DIETARY LIPID FACTORS INFLUENCING STEROL AND FATTY ACID METABOLISM IN LAYING HENS

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ABSTRACT

Two feeding trials were conducted with 30-week-old Single Comb White Leghorns fed two basal diets containing 8% of hydrogenated coconut oil or safflower oil. These basal diets were fed with or without supplements of 1% cholesterol (CH), 2% soysterols (ST) or in combination (CH+ST).

Trial 1 was designed to study the sensitivity of laying hens in response to dietary lipid factors and to compare the mode of changes in serum and egg yolk concentrations by weekly determination of their sterol levels during a feeding period with no soysterol supplementation followed by a period with soysterol supplementation. Objectives of Trial 2 were to study the effects of dietary lipid factors on the serum and egg yolk levels and to delineate their roles or mechanisms.

Changes in egg yolk sterol levels caused by dietary lipid factors were generally parallel to, and preceded by, those in serum sterol levels. This indicated that the egg sterols originated from the circulating labile sterol pool. Safflower oil suppressed and hydrogenated coconut oil elevated the sterol levels in both serum and egg yolk.

Cholesterol feeding with dietary safflower oil increased the absorption of cholesterol, resulting in an increase of serum and egg sterol levels as compared to sterol levels of hens fed

HYDROGENATED COCONUT OIL.

DIETARY SOYSTEROLS SUPPLEMENTATION RESULTED IN A DECREASE IN BOTH SERUM AND EGG YOLK STEROLS WHICH WAS DEMONSTRATED IN THE PRESENCE OF DIETARY CHOLESTEROL AS WELL AS CHOLESTEROL-FREE TREATMENTS. THE APPARENT ABSORPTION OF CHOLESTEROL WAS NOT RETARDED BY THE SIMULTANEOUS FEEDING OF CHOLESTEROL AND SOYSTEROLS. HOWEVER, SOYSTEROL FEEDING ACCELERATED THE FECAL EXCRETION OF BILE ACIDS AND CATABOLIC PRODUCTS OF NEUTRAL STEROLS.

THE APPARENT ABSORPTION OF PLANT STEROLS WAS 77%. ABSORBABILITY OF PLANT STEROLS IN LAYING HENS WAS FURTHER SUPPORTED BY DETECTION OF THESE STEROLS IN TISSUE AND EGG YOLK. THE ABSORPTION OF THE PLANT STEROLS WAS, HOWEVER, SLIGHTLY DECREASED WHEN CHOLESTEROL WAS FED SIMULTANEOUSLY.

CHOLESTEROL FEEDING INCREASED TOTAL LIPID CONTENT IN LIVER AND SERUM, WHEREAS SOYSTEROL FEEDING REDUCED OR DIMINISHED LIPID ACCUMULATION CAUSED BY THE CHOLESTEROL TREATMENT.

BOTH DIETARY CHOLESTEROL AND SOYSTEROLS ALTERED THE FATTY ACID COMPOSITION OF LIVER, SERUM AND EGG YOLK LIPIDS BY INCREASING OLEIC ACID AND DECREASING PALMITIC AND/OR STEARIC ACIDS. THESE CHANGES WERE SIGNIFICANTLY GREATER UPON FEEDING CHOLESTEROL THAN SOYSTEROLS. HOWEVER, THE SIMULTANEOUS FEEDING OF CHOLESTEROL WITH SOYSTEROLS EXERTED THE LEAST EFFECT ON THE FATTY ACID COMPOSITION IN LIVER. THE POSSIBILITY THAT SOYSTEROLS AFFECTED BIOSYNTHESIS AND/OR OXIDATIVE CATABOLISM OF FATTY ACIDS IN THE LIVER OF LAYING HENS IN A SIMILAR FASHION AS CHOLESTEROL WAS DISCUSSED.

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INTRODUCTION

ELEVATED SERUM CHOLESTEROL LEVELS HAVE LONG BEEN IMPLICATED AS ONE OF THE MAJOR CAUSES OF ATHEROSCLEROSIS SINCE RESEARCH WORKERS DISCOVERED THAT VARIOUS ANIMALS FED CHOLESTEROL EASILY BECOME HYPERCHOLESTEROLEMIC AND ATHEROSCLEROTIC. FOR THIS REASON, STUDIES IN THE GENERAL FIELD OF CHOLESTEROL METABOLISM AND SOME ANTI-HYPERCHOLESTEROLEMIC EFFECTS OF MANY DIETARY FACTORS HAVE PREDOMINATED FOR LAST SEVERAL DECADES.

STUDIES ON MANY EXPERIMENTAL ANIMAL SPECIES, INCLUDING MAN, LED TO A REASONABLE DEGREE OF AGREEMENT UPON THE GENERAL CONCEPT THAT DIETARY CHOLESTEROL IS A FACTOR GOVERNING THE SERUM CHOLESTEROL LEVEL BUT THAT UNDER THE USUAL CONDITIONS IT IS NOT AS IMPORTANT AS THE KIND OR AMOUNT OF DIETARY FAT. ALTHOUGH AGREEMENT HAS NOT YET BEEN REACHED ABOUT THE EFFECT OF SPECIFIC FATTY ACIDS, IT SEEMS CLEAR THAT HIGHLY UNSATURATED FATS CONTAINING SUBSTANTIAL PROPORTIONS OF POLYUNSATURATED FATTY ACIDS LOWER SERUM CHOLESTEROL LEVEL, WHEREAS HIGHLY SATURATED FATS RAISE IT.

DIETARY PLANT STEROLS, CONSIDERED NON-ABSORBABLE FORTY YEARS AGO, NOW HAVE RECEIVED A GREAT DEAL OF ATTENTION BECAUSE OF THE ABILITY OF THESE DIETARY FACTORS TO DECREASE SERUM CHOLESTEROL WHEN FED OR INJECTED INTO A NUMBER OF ANIMAL SPECIES. ALTHOUGH THE MECHANISMS OF THE ANTI-CHOLESTEROLEMIC ACTIVITY OF EITHER POLYUNSATURATED FATTY ACIDS OR PLANT STEROLS ARE NOT KNOWN, RECENT

EXPERIMENTAL OBSERVATIONS INDICATE THAT THE SERUM CHOLESTEROL-LOWERING EFFECT INDUCED BY FATTY ACIDS OR PLANT STEROLS ARE ACCOMPANIED BY AN INCREASE IN FECAL ACIDIC AND NEUTRAL CATABOLIC STEROLS.

THEREFORE, THE OBJECTIVES OF THE RESEARCH HEREIN REPORTED WERE TO INVESTIGATE THE EFFECTS OF DIETARY HYDROGENATED COCONUT OIL AND SAFFLOWER OIL, CHOLESTEROL AND SOYSTEROLS, AND DIETARY COMBINATIONS OF THESE INGREDIENTS ON THE CHOLESTEROL AND FATTY ACID METABOLISMS OF THE LAYING HEN.

LITERATURE REVIEW

Cholesterol Metabolism in Laying Hen

Studies in the general field of experimental atherosclerosis have been dominated by the development of diets or other conditions which will produce atherosclerotic lesions in the experimental animals used. Elevated serum cholesterol has been implicated as one of the major causes of susceptibility to atherosclerosis. Rabbits, swine, monkeys, chickens and pigeons are quite susceptible to hypercholesterolemia and atherosclosis, whereas dogs and rats are quite resistant (Roberts et al., 1965).

It is the opinion of many investigators that body cholesterol metabolism can be controlled by means of dietary lipids, although the mechanisms of action proposed by many investigators are not in complete accord. Dietary lipids can affect the body cholesterol pool by altering the mode of absorption, biosynthesis, excretion or redistribution of the cholesterol pool within body compartments.

Cholesterol is continuously turning over according to knowledge of the intermediary metabolism and steady state of body cholesterol pool. Some of the cholesterol is degraded and excreted while new molecules are synthesized and released for body use (Wilson and Lindsey, 1965; Andrews et al., 1968). When the animal is in a steady state in regard to cholesterol content, the rate of influx of new cholesterol is equal to the rate of excretion.

Dietary cholesterol is one of the major inputs and the amount varies from species to species due to the fact that intestinal absorption is limited in some species (Borgstrom, 1969; Grundy et al., 1969). However, the ability of laying hens to absorb dietary cholesterol was found to be dependent upon the nature of dietary components that aid in cholesterol absorption. Dietary fat in conjunction with cholesterol has been shown to increase the absorption and transport (March and Biely, 1959; Chung et al., 1965; Hulett et al., 1964; and Weiss et al., 1967a). Weiss et al. (1967a) observed that highly unsaturated fat such as safflower oil has a synergistic effect on cholesterol absorption and transport into egg yolk.

The actual mechanism of cholesterol transfer across the intestinal cell wall is not known. Cholesterol, once across the cell wall, is exclusively transported via lymph, and not via the portal vein in most mammals (Chaikoff et al., 1952; Borgstrom, 1960, 1967; and Simmonds et al., 1967). The intestinal lymphatic system in the fowl, however, is less well developed and differs somewhat in its structure and does not represent an important pathway for lipid absorption (Noyan et al., 1964). These workers showed that the portal blood is a significant pathway for lipid absorption, by showing that 95% of the absorbed lipids entered the portal system as a very low density lipoprotein in the fowl.

After absorption, cholesterol enters the liver and systemic circulation. The cholesterol in the plasma is rapidly exchanged with that of liver, and is therefore, considered as one continuous

CHOLESTEROL POOL (CHEVALLIER, 1967). THIS WAS ALSO SHOWN IN LAYING HENS, ORALLY ADMINISTERED RADIOACTIVE CHOLESTEROL WAS RAPIDLY BUILT UP IN THE LIVER AND PLASMA. HOWEVER, THE MAXIMUM UPTAKE OF RADIOACTIVE CHOLESTEROL IN LIVER WAS WITHIN 12 HOURS FOLLOWED BY A PLASMA PEAK WITHIN 24 HOURS (ANDREWS ET AL., 1968).

LIVER ALSO FUNCTIONS AS AN ACTIVE SITE OF CHOLESTEROL INPUT BY CHOLESTEROGENESIS (BLOCH AND RITTENBERG, 1945; ANKER, 1948). HEPATIC CHOLESTEROL BIOSYNTHESIS IS, HOWEVER, SENSITIVE TO THE AMOUNT OF DIETARY CHOLESTEROL INPUT (SCHOENHEIMER AND BREUSCH, 1933). THIS NEGATIVE FEEDBACK MECHANISM WAS REPORTED IN GROWING CHICKS (CHUNG ET AL., 1970; LUPIEN AND MIGICOVSKY, 1964), IN LAYING HENS (WEISS ET AL., 1967B) AND ADULT MALE CHICKENS (SAKAKIDA ET AL., 1963).

THE LIVER IS THE MAJOR ORGAN AT WHICH CHOLESTEROL OUTPUT TAKES PLACE BY CONVERSION TO BILE ACIDS AND SECRETION INTO THE INTESTINE WHERE SOME ARE LOST IN THE FECES, AND BY SECRETION OF CHOLESTEROL OR ITS METABOLITES INTO BILE AS NEUTRAL STEROLS (DANIELSON AND TCHEN, 1969; MIETTINEN ET AL., 1965). SINCE MOST EXTRAHEPATIC TISSUES ARE NOT ABLE TO DEGRADE CHOLESTEROL APPRECIABLY, IT WAS SUGGESTED THAT THERE IS A CONTINUOUS FLUX OF CHOLESTEROL FROM THE VARIOUS EXTRAHEPATIC SITES THROUGH THE PLASMA TO THE LIVER (MASORO, 1968).

THE FORMATION OF BILE ACIDS BY LIVER IS UNDER NEGATIVE FEEDBACK CONTROL (ABELL ET AL., 1956; WILSON, 1964). THE BILE ACID REABSORPTION FROM THE INTESTINE IS TRANSPORTED TO THE LIVER BY THE PORTAL CIRCULATION AND INHIBITS THE SYNTHESIS OF NEW BILE ACIDS

IN THE LIVER (DANIELSON AND TCHEN, 1969). STUDIES ON FECAL BILE ACID AND NEUTRAL STEROL OUTPUT DURING DIFFERENT LEVELS OF DIETARY INTAKE OF CHOLESTEROL HAVE BEEN REPORTED IN DOGS (ABELL ET AL., 1956) AND RATS (WILSON, 1964). THESE ANIMALS ARE ABLE TO COMPENSATE FOR INCREASED INTAKE OF DIETARY CHOLESTEROL BY MARKED INCREASES IN EXCRETION OF ACIDIC AND NEUTRAL STEROLS IN THE FECES. THE MECHANISM CONCERNING THE INHIBITION OF STEROL REABSORPTION AT THE SITE OF THE ENTEROHEPATIC CYCLE IS NOT KNOWN (DANIELSON, 1968).

