and from 155 to 111 and 105 mg/dl plasma in experiment 2. The first experiment values could have been an artifact of the random distribution of the hens, as could the free plasma cholesterol values from experiment 1.
   B. CI-720 caused a decline in free plasma cholesterol from 107 to 79 mg/dl plasma in the first experiment, and from 85 to 50 and 38 mg/dl plasma during the second.
   C. The percent free of total plasma cholesterol was similar in response as the free plasma cholesterol. A larger percentage was found to be in the free form in the control hens than in drug treated hens, during both experiments.

D. Plasma triglycerides were not determined in the first experiment, but were found to be significantly depressed by the drug in the second (1524;613;228 mg/dl plasma, for treatment of 0, 2600, and 5200 ppm of the diet, respectively).

E. The total yolk cholesterol was increased in the first experiment, but no difference was found between treatment means during the second.

F. Hen body weight was not changed by the drug treatment in experiment 1, but was lowered in experiment 2.

G. The percent change in body weight was not changed in experiment 1 by CI-720, but the drug treated hens showed more variation during experiment 2.

H. Weekly percent egg production declined to zero for some drug treated hens, but not all, during both experiments.

I. There was no difference between treatments in the amount of change in weekly percent egg production during experiment 1. In the second experiment, the drug treated hens were shown to have changed more in weekly percent egg production, than those given control treatments.

J. Total egg weight; yolk, albumen, and shell weights, plus their percent of total egg weight was not altered by drug administration during either experiment. The drug treated hens of

experiment 2 showed a trend toward lowered egg weight and yolk weight compared to that of control hens.

K. Feed consumption was lowered by the drug, during both experiments.

2. The conclusion drawn from these experiments was that CI-720 probably had an antilipemic effect in the chicken, but that this effect was confounded with the lowered feed intake. Also, CI-720 did not lower the egg cholesterol. Therefore, this drug probably has little direct application for the poultry industry.

APPENDICES

APPENDIX A

FOOTNOTES

1. Three classes of hypocholesterolemic compounds:
   A. 2 (p-(2-diethylaminoethoxy) phenyl) benzimidazole monohydrochloride
   B. 1'-(2-(1-naphthylamino)ethyl)-1,4'-bipiperidine dihydrochloride
   C. 4-(2-chlorophenyl)-alpha-(p-methoxyphenoxymethyl)-1-piperazine ethanol

2. SC-12937 is 20,25-diazocholestenol dihydrochloride

3. SC-10644 is 2-(1-pyrrolidinyl) ethyl triphenyl methane-4 carboxylate hydrochloride

4. beta-diethylaminoethyl diphenyl propylacetate hydrochloride

APPENDIX B

RECOVERIES

Parameter	Percent Recovered
Total Plasma Cholesterol	86.7
Free Plasma Cholesterol	86.6
Plasma Triglycerides	91.4
Total Yolk Cholesterol	102.9

An example of the method used for these determinations was to split a sample of plasma, and to one aliquot 100 mg crystalline cholesterol (or triglyceride aliquot) was added. The samples were processed as outlined in Flow Chart 1 (for total plasma cholesterol, as an example). The difference between the calculated amount of cholesterol of the "spiked" and the original sample was 86.7 mg cholesterol. This represents 86.7 percent of the added cholesterol recovered.

APPENDIX C

Appendix Table 1. Split plot analysis of variance table--a comparison between the computer and hand calculated results.*

Source of Variation	d.f.	Mean Square		f-Statistic	
		com.	man.	com.	man.
Among Subjects					
method of administration (α)	1	3727	3727	3.24	3.24
drug/no drug (β)	1	6421	6422	5.6	5.9
x interaction	1	251	249	.21	.22
error a (hens)	8	1150	1149		
total	11				
Within Subjects					
time (γ)	5	972	972	1.1	1.5
α x γ interaction	5	751	751	1.2	1.2
β x γ interaction	5	418	418	.66	.66
α x β x γ "	5	1037	1038	1.6	1.6
error b	40	630	630		
subtotal	60				
total	71				

*Total plasma cholesterol was the data used.

APPENDIX D

SPLIT PLOT STATISTICAL MODEL

Split Plot Model:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + D_{(ij)k} + (\alpha\beta)_{ij} + \gamma_l + (\alpha\gamma)_{il} + (\gamma D)_{(ij)kl} + (\alpha\beta\gamma)_{ijl} + E_{(ijkl)}$$

Where:

μ	= population mean
α_i	= method of drug administration
β_j	= drug; no drug treatments
$D_{(ij)k}$	= Error a---hens nested in treatment
$(\alpha\beta)_{ij}$	= method x drug interaction
γ_l	= time effect
$(\alpha\gamma)_{il}$	= method x time interaction
$(\beta\gamma)_{jl}$	= drug; no drug x time interaction
$(\alpha\beta\gamma)_{ijl}$	= method of drug administration x drug; no drug x time interaction
$E_{(ijkl)}$	= random experimental error---confounded with this is the hen x time interaction

APPENDIX E

SPLIT PLOT STATISTICAL ANALYSIS, SUMS OF SQUARES

$$SS_\alpha = \left(\sum_{i=1}^{2} y_{i\ldots}^2/dht\right) - \underbrace{\left(\sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{k=1}^{3}\sum_{l=1}^{6} y_{ijkl}\right)^2/N}_{\text{or, } y_{\ldots\ldots}^2/N}$$

$$SS_\beta = \left(\sum_{j=1}^{2} y_{\cdot j\cdot\cdot}^2/mht\right) - y_{\ldots\ldots}^2/N$$

$$i = 1,2\ (m)$$
$$j = 1,2\ (d)$$
$$k = 1,2,3\ (h)$$
$$l = 1,2,\ldots,6\ (t)$$

$$SS_{\alpha\beta} = \left(\sum_{i=1}^{2}\sum_{j=1}^{2} y_{ij\cdot\cdot}^2/ht\right) - \left(\sum_{i=1}^{2} y_{i\ldots}^2/dht\right) - \left(\sum_{j=1}^{2} y_{\cdot j\cdot\cdot}^2/mht\right) + y_{\ldots\ldots}^2/N$$

$$SS_D = \left(\sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{k=1}^{3} y_{ijk\cdot}^2/t\right) - \left(\sum_{i=1}^{2}\sum_{j=1}^{2} y_{ij\cdot\cdot}/ht\right)$$

$$SS_\gamma = \left(\sum_{l=1}^{6} y_{\ldots l}^2/mdh\right) - y_{\ldots\ldots}^2/N$$

$$SS_{\alpha\gamma} = \left(\sum_{i=1}^{2}\sum_{l=1}^{6} y_{i\ldots l}^2/hd\right) - \left(\sum_{i=1}^{2} y_{i\ldots}^2/dht\right) - \left(\sum_{l=1}^{6} y_{\ldots l}^2/mdh\right) + y_{\ldots\ldots}^2/N$$

$$SS_{\beta\gamma} = \left(\sum_{j=1}^{2}\sum_{l=1}^{6} y_{\cdot j\cdot l}^2/mh\right) - \left(\sum_{j=1}^{2} y_{\cdot j\cdot\cdot}^2/mht\right) - \left(\sum_{l=1}^{6} y_{\ldots l}^2/mdh\right) + y_{\ldots\ldots}^2/N$$

$$SS_{\alpha\beta\gamma} = \left(\sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{l=1}^{6} y_{ij\cdot l}^2/K\right) - \left(\sum_{i=1}^{2}\sum_{l=1}^{6} y_{i\cdot\cdot l}^2/jK\right) - \left(\sum_{i=1}^{2}\sum_{j=1}^{2} y_{ij\cdot\cdot}^2/Kl\right) -$$

$$- \left(\sum_{j=1}^{2}\sum_{l=1}^{6} y_{\cdot j\cdot l}^2/mK\right) + \left(\sum_{i=1}^{2} y_{i\ldots}^2/jKl\right) + \left(\sum_{l=1}^{6} y_{\ldots l}^2/mdh\right) +$$

$$+ \left(\sum_{j=1}^{2} y_{\cdot j\cdot\cdot}^2/mht\right) - y_{\ldots\ldots}^2/N$$

$$SS_E = SS_\gamma - SS_\alpha - SS_\beta - SS_{\alpha\beta} - SS_D - SS_\gamma - SS_{\alpha\gamma} -$$

$$- SS_{\beta\gamma} - SS_{\alpha\beta\gamma}$$

SS_Y ; conventional formula : $\sum_{i=1}^{2}\sum_{j=1}^{2}\sum_{K=1}^{3}\sum_{l=1}^{6} y_{ijkl}^2 - \left(y_{\ldots\ldots}^2/N\right)$

APPENDIX F

TUKEY HSD, MODIFIED TUKEY HSD, AND DUNNETT TEST OF MEANS PLUS f_{max}-TEST FOR HOMOGENOUS VARIANCE

Tukey HSD Test of Means:

test statistic: $$\frac{\bar{y}_{i\cdot} - \bar{y}_{i\cdot}'}{\sqrt{MS_E/r}}$$

where: t = the number of means being compared

n - t = the degrees of freedom for error

critical value: $\pm q_{\alpha, t, n-t}$

from table A.8---Gill

Modified Tukey HSD Test of Means:

test statistic: $$\frac{\bar{y}_{i\cdot} - \bar{y}_{i\cdot}'}{\sqrt{MS_E/r_s}}$$

where: r_s = the smaller of the r_i being compared

critical value: same as above

Dunnett Test of Means:

test statistic: $$\frac{\bar{y}_{i\cdot} - \bar{y}_{i\cdot}'}{\sqrt{MS_E\left(\frac{1}{r_i} + \frac{1}{r_i'}\right)}}$$

where: m = the number of experimental groups

ν = the degrees of freedom for error

critical value: $t_{D, \alpha, m, \nu}$

from table A.9.1---Gill

f_{max}-Test for Homogenous Variance:

test statistic: $\frac{S^2 \text{ max}}{S^2 \text{ min}}$

where: t = the number of groups

ν = the degrees of freedom for error = $n-1$

critical value: $f_{max, \alpha, t, \nu}$

from table 6.1---Gill, or Table T from Rohlf and Sokal

APPENDIX G

Appendix Table 2. Re-evaluation of free plasma cholesterol values of experiment 2.*