AS THE FEMALE CHICKEN APPROACHES MATURITY, A CHANGE OCCURS IN ITS LIPID METABOLISM TO MEET THE INCREASED DEMAND FOR LIPID SYNTHESIS NECESSARY FOR EGG PRODUCTION. THE LAYING HEN HAS A GREATER ABILITY TO INCORPORATE ACETATE INTO LIVER LIPIDS THAN THE NON-LAYER, AND THE SYNTHESIS OF CHOLESTEROL APPEARS TO BE PREFERENTIALLY STIMULATED IN LAYING HENS (HUSBANDS AND BROWN, 1965). EGG LAYING ALSO PROVIDES AN EXCRETORY MECHANISM FOR THE ELIMINATION OF DIETARY CHOLESTEROL (WEISS ET AL., 1967B; CHEN ET AL., 1965; ANDREWS ET AL., 1968).

POPJAK AND TIETZ (1953) DEMONSTRATED THAT OVARIAN TISSUE COULD ALSO SYNTHESIZE CHOLESTEROL AND CONTRIBUTE TO EGG YOLK CHOLESTEROL DEPOSITION. HOWEVER, IT HAS BEEN CONFIRMED THAT EGG CHOLESTEROL ARISES MAINLY FROM THE PLASMA AND ULTIMATELY ORIGINATES IN THE LIVER. WHEN LABELLED CHOLESTEROL WAS ADMINISTERED TO HENS, THE SPECIFIC ACTIVITY OF CHOLESTEROL IN EGG YOLK WAS SIMILAR TO THAT OF PLASMA CHOLESTEROL (CONNOR ET AL., 1965; ANDREWS ET AL., 1965 AND 1968). THE HYPOTHESIS THAT THE LIVER IS LARGELY RESPONSIBLE FOR EGG LIPID SYNTHESIS IS SUPPORTED BY THE WORK OF SCHJEIDE (1963)

WHICH INDICATES THAT LIPOPROTEINS FORMED IN THE LIVER THROUGH ESTROGEN INDUCTION ARE TRANSFERRED TO THE DEVELOPING OVA VIA THE CIRCULATION.

IT WAS ALSO REPORTED THAT EGG CHOLESTEROL LEVELS PARALLEL THE BLOOD LEVEL, AND THAT CHANGES IN EGG YOLK WERE PRECEDED BY THE CHANGES IN BLOOD CHOLESTEROL CONCENTRATIONS. THESE CHANGES ARE PARTICULARLY APPARENT WHEN THE LAYING HEN DIET CONTAINS BOTH FAT AND CHOLESTEROL (WOOD ET AL., 1961; HULETT ET AL., 1964). DUE TO THIS EXTRA-ACTIVE EXCRETORY PATHWAY OF CHOLESTEROL, THE RELATIVELY GREAT RESISTANCE OF LAYING HENS TO DIETARY-INDUCED HYPERCHOLESTEROL-EMIA WAS ATTRIBUTED TO THE EXCRETORY MECHANISM OF PLASMA CHOLESTEROL INTO EGG YOLK (STAMLER ET AL., 1954).

COMBS AND HELBACKA (1960) REPORTED THAT EGG YOLK CHOLESTEROL WAS INCREASED UPON FEEDING CORN OIL. WEISS ET AL. (1964) FOUND AN INCREASE IN CHOLESTEROL CONTENT AS THE UNSATURATED FATTY ACIDS IN EGG YOLK LIPIDS INCREASED WHEN HENS WERE FED DIETS CONTAINING EITHER SAFFLOWER OIL OR LINSEED OIL. IT WAS SUGGESTED THAT THE INCREASED CHOLESTEROL DEPOSITION AS A RESPONSE TO DIETARY UN UNSATURATED FATTY ACIDS WAS DUE TO THE INCREASED TRANSPORT OF CHOLESTEROL FROM LIVER TO EGG YOLK (WEISS ET AL., 1967A).

DIETARY LIPID FACTORS INFLUENCING CHOLESTEROL METABOLISM

UNSATURATED FAT.

THE ORIGINAL OBSERVATION BY KINSELL ET AL. (1953) THAT FEEDING SATURATED FATS RESULTED IN AN ELEVATION WHEREAS FEEDING UNSATURATED FATS CAUSED A LOWERING OF SERUM CHOLESTEROL, HAS BEEN

CONFIRMED REPEATEDLY IN MAN (KINSELL ET AL., 1953; AHRENS, 1957), SWINE (ROWELL ET AL., 1965), MONKEYS (JAGANATHAN, 1962), GERBILS (HEGSTED AND GALLAGHER, 1967) AND CHICKENS (HEGSTED ET AL., 1960; FISHER AND LEVEILLE, 1957; DAGHIR ET AL., 1960; WEISS AND FISHER, 1957; LEVEILLE AND FISHER, 1958).

ALTHOUGH AGREEMENT HAS NOT YET BEEN REACHED ABOUT THE EFFECT OF SPECIFIC FATTY ACIDS ON THE LEVEL OF SERUM CHOLESTEROL, IT SEEMS CLEAR THAT HIGHLY UNSATURATED FATS CONTAINING SUBSTANTIAL PROPORTIONS OF POLYUNSATURATED FATTY ACIDS LOWER THE SERUM CHOLESTEROL LEVEL, HIGHLY SATURATED FATS RAISE IT, AND FAT HIGH IN MONO-UNSATURATED FATTY ACIDS FALL IN BETWEEN AND HAVE RELATIVELY LITTLE EFFECT (HEGSTED ET AL., 1965; KEYS ET AL., 1965; KEYS AND PARLIN, 1966). THE MECHANISM BY WHICH CHANGES IN SERUM CHOLESTEROL ARE INDUCED BY DIETARY FAT IS NOT KNOWN. THEORETICALLY, UNSATURATED FATS PRODUCE A LOWERING OF SERUM CHOLESTEROL IN AT LEAST FOUR WAYS: BY INCREASING THE EXCRETION OR DEGRADATION OF CHOLESTEROL; BY INHIBITING CHOLESTEROL SYNTHESIS; BY INTERFERING WITH THE ABSORPTION OF DIETARY CHOLESTEROL; BY AFFECTING A REDISTRIBUTION OF CHOLESTEROL BETWEEN SERUM AND TISSUES EITHER DIRECTLY OR VIA AN INFLUENCE ON METABOLISM. PREVIOUS ATTEMPTS IN HUMAN STUDIES TO ELUCIDATE THE MECHANISM RESPONSIBLE FOR THIS ACTION HAVE LED TO A REASONABLE DEGREE OF AGREEMENT. BY DETERMINING FECAL BILE ACIDS WITH THE TITRATION TECHNIQUE AFTER SILICIC ACID COLUMN CHROMATOGRAPHY, GOLDSMITH ET AL. (1960) FOUND THAT THE EXCRETION OF BILE ACIDS IN HUMANS INCREASED 20-25% CONCOMITTANTLY WITH A DECREASE IN SERUM CHOLESTEROL, WHEN THE DIETARY SUPPLEMENT WAS CHANGED FROM A SATURATED TO AN UNSATURATED

FAT. THE INTERPRETATION WAS THAT POLYUNSATURATED FATTY ACIDS APPEARED TO FAVOUR THE TRANSFORMATION OF CHOLESTEROL INTO BILE ACIDS. THIS HYPOTHESIS WAS SUPPORTED BY THE FINDINGS OF MANY RECENT INVESTIGATORS USING QUANTITATIVE TECHNIQUES SUCH AS ISOTOPE DILUTION AND GAS CHROMATOGRAPHY. THESE INVESTIGATIONS ALSO DEMONSTRATED AN INCREASE IN FECAL OUTPUT OF TOTAL NEUTRAL STEROLS WITH POLYUNSATURATED FATTY ACIDS (GRUNDY AND AHRENS, 1966; MOORE ET AL., 1968; WOOD ET AL., 1966; CONNOR ET AL., 1969).

FURTHERMORE, MOORE ET AL. (1968) FOUND A RECIPROCAL RELATIONSHIP BETWEEN THE CHANGES IN SERUM CHOLESTEROL CONCENTRATION AND FECAL NEUTRAL PLUS ACIDIC STEROID EXCRETION SHOWING THAT THE TOTAL DECREASE IN THE SERUM CHOLESTEROL CONTENT WAS MORE THAN COULD BE ACCOUNTED FOR BY STEROID EXCRETION. THE CHANGES IN EXCRETION WERE RAPID AND WERE MAINTAINED EVEN WHEN NO FURTHER REDUCTION IN SERUM CHOLESTEROL OCCURRED, SUGGESTING A SECONDARY INCREASED FLOW OF CHOLESTEROL INTO THE SERUM AND THENCE OUT INTO THE FECES.

ALTHOUGH THE EXACT MECHANISM OF THE ACTION OF POLYUNSATURATED FATTY ACIDS IN CHOLESTEROL DEGRADATION IS UNKNOWN, SOME INDIRECT EVIDENCE HAS APPEARED IN THE LITERATURE. IN ESSENTIAL FATTY ACID (EFA)-DEFICIENT RATS, CHOLESTEROL IS FOUND TO BE TAKEN UP PROGRESSIVELY BY ELEMENTS OF THE RETICULOENDOTHELIAL SYSTEM AND THIS PROCESS HAS BEEN FOUND REVERSIBLE UPON ADDITION OF LINOLEIC ACID TO THE FAT-FREE DIET (BERNICK AND ALFIN-SLATER, 1963). SWELL ET AL. (1953) SHOWED THAT A DIET HIGH IN LINOLEIC ACID PRODUCED A MARKED INCREASE WITH PROPORTION OF LINOLEIC ACID IN THE SERUM CHOLESTEROL ESTERS, CONCOMITANT WITH A FALL IN PLASMA

CHOLESTEROL LEVEL. ON THE ASSUMPTION THAT THE REDUCTION IN CIRCULATING CHOLESTEROL INVOLVES WITHDRAWAL OF CHOLESTEROL ESTERS FROM THE RETICULOENDOTHELIAL SYSTEM AND THAT THERE IS PREFERENTIAL CATABOLISM OF THE UNSATURATED FATTY ESTERS OF CHOLESTEROL, BOYDE (1962) ADVANCED THE HYPOTHESIS THAT CHOLESTEROL LINOLEATE IS MORE RAPIDLY METABOLIZED TO BILE ACID THAN ARE OTHER ESTERS. ACCORDING TO THIS HYPOTHESIS, 7A-HYDROXYLATION OF THE STEROID NUCLEUS MIGHT BE FACILITATED BY THE INTERMEDIATE FORMATION OF A HYDROPEROXIDE OF THE FATTY ACID.

HOWEVER, IN CONTRADICTION TO THE REPORTS CITED ABOVE, A NUMBER OF OTHER RECENT STUDIES HAVE FAILED TO DEMONSTRATE AN INCREASE IN STEROID EXCRETION OR TURNOVER WITH POLYUNSATURATED FATTY ACID DIETS (AVIGAN AND STEIBERG, 1965; SPRITZ ET AL., 1965; LINDSTEDT ET AL., 1965; HELLSTROM AND LINDSTEDT, 1966). WHEN HIGHLY UNSATURATED FATS WERE FED TO RATS, GERSON ET AL. (1961) REPORTED THAT A DECREASE IN THE CHOLESTEROL CONCENTRATION OF BLOOD PLASMA WAS ACCOMPANIED BY AN INCREASE IN THE CHOLESTEROL CONTENT OF HEART, AORTA, LIVER, INTESTINE AND MUSCLE TISSUES. ON THE BASIS OF THESE OBSERVATIONS, TOGETHER WITH THE FAILURE TO OBSERVE CHANGES IN CHOLESTEROL OR BILE ACID EXCRETION WHEN PLASMA CHOLESTEROL WAS LOWERED, IT WAS POSTULATED THAT THE ACTION OF UNSATURATED FAT IN REDUCING SERUM CHOLESTEROL MIGHT REFLECT A SHIFT IN PART OF THE SERUM CHOLESTEROL POOL TO OTHER TISSUE COMPARTMENTS (AVIGAN AND STEINBERG, 1965; HELLSTROM AND LINDSTEDT, 1966).

Plant Sterols.

Dietary plant sterols, considered nonabsorbable 40 years ago, now have received a great deal of attention because of their ability to decrease serum cholesterol levels when administered orally (Peterson, 1951; Beveridge et al., 1958), or by injection (Gerson et al., 1964; Konlande and Fisher, 1969) to a number of animal species. Interest in the metabolism of plant sterols in animals actually stemmed from the observation by Peterson (1951) that the addition of soysterols (1%) to a diet rich in cholesterol prevented the increase in plasma cholesterol which usually occurs after cholesterol feeding in chicks. This observation has been confirmed in rabbits (Pollak, 1953a) and in human subjects (Pollak, 1953b).

The mechanism of its anti-cholesterolemic action was postulated by Davis (1955) to involve the formation of a non-absorbable complex of plant sterols and cholesterol at the intestinal absorption site. Other workers (Hernandez et al., 1953) thought that plant sterols might be inhibiting cholesterol esterification and thus decrease its rate of uptake by the intestine. Glove and Green (1957) proposed an interaction between the sterols and a lipoprotein of the intestinal surface, with formation of a complex with lipoproteins or mucoprotein which is essential for cholesterol absorption. They suggested that the plant sterols might be participating in this complex formation to a less degree than cholesterol, but that once the plant sterols became attached to this protein carrier, cholesterol absorption was retarded.

Recent studies, however, do not indicate competition between

CHOLESTEROL AND PLANT STEROLS FOR THE ABSORPTION SITES (BORGSTROM, 1967, 1968; SYLVEIN AND BORGSTROM, 1969). USING MICELLAR SOLUTIONS OF THE INDIVIDUAL STEROLS OR MIXTURES, RESULTS SHOWED THAT CHOLESTEROL AND PLANT STEROLS WERE ABSORBED INDEPENDENTLY BY BOTH INTESTINAL MUCOSA IN VIVO AND INTESTINAL SLICES IN VITRO. GERSON ET AL. (1964) ALSO OBSERVED THAT BETA-SITOSTEROL INJECTED INTRAPERITONEALLY IN RATS PRODUCED A 78 TO 84% REDUCTION OF CHOLESTEROL IN AORTA AND ADRENALS, AND 7 TO 12% IN LIVER AND PLASMA. THESE POST-ABSORPTIVE ACTIONS OF PLANT STEROLS WERE FURTHER CONFIRMED WITH CHICK STUDIES (KONLANDE AND FISHER, 1969; ZILLETTI, 1970).

ON EXAMINING VARIOUS PLANT STEROLS FOR THEIR EFFICACY IN ELICITING THE BLOOD CHOLESTEROL-LOWERING EFFECT, IT WAS DEMONSTRATED THAT THE ACTIVITY OF THE PLANT STEROL MIXTURES WAS DIRECTLY PROPORTIONAL TO THEIR CAMPESTEROL CONTENT (KONLANDE AND FISHER, 1969). SINCE CAMPESTEROL IS ABSORBED MORE READILY THAN BETA-SITOSTEROL (KUKSIS AND HUANG, 1962), IT WAS CONCLUDED BY KONLANDE AND FISHER (1969) THAT THE ANTI-HYPERCHOLESTEROLEMIC ACTION OF PLANT STEROLS WAS PROBABLY DUE TO SOME EXTRA-ABSORPTIVE EFFECT.

THERE IS SOME EXPERIMENTAL EVIDENCE INDICATING THAT PLANT STEROLS INCREASE THE TURNOVER RATE OF CHOLESTEROL IN LIVER AND BLOOD. SPECIFIC ACTIVITY OF LIVER CHOLESTEROL INCREASED 140% AFTER INJECTION OF ACETATE-1-^{14}C IN ANIMALS THAT HAD BEEN PRETREATED WITH INJECTIONS OF PLANT STEROLS (GERSON ET AL., 1964). LINDSEY ET AL. (1969) REPORTED THAT FEEDING BETA-SITOSTEROL TO COCKERELS INCREASED TOTAL BILE ACID EXCRETION IN FECES. GRUNDY ET AL. (1969) HAVE

SHOWED THAT THE REABSORPTION OF ENDOGENOUS NEUTRAL AND ACIDIC STEROLS DERIVED FROM LIVER AND INTESTINE WAS REDUCED, AND FECAL EXCRETION INCREASED WHEN PLANT STEROLS WERE FED TO HUMAN SUBJECTS. HOWEVER, LITTLE INFORMATION IS AVAILABLE IN THE LITERATURE ON PLANT STEROL ABSORPTION AND METABOLISM IN LAYING HENS. WOOD ET AL. (1961) COULD NOT DETECT BETA-SITOSTEROL BY PAPER CHROMATOGRAPHIC ANALYSIS IN THE SERUM FROM HENS FED DIETS CONTAINING CORN OIL AT A LEVEL OF 10%. WEISS ET AL. (1967A) REPORTED THAT ADDING 1% OF BETA-SITOSTEROL TO A LOW FAT BASAL DIET FOR LAYING HENS HAD NO EFFECT OTHER THAN TO RETARD THE INCREASE IN PLASMA AND EGG YOLK CHOLESTEROL LEVELS CAUSED BY DIETARY CHOLESTEROL. AT THE SAME TIME, THE ADDITION OF 1% BETA-SITOSTEROL TO A DIET CONTAINING 29% OF SAFFLOWER OIL LOWERED THE CHOLESTEROL LEVEL IN BLOOD AND INCREASED IT IN THE EGG YOLK. THESE INVESTIGATORS DID NOT DETECT THE PLANT STEROL IN EGGS BUT REFERRED TO A PERSONAL COMMUNICATION FROM T. A. MIETTINEN[1] WHO HAD OBSERVED 1.2% PLANT STEROLS IN COMMERCIAL EGGS.

CLARENBERG ET AL. (1971) ALSO SHOWED THAT PLANT STEROL WAS ABSORBED 60% IN LAYING HENS AND 85% IN NON-LAYERS WHEN VARIOUS DIETARY LEVELS OF ^{3}H-SITOSTEROL WERE FED. A SIGNIFICANT REDUCTION WAS ALSO OBSERVED IN CHOLESTEROL CONCENTRATION CONCOMITANT WITH SIGNIFICANT DEPOSITION OF PLANT STEROL IN EGG YOLK.

IN EARLY STUDIES OF PLANT STEROL ABSORPTION, SCHOENHEIMER

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[1] INSTITUTE OF MEDICAL CHEMISTRY, UNIVERSITY OF HELSINKI, HELSINKI, FINLAND.

(1931) USED THE INCREASE IN LIVER STEROL CONCENTRATION AS A CRITERION OF ABSORPTION, AND CONCLUDED THAT ABSORPTION OF PLANT STEROL WAS NEGLIGIBLE IN MAN. HOWEVER, THE REPORTED RATES OF PLANT STEROL ABSORPTION IN THE RAT HAVE BEEN MUCH HIGHER (SWELL ET AL., 1956). THE FIRST DEMONSTRATION OF DIFFERENTIAL ABSORPTION RATES OF PLANT STEROLS IN ANIMALS WAS BY KUKSIS AND HUANG (1962). THEY IDENTIFIED THE STEROLS APPEARING IN THE THORACIC DUCT OF DOGS AFTER AN ORAL DOSE OF A CONCENTRATED MIXTURE OF CAMPESTEROL AND BETA-SITOSTEROL. THERE WAS MORE CAMPESTEROL THAN BETA-SITOSTEROL OBSERVED IN LYMPH. THIS WAS LATER CONFIRMED BY SUBBIAH ET AL. (1970) IN THE PIGEON AND KOLANDE AND FISHER (1969) IN CHICKS.

THE DEMONSTRATION THAT PLANT STEROLS ARE ABSORBED BY ANIMALS STIMULATED INTEREST IN THE FATE OF THESE STEROLS THAT HAD CROSSED THE INTESTINAL BARRIER. MOST OF THE INJECTED OR ABSORBED BETA-SITOSTEROL WAS EXCRETED IN THE BILE AS NEUTRAL STEROLS (49%) AND BILE ACIDS (60%) IN RAT (SUBBIAH ET AL., 1969). IT WAS ALSO REPORTED THAT THE TURNOVER RATE FOR THE LABELLED PLANT STEROL IN PLASMA WAS MUCH FASTER THAN FOR CHOLESTEROL AND THAT PLANT STEROLS DO NOT ACCUMULATE IN THE BODY BECAUSE OF RAPID DEGRADATION AND EXCRETION (GOULD, 1954; GOULD ET AL., 1955).

CHOLESTEROL.

IT IS GENERALLY ACCEPTED THAT CERTAIN HOMEOSTATIC MECHANISMS COMPENSTATE FOR THE LOAD OF DIETARY CHOLESTEROL AT VARIOUS MAGNITUDES DEPENDING ON THE ANIMAL SPECIES.

THE PLASMA CHOLESTEROL CONCENTRATION IS ALSO INFLUENCED BY

DIETARY CHOLESTEROL (CONNOR ET AL., 1961, 1964; ERIKSON ET AL., 1964). THE FEEDING OF CHOLESTEROL TO ANIMALS OF CERTAIN SPECIES SUCH AS RABBITS (WELLS AND ANDERSON, 1959), FOWL (KATZ AND PICK, 1961), AND SWINE (DOWNIE ET AL., 1963) PRODUCES A MARKED HYPERCHOLESTEROLEMIA, WHEREAS IN OTHER SPECIES, RAT, DOG, AND HUMAN, THE RESPONSE OF PLASMA CHOLESTEROL IS MUCH LESS.

GRUNDY ET AL. (1969) AND QUINTAO ET AL. (1971A&B) DEMONSTRATED THAT TOTAL DAILY SYNTHESIS OF CHOLESTEROL IN HUMAN LIVER WAS SIGNIFICANTLY SUPPRESSED WHEN CHOLESTEROL WAS INCORPORATED IN THE DIET. THIS SUGGESTS A FEEDBACK CONTROL MECHANISM IN CHOLESTEROL SYNTHESIS TO COMPENSATE FOR THE EXOGENOUS CHOLESTEROL INPUT. THE MECHANISM WAS ALSO EXTENSIVELY STUDIED IN RATS (TOMKINS ET AL., 1953; SIPERSTEIN AND GUEST, 1960). THE PRIMARY SITE OF SUPPRESSION OF CHOLESTEROGENESIS BY EXOGENEOUS CHOLESTEROL IS THE REDUCTION OF BETA-HYDROXYL-BETA-METHYLGLUTARYL-COA TO MEVALONIC ACID (SIPERSTEIN, 1960). HOWEVER, THE NEGATIVE FEEDBACK REGULATION IS IN THE LIVER, AND THE EXTRAHEPATIC TISSUES LACK SUCH A FEEDBACK SYSTEM AND CONSEQUENTLY SYNTHESIZE CHOLESTEROL EVEN WHEN THE ANIMAL IS ON A HIGH CHOLESTEROL DIET AS SHOWN BY TAYLOR AND GOULD (1950) AND GOULD (1953).

STUDIES ON THE FECAL BILE ACIDS AND NEUTRAL STEROL OUTPUT AT DIFFERENT LEVELS OF DIETARY CHOLESTEROL INTAKE ALSO INDICATE THAT ANIMALS ARE ABLE TO COMPENSATE FOR THE INCREASED EXOGENOUS INPUT OF CHOLESTEROL BY A REDUCTION IN THE RESORPTION RATE OF ACIDIC AND NEUTRAL STEROLS AT THE SITE OF ENTEROHEPATIC CYCLE AND BY EXCRETION OF INCREASED AMOUNTS OF TOTAL STEROLS INTO FECES

(Wilson, 1964; Wilson and Lindsey, 1965; Grundy and Ahrens, 1969; Grundy et al., 1969).

Dietary Fatty Acids and Lipid Metabolism in Liver

The addition of fat to the diet of most animals causes the body fat or egg yolk to take on a fatty acid composition similar to that found in the diet (Hegsted et al., 1960; Chen et al., 1965). This effect has been attributed to the direct deposition of fatty acids derived from the dietary source (Cruikshank, 1934). However, the fatty acid composition of body tissue never actually duplicates that of dietary fat (Di Giorgio et al., 1962).

Many factors may directly or indirectly influence the fatty acid composition of body fat after feeding dietary fat. The amount and rate of oxidation of ingested fatty acids to CO_2 and the amount of fatty acids synthesis either de novo or by interconversion has an influence on the body fatty acid composition. Short chain fatty acids are oxidized to CO_2 at a much faster rate than are longer chain fatty acids or converted to longer chain fatty acid by two carbon elongation (Kirschner and Harris, 1961).

Another factor regulating the quantity and type of fatty acids deposited is the influence of the nature of the dietary fat on fatty acid synthesis. Feedback inhibition of fatty acid synthesis in liver (Reiser et al., 1963; Hill et al., 1960), and adipose tissue (Di Giorgio et al., 1962; Bottino et al., 1965) is greater during ingestion of unsaturated than saturated fat. Polyunsaturated fatty acids derived from dietary fat were found to be potent in the suppression of the activity of liver enzymes which

PARTICIPATE IN THE SYNTHESIS OF SATURATED AND MONO-UNSATURATED FATTY ACIDS (ALLMAN AND GIBSON, 1965; MUTO AND GIBSON, 1970; SABINE ET AL., 1969; BORTZ AND LYNEN, 1963). WHEN UNSATURATED FAT, SUCH AS SAFFLOWER OIL, WAS FED, THE TOTAL LIPID SYNTHESIS WAS DIMINISHED TO A GREATER EXTENT, THAN WHEN SATURATED FAT, SUCH AS HYDROGENATED OIL, WAS FED (CHUNG ET AL., 1970).