Hen #	Rep. 1	Rep. 2	$\bar{y}$	Initial Determination
Control Treatment (CI-720 @ 0 ppm) in mg/dl				
99	93	92	93	90
102	96	95	96	97
103	88	82	85	86
115	83	81	82	81
121	88	94	91	91
122	133	128	131	113
124	55	55	55	54
125	50	48	49	48
Low Drug Level Treatment (CI-720 @ 2600 ppm) in mg/dl				
105	34	32	33	29
106	60	63	62	61
107	52	51	52	52
108	--	95	95	95
109	31	31	31	30
110	60	61	61	60
136	36	36	36	33
139	40	37	39	37
High Drug Level Treatment (CI-720 @ 5200 ppm) in mg/dl				
104	74	78	76	71
116	40	40	40	36
117	36	38	37	34
131	36	32	34	32
137	37	33	35	35
138	44	41	43	38
141	30	27	29	28
142	33	31	32	30

*Data is in mg/free cholesterol/dl plasma. There were eight hens in each of the three treatments. Values labelled Rep. 1 and 2 were calculated from different curves, on the same day. The values labelled initial determination are the means obtained as a final value at a previous time. All determinations were from the same plasma sample.

APPENDIX H

Appendix Table 3. Coefficient of variation* for parameters of experiments one and two.

Parameter	Experiment 1	Experiment 2
Total plasma cholesterol	23.7%	21.4%
Free plasma cholesterol	31.1	34.6
Percent free of total plasma cholesterol	17.8	21.5
Plasma triglycerides	----**	59.8
Body weight (in grams)	10.9	12.3
Weekly egg production	44.3	49.9
Total yolk cholesterol	10.0	11.9
Total egg weight	6.1	8.8
Shell weight	11.0	13.1
Albumen weight	6.8	11.3
Yolk weight	7.5	5.9

*calculated as: $\frac{\text{(standard deviation) x (100)}}{\bar{y}}$

**data discarded

APPENDIX I

Appendix Table 4. Plasma triglyceride values (in mg/dl) from experiment one.

Hen #	day 0	day 4	day 7	day 14	day 21	day 7	mean
Control Feed Treatment (CI-720 @ 0 ppm)							
1 R1	1674	2205	2038	1648	1181	cn	
R2	1599	2150	1936	1572	cn	cn	
mean	1637	2178	1987	1610	1181	--	1853
2 R1	1073	994	1744	930	55	cn	
R2	1245	1078	1566	790	36	ns	
mean	1159	1036	1655	860	46	--	1178
3 R1	2005	2295	3049	1854	37	n	
R2	2123	2098	3060	1763	51	cn	
mean	2064	2197	3055	1809	44	--	2281
date mean	1620	1804	2232	1426	----	--	1771

R1 = replicate 1; R2 = replicate 2; cn = calculated a negative number; n = negative number obtained when blank was subtracted; ns = no sample; days 0, 4, 7, 14, and 21 represent days on treatment, the day 7 of the right column represents day 7 of post-treatment

Appendix Table 4 (cont'd.).

Hen #	day 0	day 4	day 7	day 14	day 21	day 7	mean
Drug Feed Treatment (CI-720 @ 2600 ppm)							
6 R1	1136	1040	409	154	cn	n	
R2	1197	1599	19	cn	n	cn	
mean	1167	1320	214	154	--	--	1244
7 R1	267	29	308	cn	n	n	
R2	298	47	360	cn	n	n	
mean	283	38	334	--	--	--	309
8 R1	1477	840	466	579	cn	cn	
R2	1638	970	685	785	154	87	
mean	1558	905	576	682	154	87	930
date mean	1003	1113	455	682	--	--	828
Control per os Treatment (starch-containing capsule)							
9 R1	2324	2309	1440	1863	149	cn	
R2	2165	2128	1606	2124	210	4	
mean	2245	2169	1523	1994	180	4	1983
10 R1	ns	1496	1054	1488	cn	n	
R2	867	2269	925	1613	cn	n	
mean	867	1883	990	1551	--	--	1323
11 R1	807	1336	1077	751	cn	23	
R2	967	1405	1243	1050	137	214	
mean	887	1371	1160	901	137	110	1080
date mean	1333	1808	1224	1482	--	--	1462

Appendix Table 4 (cont'd.).

Hen #	day 0	day 4	day 7	day 14	day 21	day 7	mean
Drug per os Treatment (capsule contained CI-720 @ 150 mg/kg body weight)							
14 R1	776	453	639	1871	cn	cn	
R2	896	524	429	1908	cn	cn	
mean	836	489	534	1890	--	--	937
15 R1	240	538	625	432	n	n	
R2	502	484	706	422	n	n	
mean	371	511	666	427	--	--	494
16 R1	2146	1355	698	623	cn	n	
R2	1847	1462	791	858	237	n	
mean	1997	1409	745	741	237	--	1223
date mean	1068	803	648	1019	--	--	885

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