A NUMBER OF REPORTS INDICATE THAT TRANSPORT OF LIVER LIPIDS TO OTHER TISSUE COMPARTMENTS OR TO EGG YOLK MIGHT BE AFFECTED BY THE NATURE OF THE DIETARY FATTY ACIDS. MORTON AND HORNER (1961) REPORTED THAT ESSENTIAL FATTY-ACID-DEFICIENCY LED TO AN ACCUMULATION OF FAT IN THE LIVER. THIS FAT ACCUMULATION IS ASSOCIATED WITH AN INCREASE IN TRIGLYCERIDES AND SATURATED CHOLESTEROL ESTERS, GREATER CHANGES IN THE FATTY ACID PATTERN OF LIVER LIPIDS (INCREASING OLEIC ACID AND DECREASING LINOLEIC ACID), AND LOW LEVELS OF PLASMA TRIGLYCERIDES (SINCLAIR AND COLLINS, 1968; MEAD AND FILLERUP, 1954). SIMILAR OBSERVATIONS HAVE BEEN MADE IN ANIMALS WITH EXPERIMENTALLY INDUCED FATTY LIVERS, AND IT HAS BEEN SUGGESTED THAT AN IMPORTANT FACTOR IN THE DEVELOPMENT OF FATTY LIVER UNDER THESE CONDITIONS IS AN IMPAIRMENT IN THE SECRETION OF TRIGLYCERIDES (LOMBARDI, 1965; MADSEN, 1969). IMPAIRMENT OF THE TRANSPORT OF TRIGLYCERIDES FROM LIVER OF ESSENTIAL FATTY ACID (EFA)-DEFICIENT OR SATURATED FAT-FED ANIMALS MAY BE ATTRIBUTED TO A NUMBER OF FACTORS. EFA MAY BE REQUIRED FOR THE FORMATION OF THE PHYSICOCHEMICAL STRUCTURES OF LIPOPROTEIN FOR TRANSPORT OF LIVER LIPIDS INTO CIRCULATION (ALFIN-SLATER AND AFTERGOOD, 1968; RUDERMAN ET AL., 1968).

PEIFER AND HOLMAN (1955) NOTED THAT FEEDING CHOLESTEROL TO RATS IN THE STATE OF EFA-DEFICIENCY HASTENED THE APPEARANCE AND SEVERITY OF FATTY LIVER SYNDROME. THEY POSTULATED THAT THE ESSENTIAL FATTY ACID WOULD BE PREFERENTIALLY ESTERIFIED WITH CHOLESTEROL AND RELEASED RAPIDLY FROM LIVER, CONSEQUENTLY FOLLOWED BY RAPID DEPLETION OF ESSENTIAL FATTY ACID STORAGE IN LIVER. SINCLAIR AND COLLINS (1968) REPORTED THAT THE ANIMAL STARTS TO DEVELOP THE FATTY LIVER SNYDROME BY THE TIME THE ESSENTIAL FATTY ACIDS ARE DEPLETED IN THE LIVER.

SEVERAL WORKERS (RIDOUT ET AL., 1952; KLEIN, 1958; MORIN ET AL., 1962; DILLER ET AL., 1961) HAVE REPORTED THAT DIETARY CHOLESTEROL ACCELERATES FAT ACCUMULATION IN LIVER, AND THE COMPOSITION OF HEPATIC LIPIDS WERE FOUND TO DIFFER FROM THAT CAUSED BY THE ORDINARY DIETARY FATTY LIVER SYNDROME (LUCAS AND RIDOUT, 1967). THE LATTER CAUSES ACCUMULATION OF MAINLY TRIGLYCERIDES WITH ONLY A SMALL INCREASE IN TOTAL CHOLESTEROL, WHEREAS THE FATTY LIVER CAUSED BY FEEDING CHOLESTEROL DISPLAYS MARKED INCREASES IN BOTH TRIGLYCERIDES AND CHOLESTEROL ESTERS. FURTHERMORE, CHOLESTEROL FEEDING STIMULATES LIPOGENESIS IN THE HEPATIC TISSUES OF GROWING OR LAYING CHICKENS AND CONSEQUENTLY GREATER CHANGES IN FATTY ACID COMPOSITIONS OF TISSUE LIPIDS (CHUNG ET AL., 1970; WEISS ET AL., 1967B). CHUNG ET AL. (1967, 1966) OBSERVED THAT CHOLESTEROL TREATMENT GREATLY INCREASED OLEIC ACID LEVEL IN THE LIVER AND PLASMA LIPIDS.

FATTY LIVER MAY BE THE RESULT OF AN IMBALANCE IN THE LIPID CYCLE, HOWEVER, SEVERAL FACTORS EITHER TOGETHER OR SEPARATELY

CONTRIBUTE TO ITS DEVELOPMENT. THESE INCLUDE AN ELEVATED FLUX OF FREE FATTY ACIDS FROM PLASMA TO LIVER, A DECREASED OXIDATION IN THE LIVER OR DECREASE IN SECRETION OF LIPOPROTEINS FROM THE LIVER AND A RISE IN FATTY ACID SYNTHESIS (LOMBARDI, 1966; STEINBERG, 1963). EVEN IF EXCESS LIPOGENESIS OCCURRED IN THE LIVER DUE TO EITHER EFA-DEFICIENCY OR CHOLESTEROL FEEDING, THERE WOULD BE NO EXCESSIVE ACCUMULATION OF FAT IF A SUFFICIENT AMOUNT OF THE PROPER LIPOPROTEINS WERE FORMED (RUDERMAN ET AL., 1968; WINDMUELLER AND SPAETH, 1967). HOWEVER, IF THE LIVER CAN ONLY PRODUCE A BASAL AMOUNT OF LIPOPROTEINS (BUT NOT ENOUGH IN RESPONSE TO THE HIGHER RATE OF LIPOGENESIS), OR IMPROPER FORMS OF LIPOPROTEINS ARE FORMED DUE TO THE LACK OF SPECIFIC FATTY ACIDS, LIPIDS WILL ACCUMULATE ACCORDINGLY (RUDERMAN ET AL., 1968; FUKAZAWA ET AL., 1970). INCREASED LIVER SIZE AND FAT ACCUMULATION HAVE BEEN REPORTED WHEN CHICKS WERE FED FAT-FREE OR DIETS CONTAINING SATURATED FATS (EDWARDS ET AL., 1962; EDWARDS, 1967; HOPKINS AND NESHEIM, 1967) AND WHEN LAYING HENS WERE FED A DIET HIGH IN SATURATED FAT (SUNDE, 1966; BRAGG ET AL., 1973) OR LOW FAT CARBOHYDRATE DIETS (BARTON ET AL., 1967; DUKE, 1968). MENGE (1967) AND BRAGG ET AL. (1973) SHOWED THAT LINOLEIC ACID IN THE DIET PREVENTED THE ACCUMULATION OF FAT IN THE LIVER OF LAYING HENS, THUS PREVENTING THE OCCURRENCE OF THE FATTY LIVER SYNDROME.

EXPERIMENTAL

MATERIALS

BIRDS: ONE HUNDRED AND SIXTY SINGLE COMB WHITE LEGHORN LAYING HENS WERE PLACED IN CAGES (2 HENS/CAGE) EQUIPPED WITH AUTOMATIC WATER SYSTEM AND MAINTAINED ON A COMMERCIAL LAYER DIET TO THIRTY WEEKS OF AGE. DURING A TWO-WEEK PRE-EXPERIMENTAL PERIOD, DAILY EGG PRODUCTION WAS RECORDED.

EXPERIMENTAL DIETS: TWO BASAL DIETS CONTAINING EITHER 8% HYDROGENATED COCONUT OIL (HCO) OR 8% SAFFLOWER OIL (SFO) WERE PREPARED (TABLE 1). ADDITIONAL DIETS WERE PREPARED BY SUPPLEMENTING EACH BASAL DIET WITH 1% OF CHOLESTEROL (CH), 2% OF SOYSTEROLS (ST) OR A COMBINATION OF CHOLESTEROL (1%) AND SOYSTEROLS (2%), (TABLE 2). ALL THE STEROL SUPPLEMENTS WERE INCORPORATED INTO DIETS AT THE EXPENSES OF STARCH BY WEIGHT.

DUE TO THE HIGH MELTING POINT OF STEROL SUPPLEMENTS, THEY WERE LIQUEFIED AND THOROUGHLY PREMIXED INTO THE HEATED OILS BEFORE INCORPORATING INTO DIETS. THE FATTY ACID COMPOSITIONS OF THE TWO BASAL DIETS (HCO AND SFO) WERE DETERMINED FROM THEIR LIPID EXTRACTS (TABLE 3). THE PURITY AND COMPOSITION OF SOYSTEROLS USED ARE PRESENTED IN TABLE 4.

TABLE 1.--COMPOSITION OF EXPERIMENTAL BASAL RATIONS[1].

INGREDIENTS	HCO RATION	SFO RATION
	PERCENT	
GROUND WHEAT (13% PROTEIN)	70.0	70.0
ISOLATED SOY PROTEIN (90% PROTEIN)	8.0	8.0
DEHYD. ALFALFA MEAL (17% PROTEIN)	1.0	1.0
GROUND LIMESTONE	5.0	5.0
DEF. ROCK PHOSPHATE	2.5	2.5
MINERAL PREMIX[2]	0.5	0.5
VITAMIN PREMIX[3]	1.0	1.0
STARCH	4.0	4.0
HYDROGENATED COCONUT OIL	8.0	---
SAFFLOWER OIL	---	8.0

[1] CALCULATED ANALYSIS OF RATION: CRUDE PROTEIN, 16.5%; METABOLIZABLE ENERGY, 3,060 KCAL/KG FEED; METHIONINE PLUS CYSTINE, 0.542%; LYSINE, 0.925%; CA, 2.77%; P, 0.542%.

[2] MINERAL PREMIX SUPPLIES THE FOLLOWING PER KILOGRAM OF RATION: MN, 31.8 MG; CU, 7.1 MG; ZN, 44.8 MG; NACL, 4.34 G.

[3] VITAMIN PREMIX SUPPLIES THE FOLLOWING PER KILOGRAM OF RATION: VITAMIN A, 9750 IU; VITAMIN D_3, 1200 ICU; VITAMIN E, 12.4 IU; VITAMIN B_{12}, 9.98 UG; RIBOFLAVIN, 2.9 MG; PANTOTHENIC ACID, 4.0 MG; FOLIC ACID, 0.87 MG; NIACIN, 6.0 MG; CHOLINE CHLORIDE, 50 MG; VITAMIN K, 2.0 MG; BUTYLATED HYDROXYTOLUENE, 1.0 MG; OLEOMYCIN, 10 MG.

TABLE 2.--OUTLINE OF DIETARY TREATMENTS.

	DIETS	SUPPLEMENTS
1.	HCO BASAL	8% OF HYDROGENATED COCONUT OIL
2.	HCO+CH	8% OF HYDROGENATED COCONUT OIL PLUS 1% CHOLESTEROL
3.	HCO+ST	8% OF HYDROGENATED COCONUT OIL PLUS 2% OF SOYSTEROLS
4.	HCO+CH+ST	8% OF HYDROGENATED COCONUT OIL PLUS 1% OF CHOLESTEROL AND 2% OF SOYSTEROLS
5.	SFO BASAL	8% OF SAFFLOWER OIL
6.	SFO+CH	8% OF SAFFLOWER OIL PLUS 1% OF CHOLESTEROL
7.	SFO+ST	8% OF SAFFLOWER OIL PLUS 2% OF SOYSTEROLS
8.	SFO+CH+ST	8% OF SAFFLOWER OIL PLUS 1% OF CHOLESTEROL AND 2% SOYSTEROLS

TABLE 3.--FATTY ACID COMPOSITION OF THE DIETARY LIPIDS EXTRACTED FROM THE BASAL RATIONS.[1]

FATTY ACID[2]	DIETARY OIL[3]	
	HCO	SFO
$C_{8:0}$	11.13	---
$C_{10:0}$	4.24	---
$C_{12:0}$	42.40	---
$C_{14:0}$	12.57	---
$C_{16:0}$	9.77	9.47
$C_{18:0}$	8.56	5.79
$C_{18:1}$	6.78	16.23
$C_{18:2}$	4.55	68.51

[1] PERCENT OF TOTAL METHYL ESTERS OF FATTY ACIDS.

[2] CARBON CHAIN LENGTH : NUMBER OF DOUBLE BONDS.

[3] THESE TWO REFINED OILS WERE PURCHASED FROM NUTRITIONAL BIOCHEMICAL CORP., CLEVELAND, OHIO (HCO) AND GARDENLAND PACKERS LTD., ALTONA, MANITOBA (SFO). CHROMOGENIC STEROL CONTENTS FOR HCO AND SFO, 0.35% AND 0.65% RESPECTIVELY.

TABLE 4.--Composition of soysterol mixture used in experiment.[1]

	Percent	
	Manufactuer's Suggested Composition	GLC Analysed Composition
Purity	65	85.3
Beta-sitosterol	32	41.6
Campesterol	17	23.8
Stigmasterol	16	19.9

[1] The soysterol mixture used in these studies were kindly supplied by Dr. N. Embree, Distillation Products Industries, Rochester, N. Y.

Feeding and Sampling Procedures

Trial 1

This trial was designed to test the laying hen's sensitivity to the dietary lipid factors in changes of serum and egg yolk sterol concentrations, and the mode of changes in serum sterol levels in relation with those in egg yolk sterol levels.

A group of thirty-two laying birds showing a similar rate of egg production, body weight and serum and egg yolk sterol levels (Table 5) were divided at random into four groups consisting of eight hens each (one bird/cage). Four soysterol-unsupplemented experimental diets (**HCO, HCO+CH, SFO, SFO+CH**) were assigned at random to each hen group and fed for three weeks. At the end of the third week-feeding period, hens fed the soysterol-free diets were shifted to soysterol supplemented diets (**HCO+ST, HCO+CH+ST, SFO+ST** and **SFO+CH+ST**), and were fed for another three weeks. Feed and water were provided ad libitum for the six week experimental period.

Eggs were collected for three consecutive days and yolks from each hen were pooled and stored at $-20^{\circ}C$ in a sealed plastic bag for the sterol determination. At the end of each week, blood was drawn from the brachial vein of bird into 10 ml-test tubes and serum was harvested by centrifugation at slow speed (1000 rpm) after clotting. Total sterol concentrations of egg yolk and serum were determined as total chromogenic sterols described in the analytical procedures.

TABLE 5.--CONCENTRATIONS OF STEROL IN SERUM AND EGG YOLK AND EGG PRODUCTION OBTAINED FROM THE SELECTED LAYING HEN GROUPS BEFORE DIETARY TREATMENT (TRIAL 1).

TREATMENT	STEROL LEVELS		AVERAGE EGG PRODUCTION (%)
	SERUM (MG %)	EGG YOLK (MG/G)	
1	74.1+18.5	9.91+0.80	71.8
2	80.2+20.1	10.17+0.90	71.8
3	67.6+18.7	10.81+1.60	71.8
4	74.3+29.2	10.84+1.43	68.9

TRIAL 2

THIS TRIAL WAS DESIGNED TO RE-EXAMINE THE EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE SERUM AND EGG YOLK STEROL LEVELS IN LAYING HENS, AND TO STUDY THE ROLES OF DIETARY LIPID FACTORS ON THE STEROL AND FATTY ACID METABOLISM DURING A NINE-WEEK FEEDING PERIOD.

THIS TRIAL WAS CARRIED OUT WITH THE REMAINING ONE HUNDRED AND TWENTY-EIGHT LAYING BIRDS WHICH WERE DIVIDED AT RANDOM INTO EIGHT EXPERIMENTAL GROUPS OF SIXTEEN BIRDS (EIGHT CAGES OF TWO BIRDS EACH). THE FOLLOWING EIGHT EXPERIMENTAL DIETS WERE ASSIGNED TO BIRD GROUPS AT RANDOM; **HCO, HCO+CH, HCO+ST, HCO+CH+ST, SFO, SFO+CH, SFO+ST** AND **SFO+CH+ST** (TABLE 2). FEED AND WATER WERE SUPPLIED AD LIBITUM THROUGHOUT THE NINE-WEEKFEEDING PERIOD.

DAILY RECORDS WERE KEPT OF EGG PRODUCTION, AND FEED CONSUMPTION WAS DETERMINED AT TWO WEEK INTERVALS. BLOOD SAMPLES WERE TAKEN FROM THE WING VEIN OF INDIVIDUAL BIRDS AT THE END OF THE EIGHTH WEEK. THE SERUM WAS HARVESTED ACCORDING TO THE METHOD DESCRIBED IN TRIAL 1. POOLED SERUM SAMPLES (EQUAL VOLUME FROM EACH BIRD) WERE COLLECTED FROM EACH EXPERIMENTAL UNIT (2 HENS/CAGE). TOTAL EGGS PER EXPERIMENTAL UNIT WERE ALSO COLLECTED FOR FOUR CONSECUTIVE DAYS DURING THE EIGHTH WEEK OF FEEDING. THE EGGS WERE BROKEN AND POOLED YOLK SAMPLES WERE SAVED FOR FURTHER ANALYSIS. THE CHROMOGENIC STEROL DETERMINATION WAS CARRIED OUT ON BOTH SERUM AND EGG YOLK SAMPLES.

AT THE BEGINNING OF THE NINTH WEEK OF THE FEEDING PERIOD, FOUR BIRDS PER TREATMENT GROUP WERE SELECTED ON THE BASIS OF

WEEKLY EGG PRODUCTION RECORDS (55 TO 65%) AND FINAL BODY WEIGHT (1.7 TO 2.2 KG) AND TRANSFERRED TO INDIVIDUAL CAGES. ONE CAGE WAS LEFT EMPTY BETWEEN TREATMENT GROUPS TO PREVENT CROSS-CONTAMINATION OF FEED AND FECES.

THE SAME EIGHT EXPERIMENTAL DIETS REMIXED WITH MARKER (0.3% CHROMIC OXIDE) WERE FED FOR SIX DAYS. FECES WERE COLLECTED FROM INDIVIDUAL BIRDS FOR THREE CONSECUTIVE DAYS AFTER A THREE-DAY ADJUSTMENT PERIOD ON THE CHROMIC OXIDE FEED. FECES WERE COLLECTED ON A SHEET OF ALUMINUM FOIL SURROUNDED BY A LARGE SIZE (40 x 40 CM) OF POLYETHYLENE BAG THAT WAS HOOKED UNDER THE CAGE. THE FECES WERE REMOVED DAILY AND STORED AT $-20^{\circ}C$. THE THREE-DAY FECAL COLLECTION WAS LYOPHYLIZED TO CONSTANT WEIGHT, HOMOGENIZED IN A WARING BLENDOR, AND STORED AT $-20^{\circ}C$ IN A SEALED POLYETHYLENE BAG FILLED WITH NITROGEN. AT THE END OF THE FECAL COLLECTION PERIOD, BIRDS WERE INDIVIDUALLY WEIGHED AND KILLED. LIVERS AND HEARTS WERE CAREFULLY REMOVED, WEIGHED AND PROCESSED IN A SIMILAR MANNER AS THE FECES.

ANALYTICAL PROCEDURES

SERUM AND EGG YOLK STEROL CONCENTRATIONS: TOTAL CHROMOGENIC STEROLS REACTING WITH FERRIC CHLORIDE REAGENT WERE DETERMINED BY A MODIFICATION OF ZLATKIS METHOD (ZLATKIS *ET AL.*, 1953).

SERUM (0.2 ML) OR FRESH YOLK (0.5 G) WAS SAPONIFIED WITH 10 ML OF A 10 **N NaOH** ETHANOL SOLUTION, AND THE UNSAPONIFIABLE STEROLS WERE EXTRACTED FROM THE SAPONIFICATION MIXTURE WITH 10 ML OF PETROLEUM ETHER (B.P. 65°-$85^{\circ}C$). THE SAPONIFICATION AND

EXTRACTION PROCEDURES ARE SIMILAR TO THOSE DESCRIBED BY ABELL ET AL. (1952) EXCEPT THAT THE SAPONIFICATION AT 50°C WAS CARRIED OUT FOR 120 MINUTES INSTEAD OF 55 MINUTES.

ALIQUOTS OF PETROLEUM ETHER EXTRACTS PLACED IN 150 x 20 MM TEST TUBES WERE EVAPORATED TO DRYNESS UNDER A STREAM OF NITROGEN. SIX-TENTHS (0.6) ML OF FERRIC CHLORIDE REAGENT ($FeCl_3$-$6H_2O$, 0.1% w/v IN ETHANOL) WAS ADDED TO EACH SAMPLE. AFTER COOLING IN AN ICE BATH, 0.4 ML OF H_2SO_4 (SP. GR. 1.84) WAS SLOWLY ADDED TO FORM A LOWER SULFURIC ACID LAYER. THE SAMPLES WERE IMMEDIATELY AGITATED AND ALLOWED TO REACT FOR 15 MINUTES IN THE ICE BATH AND FURTHER 90 MINUTES AT ROOM TEMPERATURE BEFORE THE OPTICAL DENSITY WAS READ AT 560 MU USING UNICAM MODEL SPECTROMETER. THE AMOUNTS OF STEROL WERE ESTIMATED FROM A STANDARD CURVE WHICH PASSED THROUGH THE ORIGIN AND WAS LINEAR UP TO 45 UG OF STANDARD CHOLESTEROL.

CHOLESTEROL, PLANT STEROLS AND DEGRADED STEROL PRODUCTS: ISOLATION AND QUANTIFICATION OF CHOLESTEROL, PLANT STEROLS AND THE DEGRADED STEROL METABOLITES IN FECES, TISSUES (LIVER AND HEART) AND EGG YOLK WERE CARRIED OUT ACCORDING TO THE ANALYTICAL PRINCIPLE DEVELOPED BY MIETTINEN ET AL. (1965). A FLOW SHEET INDICATING THE GENERAL ASPECTS OF THE ANALYTICAL STEPS IS SUMMARIZED IN FIGURE 1.

ALL SAMPLES PER TREATMENT WERE POOLED AND ANALYZED IN TRIPLICATES, SINCE THE PROCEDURE IS LONG AND CUMBERSOME AND DOES NOT LEND ITSELF TO ROUTINE ANALYSIS. SAMPLES WERE WEIGHED INTO 150 ML POLYETHYLENE BOTTLES WITH SCREW CAPS AND SAPONIFIED WITH

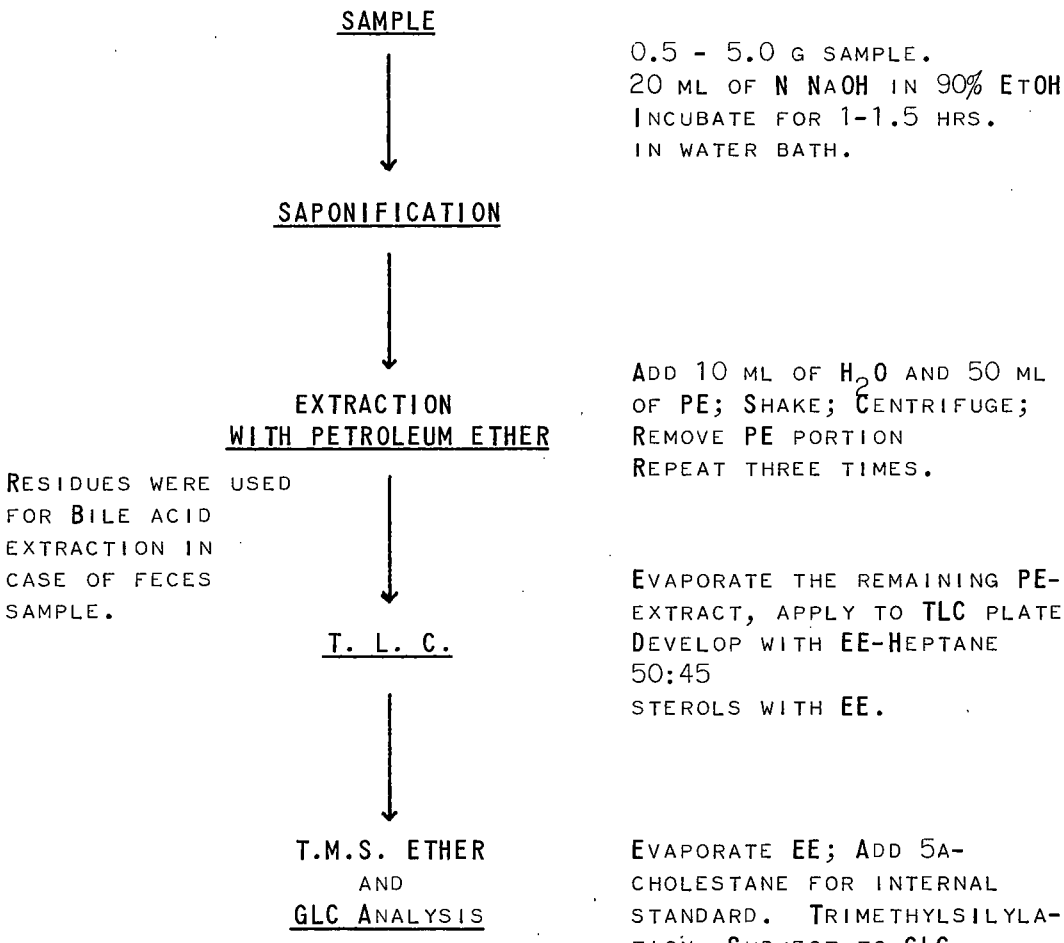

FIG. 1.--FLOW SHEET FOR DETERMINATION AND ANALYTICAL STEPS OF TISSUE, EGG YOLK AND FECAL STEROLS.

20 ml of NaOH in 90% ethanol by incubating in a water bath at 70°C for one hour. The unsaponifiable portion was extracted with petroleum ether (PE) by centrifugation for five minutes at 1000 rpm (Sorvall General Laboratory, Model 1), and repeated extraction was made until the reaction of PE extract with acid dichromate reagent (Amenta, 1964) was negative.

Total PE-extracts containing unsaponifiable sterols were evaporated to approximately one ml volume and quantitatively transferred to a 20 x 20 cm glass TLC-plate (thin layer chromatography) precoated with 0.5 mm silica-gel. The sample was applied in a streak at the base of the plate. The plates were developed to full length by an ethyl ether:heptane, 55:45, solvent system and then sprayed with a 50%-saturated aqueous solution of Rhodamine G (Applied Science Inc.). The plates were visualized under ultraviolet light.

Most of the tissue or egg yolk sterols migrate to form one thick sterol band, whereas fecal sterols forms three distinct sterol bands (Fig. 2). The sterol fraction (Band 1) which has the same Rf value as the standard mixture (cholesterol, campesterol, stigmasterol and beta-sitosterol) and the unknown sterol fractions (Band 2 and 3) were separately removed from TLC plate into a 15 ml centrifuge tube. The sterols were dissolved in ethyl ether overnight and extracted repeatedly by centrifugation from the TLC adsorbent. The ethyl ether elution mixture containing sterols were dried under a nitrogen stream and sterol residues were dissolved in a known volume of ethyl acetate containing a known

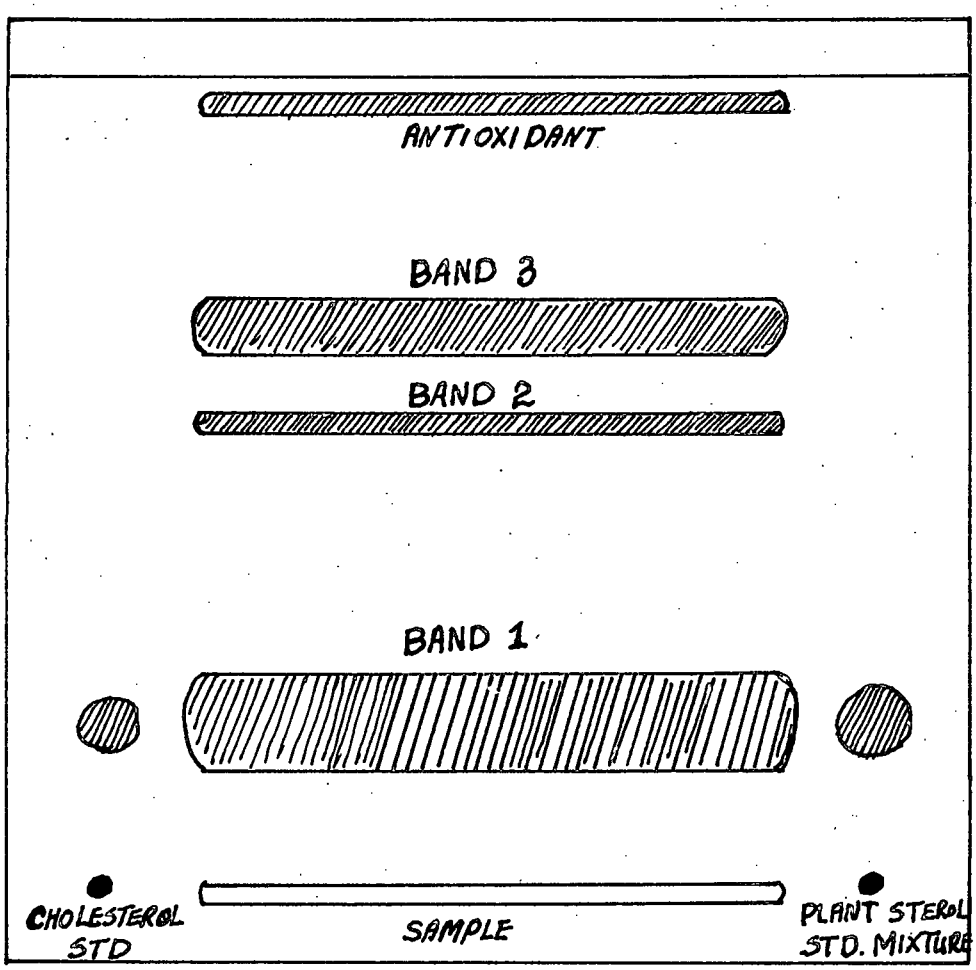

FIG. 2.--FECAL STEROL MIGRATION PATTERN ON TLC PLATE (20 x 20 CM, 0.5 MM THICKNESS) DEVELOPED WITH ETHYLETHER:HEPTANE, 55:45, SOLVENT SYSTEM.

CONCENTRATION OF 5A-CHOLESTANE AS AN INTERNAL STANDARD FOR GLC ANALYSIS (GAS LIQUID CHROMATOGRAPHY).

AN ALIQUOT OF THE ETHYL ACETATE SOLUTION WAS PIPETTED INTO A DISPOSABLE-CAP½STOPPERED GLASS VIAL (3 ML SIZE). THE SOLUTION WAS DRIED COMPLETELY UNDER NITROGEN GAS, AND THEN 1 ML OF THE TMS REAGENT (TRIMETHYLSILYLATION) WAS ADDED (SUPPLIED BY APPLIED SCIENCE INC.). AFTER 30 MINUTES REACTION TIME AT ROOM TEMPERATURE, THE TMS-STEROL ETHER WAS IMMEDIATELY SUBJECTED TO GLC ANALYSIS.

A GLC INSTRUMENT (F & M SCIENTIFIC, MODEL 5750, HEWLETT PACKARD) EQUIPPED WITH A HYDROGEN IONIZATION DETECTOR WAS EMPLOYED. A SIX-FOOT GLASS COLUMN, 4 MM INSIDE DIAMETER PACKED WITH SILANIZED GAS CHROM P (100-120 MESH SIZE) COATED WITH 1-2% FILM OF SE-30 (APPLIED SCIENCE) WAS UTILIZED IN THE GLC ANALYSIS. THE OPERATING TEMPERATURES WERE 240°C FOR THE COLUMN OVEN, 300°C FOR THE INJECTION PORT AND 290°C FOR THE DETECTOR. NITROGEN GAS WAS USED AS A CARRIER AT A FLOW RATE OF 30-60 ML/MIN. AND AN INLET PRESSURE OF 20-30 PSI.

FECAL BILE ACIDS: TOTAL BILE ACIDS IN FECES SAMPLES WERE DETERMINED WITH A SLIGHT MODIFICATION OF GRUNDY'S GLC METHOD (GRUNDY ET AL., 1965). FECAL SAMPLE RESIDUE AFTER REMOVAL OF NEUTRAL STEROLS WERE USED FOR EXTRACTION OF BILE ACIDS. A FLOW SHEET INDICATING THE ANALYTICAL STEPS ARE SUMMARIZED IN FIGURE 3.

TWO ML OF 10 N NAOH IN ETHANOL WAS ADDED TO THE SAMPLE AND INCUBATED IN A PRESSURIZED CHAMBER AT 2 ATMOSPHERES (15 PSI) FOR THREE HOURS. AFTER THE RIGOROUS SAPONIFICATION PROCESS, THE

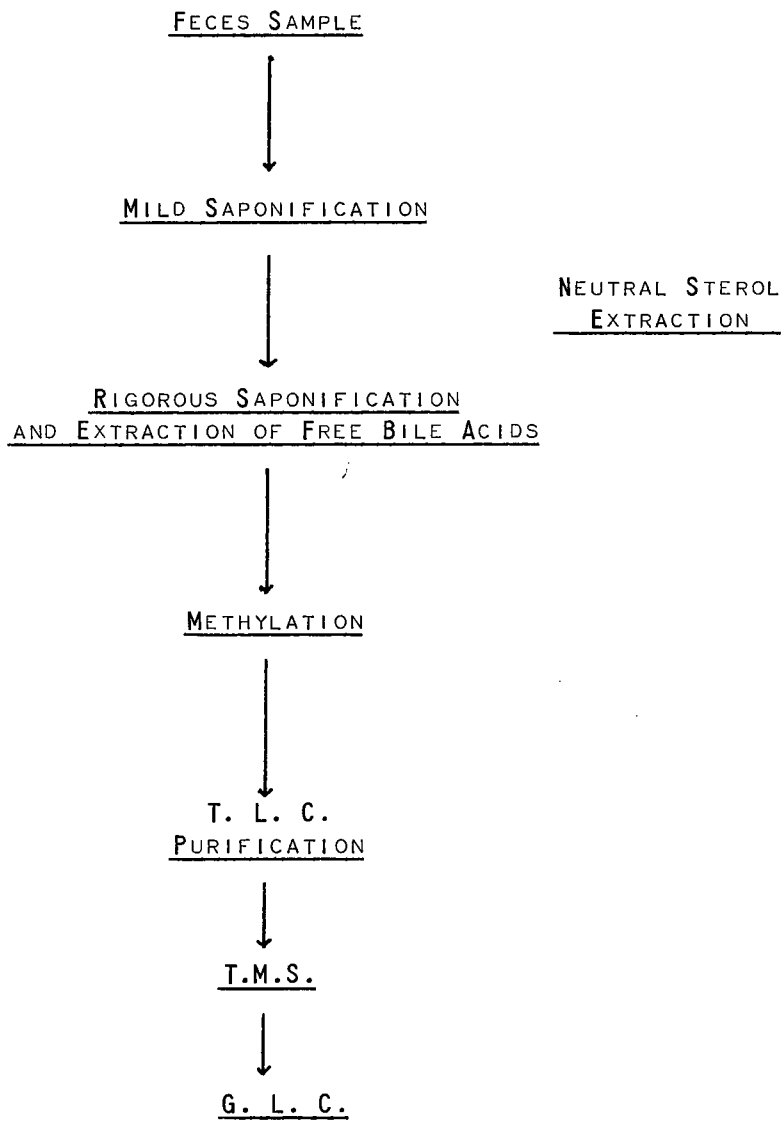

FIG. 3.--FLOW SHEET OF FECAL BILE ACID DETERMINATION AND ANALYTICAL STEPS.

MIXTURE WAS ACIDIFIED WITH CONCENTRATED HCL TO PH 2 AND QUANTITATIVELY TRANSFERRED TO A SEPARATORY FUNNEL AND BILE ACIDS WERE REPEATEDLY EXTRACTED WITH CHLOROFORM:METHANOL, 2:1 SOLVENT SYSTEM.

THE LOWER CHLOROFORM PHASE CONTAINING BILE ACIDS WAS COLLECTED INTO A 250 ML ROUND BOTTOM FLASK. THESE CHLOROFORM EXTRACTS WERE DRIED BY ROTATORY EVAPORATOR, AND THE RESIDUES WERE DISSOLVED IN A KNOWN VOLUME OF CHLOROFORM:METHANOL (2:1) SOLVENT. AN ALIQUOT OF THIS SOLUTION WAS PIPETTED TO A GLASS-STOPPERED TEST-TUBE AND SOLVENT WAS EVAPORATED UNDER NITROGEN STREAM.

THREE ML OF 5% HCL IN SUPER-DRY METHANOL WAS ADDED AND ALLOWED TO STAND AT ROOM TEMPERATURE OVERNIGHT FOR COMPLETE METHYLATION OF BILE ACIDS AND FATTY ACIDS. AFTER EVAPORATION OF THE SOLUTION, THE RESIDUE WAS APPLIED ALONG THE BASE OF A TLC PLATE WITH REFERENCES OF METHYL CHOLATE ANDMMETHYL OLEATE. THE PLATE WAS DEVELOPED IN BENZENE AND EXPOSED TO IODINE VAPOR TO LOCATE THE FATTY ACID METHYL ESTER ZONE, AND A LINE WAS DRAWN BELOW THE FATTY ACID ZONE AS A MARKER TO PREVENT FATTY ACID CONTAMINATION. THE PLATE WAS THEN DEVELOPED AGAIN IN ISOOCTANE-ISOPROPANOL-ACETIC ACID, 120:40:1, SOLVENT SYSTEM UP TO THE LINE DRAWN FOR THE FATTY ACID ZONE AND THE CHROMATOGRAM WAS EXPOSED TO IODINE VAPOR. THE AREA BETWEEN THE FATTY ACID ZONE AND THE CHOLIC METHYL ESTER ZONE WAS QUANTITATIVELY REMOVED AND ELUTED WITH METHANOL. TMS-BILE ACID ETHER PREPARATION AND GLC ANALYSIS FOR BILE ACID WERE CARRIED OUT IN A SIMILAR MANNER TO THAT PREVIOUSLY DESCRIBED IN THE NEUTRAL STEROL DETERMINATION.

Chromic Oxide Analysis: Chromic oxide was included in the ration at 0.3% as an index. Chromic oxide in the feed and feces samples was determined by the method described by Williams et al. (1962), using a Jarrel Ash atomic absorption spectrophotometer. Daily fecal excretions of bile acids, degraded sterol products, cholesterol and plant sterols were calculated by the following formular:

$$A = B\ (C \times \frac{D}{E})$$

A = mg of fecal sterols excreted per hen per day.

B = Concentration of particular sterol in dried feces (mg or ug / g feces).

C = Moisture-free feed consumed per hen per day.

D = Chromic oxide content of dried feed (%).

E = Chromic oxide content of dried feces (%).

Lipid Extraction and Fatty Acid Analysis: Total lipids of liver, egg yolk and serum were extracted by the method of Folch et al. (1957). Approximately 1 g of dried liver or fresh egg yolk sample, and 5 ml of serum sample were placed into Erlenmyer flasks and 20 ml of chloroform:methanol, 2:1, was added, and lipids were extracted overnight at room temperature. The lipid extracts were filtered through a fluted filter paper. Flask and filter paper were repeatedly washed with the same solvent system while monitoring the extractability with acid dichromate reagent (Amenta, 1964).

The filtrate was further purified by washing with saline, and chloroform:methanol:salinemixture, 3:47:48, and the chloroform

LAYER CONTAINING LIPIDS WAS EVAPORATED AND LIPID CONCENTRATION OF SAMPLES WERE DETERMINED BY WEIGHT. AN ALIQUOT OF THE LIPID SAMPLES WAS MIXED WITH 3 ML OF BORON TRIFLORIDE-METHANOL REAGENT IN A SCREW-CAPPED TEST TUBE, AND FATTY ACID METHYL ESTERS WERE PREPARED ACCORDING TO THE PROCEDURE OF METCALFE ET AL. (1961).

THE FATTY ACID METHYL ESTERS WERE SUBJECTED TO GLC ANALYSIS FOR THE FATTY ACID COMPOSITION. A GLC INSTRUMENT (F & M SCIENTIFIC, MODEL 5750, HEWLETT PACKARD), EQUIPPED WITH A HYDROGEN FLAME IONIZATION DETECTOR, SIX-FOOT STAINLESS STEEL COLUMNS (ONE-EIGHTH INCH INSIDE DIAMETER) WERE USED. COLUMNS WERE PACKED WITH CHROMOSORB P (MESH SIZE, 100-120) PRE-COATED WITH 10% ETHYLENE SUCCINATE METHYLSILICONE POLYMERS (APPLIED SCIENCE INC.).

OPERATING TEMPERATURES WERE 180°C FOR THE COLUMN, 210°C FOR THE DETECTOR AND INJECTION PORT. NITROGEN GAS WAS THE CARRIER AT A FLOW RATE OF 40 ML PER MINUTE.

EACH CHROMATOGRAM PEAK WAS IDENTIFIED BY COMPARISON OF RETENTION TIME TO A STANDARD FATTY ACID MIXTURE WITH A KNOWN COMPOSITION (APPLIED SCIENCE LAB.). PERCENT FATTY ACID WAS CALCULATED AS THE RATIO OF PEAK AREA TO THE TOTAL CHROMATOGRAM MEASURED BY TRIANGULATION.

DATA FROM TRIAL 2 WERE TESTED BY ANALYSIS OF VARIANCE DESCRIBED BY SNEDECOR (1956) AND MULTIPLE RANGE COMPARISON WAS MADE ACCORDING TO DUNCAN (1955) TO DETERMINE SIGNIFICANT DIFFERENCES AMONG TREATMENT MEANS.

RESULTS AND DISCUSSION

Effect of Dietary Oil, Cholesterol and Soysterols on the Serum and Egg Yolk Sterol Concentrations in Laying Hens

Trial 1

Weekly responses of laying hen's serum and egg yolk sterol levels to the dietary lipid factors and the physiological relationship between serum and egg yolk sterol metabolism were assessed. Average sterol concentrations in serum and egg yolk from eight birds per treatment (weekly intervals) during the 6-week feeding period are summarized in Table 6. The pattern of change in sterol levels due to dietary treatment was expressed by plotting sterol levels of serum (Fig. 4) and egg yolk (Fig. 5) against time.

Serum sterol levels in laying hens were elevated due to feeding the experimental rations for one week when compared to the initial levels estimated before feeding the experimental diets (Table 5). This general increase in serum sterol levels suggests that high fat diets (8%) are hypersterolemic to the laying hen irrespective of the type of dietary oil fed (Table 5 and 6).

It is apparent, however, that when cholesterol-free diets were fed, the type of dietary oil exhibited a marked influence on changes in serum and egg yolk sterol levels (Table 6). Laying hens fed the SFO diet maintained a lower sterol level in serum

TABLE 6.--EFFECT OF DIETARY OIL, CHOLESTEROL, AND SOYSTEROLS ON THE WEEKLY CHANGES IN SERUM AND EGG YOLK STEROL LEVELS OF LAYING HENS (TRIAL 1).

TREATMENTS	WEEKLY PERIOD[1]							
	WITHOUT SOYSTEROLS				WITH SOYSTEROLS			
	1	2	3	MEAN	4	5	6	MEAN
	SERUM (MG %)[2]							
HCO	127.0±19.2	153.6± 5.9	240.8±10.6	173.2	221.0±10.2	153.2±25.3	158.8±26.8	177.6
HCO+CH	173.9±37.9	217.7±22.9	226.0±33.5	205.8	257.0±93.5	190.0±39.6	218.8±21.2	221.9
SFO	100.0±13.6	98.4±15.6	129.7±13.8	112.3	133.5±10.3	123.3± 9.2	133.1±21.4	129.9
SFO+CH	149.8±34.1	206.4±65.2	313.2±41.4	223.1	179.7±17.1	179.7±11.5	157.2±32.3	171.9
	EGG YOLK (MG/G)[2]							
HCO	11.6±1.75	10.0±1.0	15.0±1.3	12.2	18.6±0.5	14.4±1.0	16.5±0.4	16.5
HCO+CH	12.7±1.28	15.3±1.6	24.4±4.9	17.5	28.2±1.5	22.2±1.2	16.9±1.2	22.4
SFO	11.2±0.9	9.7±1.1	11.5±0.8	10.8	12.3±1.7	12.4±0.4	9.6±1.7	11.4
SFO+CH	12.5±1.7	16.0±3.3	30.42±2.0	19.5	48.0±1.6	33.6±6.9	18.9±0.6	33.5

[1] AT THE END OF THIRD WEEK OF FEEDING PERIOD, ALL THE DIETS WERE REPLACED WITH 2% OF SOYSTEROL-SUPPLEMENTED DIETS.

[2] MEAN VALUES OF 8 HENS ± S.D.

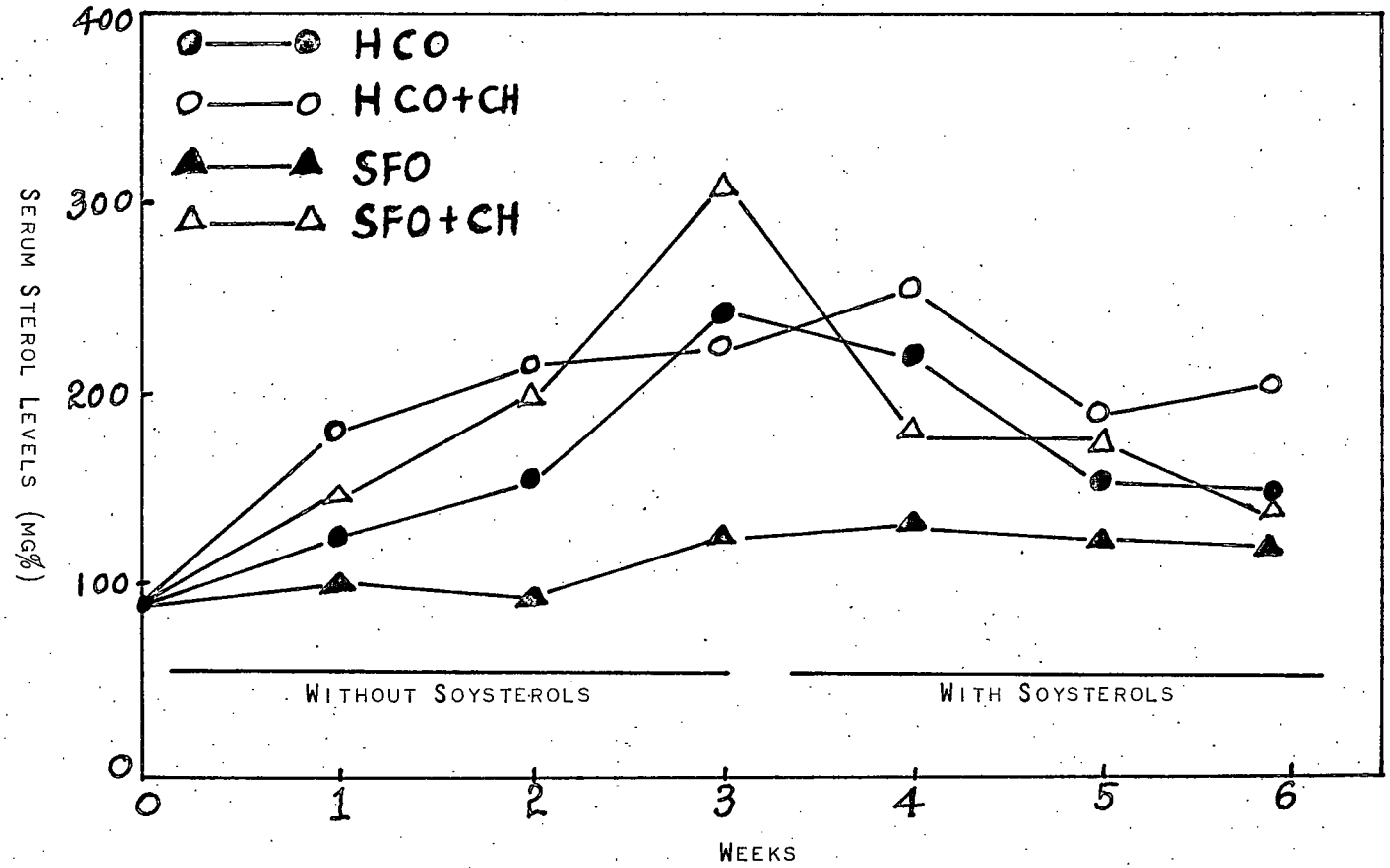

FIG. 4.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE WEEKLY CHANGES IN SERUM STEROL LEVELS OF LAYING HENS (TRIAL 1).

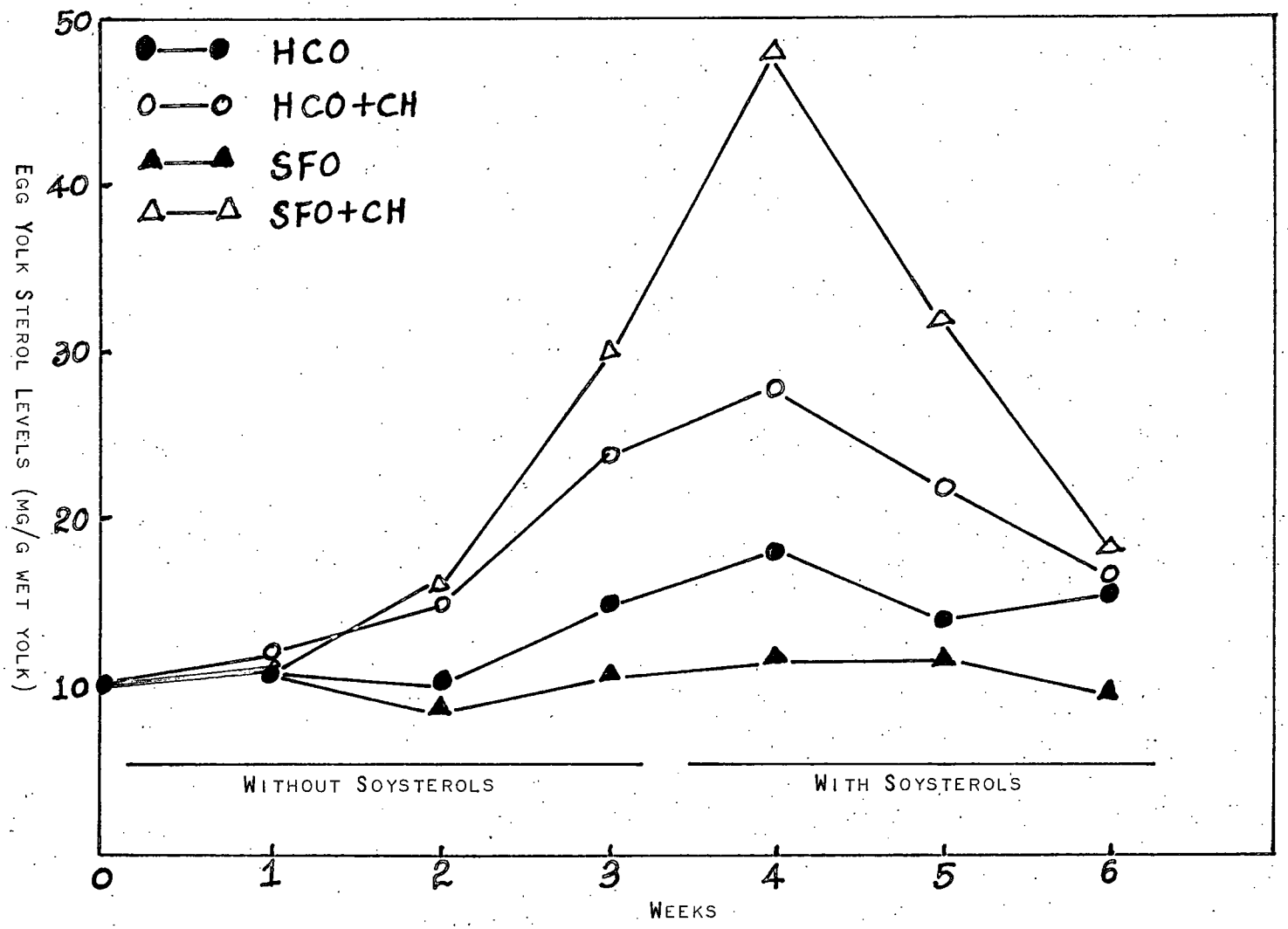

Fig. 5.--Effect of dietary oil, cholesterol and soysterols on the weekly changes in egg yolk sterol levels of laying hens (Trial 1).

AND EGG YOLK, WHICH WAS RELATIVELY CONSTANT DURING THE SIX-WEEK FEEDING PERIOD, WHEREAS LAYING HENS FED THE **HCO** DIET SHOWED A RAPID RISE IN SERUM LEVEL UNTIL DIETS WERE REPLACED WITH THE SOYSTEROL SUPPLEMENTED DIETS (FIG. 4). EGG YOLK STEROL LEVEL FROM HENS FED **HCO** DIETS ALSO SHOWED AN INCREASE BEFORE THE DIETS WERE CHANGED TO SOYSTEROL SUPPLEMENTED DIETS (FIG. 5).

THESE OBSERVATIONS DEMONSTRATE THAT FEEDING SATURATED OIL LEADS TO AN ELEVATION IN BOTH SERUM AND EGG YOLK STEROL, WHEREAS FEEDING UNSATURATED OIL CAUSED LITTLE CHANGE IN SERUM AND YOLK STEROLS AFTER ONE-WEEK ADJUSTMENT PERIOD.

THE ADDITION OF CHOLESTEROL TO DIETS CONTAINING **HCO** AND **SFO** (**SFO+CH** AND **HCO+CH** TREATMENT) PRODUCED AN ADDITIONAL INCREASE IN BOTH SERUM AND EGG YOLK STEROL LEVELS COMPARED TO **HCO** AND **SFO** TREATMENTS. THESE RESPONSES WERE GREATER WITH HENS FED **SFO+CH** DIET THAN WITH **HCO+CH** DIET DURING THE THIRD AND FOURTH WEEK OF FEEDING (FIG. 4 AND 5). MAXIMUM VALUES OBTAINED AT THE THIRD WEEK INTERVAL WERE 313.2 MG% AND 226.4 MG% IN SERUM AND 30.4 MG/G AND 24.4 MG/G IN EGG YOLK FOR THE **SFO+CH** AND **HCO+CH** DIETS RESPECTIVELY (TABLE 6).

THE RESULTS DEMONSTRATED THAT SAFFLOWER OIL HAS STEROL LOWERING OR DEPRESSING PROPERTY PER SE IN THE ABSENCE OF HIGH DIETARY CHOLESTEROL, BUT IT PRODUCED THE OPPOSITE EFFECT IN THE PRESENCE OF ADDITIONAL DIETARY CHOLESTEROL. THE STIMULATING EFFECT OF SAFFLOWER OIL IN RAISING STEROL LEVELS OF SERUM AND EGG YOLK UPON FEEDING CHOLESTEROL DOES NOT APPEAR TO HAVE RESULTED FROM THE ADDED EFFECTS OF OIL AND DIETARY CHOLESTEROL, BUT WAS

PROBABLY CAUSED BY AN INCREASED ABSORPTION OR TRANSPORT OF CHOLESTEROL. OTHER INVESTIGATORS HAVE ALSO REPORTED A SYNERGISTIC EFFECT OF DIETARY SAFFLOWER OIL OR OTHER UNSATURATED FATS ON INCREASING STEROL LEVELS IN SERUM IN THE PRESENCE OF DIETARY CHOLESTEROL (CHUNG ET AL., 1965; HULETT ET AL., 1964; WOOD ET AL., 1961; MARCH AND BIELY, 1959; WEISS ET AL., 1967A).

SOYSTEROL INCLUSION IN DIETS AT THE END OF THE THIRD WEEK-FEEDING PERIOD RESULTED IN A DEPRESSION OF THE SERUM AND EGG YOLK STEROL CONCENTRATIONS (FIG. 4 AND 5). THIS ANTI-STEROGENIC EFFECT OF DIETARY SOYSTEROLS WAS OBSERVED IN THE CHOLESTEROL-TREATED GROUPS AS WELL AS THE CHOLESTEROL-FREE DIET CONTAINING **HCO**. HOWEVER, LAYING HENS FED THE **SFO** DIET DID NOT EXHIBIT ANY SIGNIFICANT RESPONSE IN THEIR SERUM AND EGG YOLK STEROL LEVELS DUE TO PLANT STEROL FEEDING (FIG. 4 AND 5, TABLE 6). THE MAGNITUDE OF THE REDUCTION IN STEROL LEVELS OF EITHER SERUM OR EGG YOLK WAS PROPORTIONAL TO THE DEGREE OF THE BIRD'S HYPERSTEROLEMIC STATUS OR STEROL LEVELS IN SERUM AND EGG YOLK INDUCED BY THE DIETS. THE HYPERSTEROLEMIC EFFECT IN HENS INDUCED BY FEEDING THE **SFO+CH** DIET SHOWED GREATER REDUCTION IN BOTH SERUM AND EGG YOLK STEROL LEVELS THAN WAS OBSERVED WITH HENS IN WHICH THE HYPERSTEROLEMIC EFFECT WAS INDUCED BY FEEDING EITHER **HCO+CH** OR **HCO** DIETS.

IT IS INTERESTING TO NOTE THAT CHANGES IN EGG YOLK STEROL LEVELS WERE CLOSELY RELATED TO SERUM STEROL LEVELS. IN CASES WHERE HYPERSTEROLEMIA WAS OBSERVED, THE INCREASE IN EGG YOLK STEROL LEVELS CAUSED BY DIETARY CHOLESTEROL OR DECREASE CAUSED BY DIETARY SOYSTEROLS PARALLELED THE SERUM CHANGES. THIS FACT

INDICATES THAT THE TOTAL STEROL POOL OF CIRCULATING BLOOD IS A MAJOR SOURCE OF EGG YOLK STEROLS. THEREFORE, THE EGG DEPOSITION OF STEROLS IS PROBABLY A MAJOR PATHWAY FOR THE ELIMINATION OF STEROLS FROM THE LABILE BLOOD POOL IN LAYING HENS. THE ABILITY OF HENS TO ELIMINATE DIETARY CHOLESTEROL VIA THE EGG WAS PREVIOUSLY REPORTED BY ANDREWS ET AL. (1968) AND WEISS ET AL. (1967A).

A COMPARISON OF THE STEROL PATTERN IN SERUM AND EGG YOLK (FIG. 4 AND 5), INDICATES THAT CHANGES IN EGG YOLK STEROL LEVELS WERE GENERALLY PRECEDED BY CHANGES IN SERUM LEVELS. SERUM LEVELS SHOWED AN INCREASE IN HENS RECEIVING THE **HCO, HCO+CH** AND **SFO+CH** DIETS DURING THE FIRST WEEK OF TREATMENT, HOWEVER, EGG YOLK DID NOT EXHIBIT THIS EFFECT UNTIL THE SECOND WEEK. FURTHERMORE, THE MAGNITUDE OF SERUM CHANGE WAS GREATER DURING THE FIRST WEEK THAN IN THE EGG YOLK DURING THE SECOND WEEK.

THE DECREASE IN SERUM STEROL LEVELS DUE TO THE PRESENCE OF SOYSTEROLS IN DIETS WAS OBSERVED IN ALL TREATMENTS DURING THE FOURTH WEEK, WITH THE EXCEPTION OF **HCO+CH** DIET, WHEREAS THE CORRESPONDING RESPONSE IN EGG YOLK OCCURRED ONE WEEK LATER (5TH WEEK). THIS TIME LAG CORRESPONDS TO THE TIME REQUIRED FOR EGG YOLK FORMATION (NORTH, 1972). EGG YOLK FORMATION REQUIRES APPROXIMATELY 7-10 DAYS IN WHICH 95 TO 99% OF YOLK MATERIAL IS LAID DOWN BY THE LAYING HEN (NORTH, 1972). THIS OBSERVATION IS IN AGREEMENT WITH THE REPORT BY ANDREWS ET AL. (1968) WHO SHOWED THAT MAXIMUM CHOLESTEROL INCORPORATION IN EGG YOLK TAKES 6-7 DAYS, WHEREAS SERUM CHANGES WERE IMMEDIATE WHEN RADIOACTIVE CHOLESTEROL WAS ORALLY FED TO LAYING HENS.

Trial 2

Data pertaining to the sterol concentrations in serum and egg yolk determined from 16 birds per treatment in Trial 2 following an 8-week feeding period are summarized in Table 7, and Figure 6.

Sterol concentrations in the serum and egg yolk of hens fed the **HCO** diet were significantly higher ($P < 0.01$) than those fed the **SFO** diet (239.8 mg% and 16.07 mg/g with **HCO**, and 147.9 mg% and 12.6 mg/g with **SFO** diet for serum and egg yolk respectively). These results agree with data reported by Weiss *et al.* (1967a) and Bartov *et al.* (1971) that hydrogenated coconut oil is more hypersterogenic than safflower oil in laying hens. Similar observations were reported with human subjects (Hegsted *et al.*, 1965), and in the gerbil (Hegsted and Gallagher, 1967).

The safflower oil diet increased significantly ($P < 0.01$) both serum and egg yolk sterol levels when cholesterol was fed (**SFO+CH**). Feeding **HCO+CH** diet resulted in an elevated egg yolk sterol level, however, no significant ($P < 0.01$) effect on the serum level was observed (Table 7). Both serum and egg yolk sterol levels were significantly ($P < 0.01$) higher from hens fed the **SFO+CH** diet compared to those fed the **HCO**, **HCO+CH** and **SFO** treatments (Fig. 6). These results demonstrated that the response to cholesterol ingestion is influenced by the composition of the diet and dietary oil used.

Cholesterol addition to the **SFO** diet increased the sterol levels in serum to 140% and in egg yolk to 130% of the **SFO**

TABLE 7.--SERUM AND EGG YOLK STEROL CONCENTRATIONS OF LAYING HENS FED DIETARY OIL, CHOLESTEROL AND SOYSTEROLS (TRIAL 2).

TREATMENTS	STEROL CONCENTRATIONS	
	SERUM	EGG YOLK
	(MG %)	(MG/G)
HCO	239.8^{F}[1]	16.07^{D}[1]
SFO	147.9^{B}	12.60^{B}
HCO+CH	244.8^{F}	20.10^{F}
SFO+CH	374.0^{G}	28.96^{G}
HCO+ST	158.8^{C}	14.58^{C}
SFO+ST	123.0^{A}	9.65^{A}
HCO+CH+ST	218.8^{E}	17.06^{D}
SFO+CH+ST	179.7^{D}	17.95^{D}

[1] MEANS WITHIN A COLUMN FOLLOWED BY THE SAME SUPERSCRIPT ARE NOT SIGNIFICANTLY DIFFERENT.

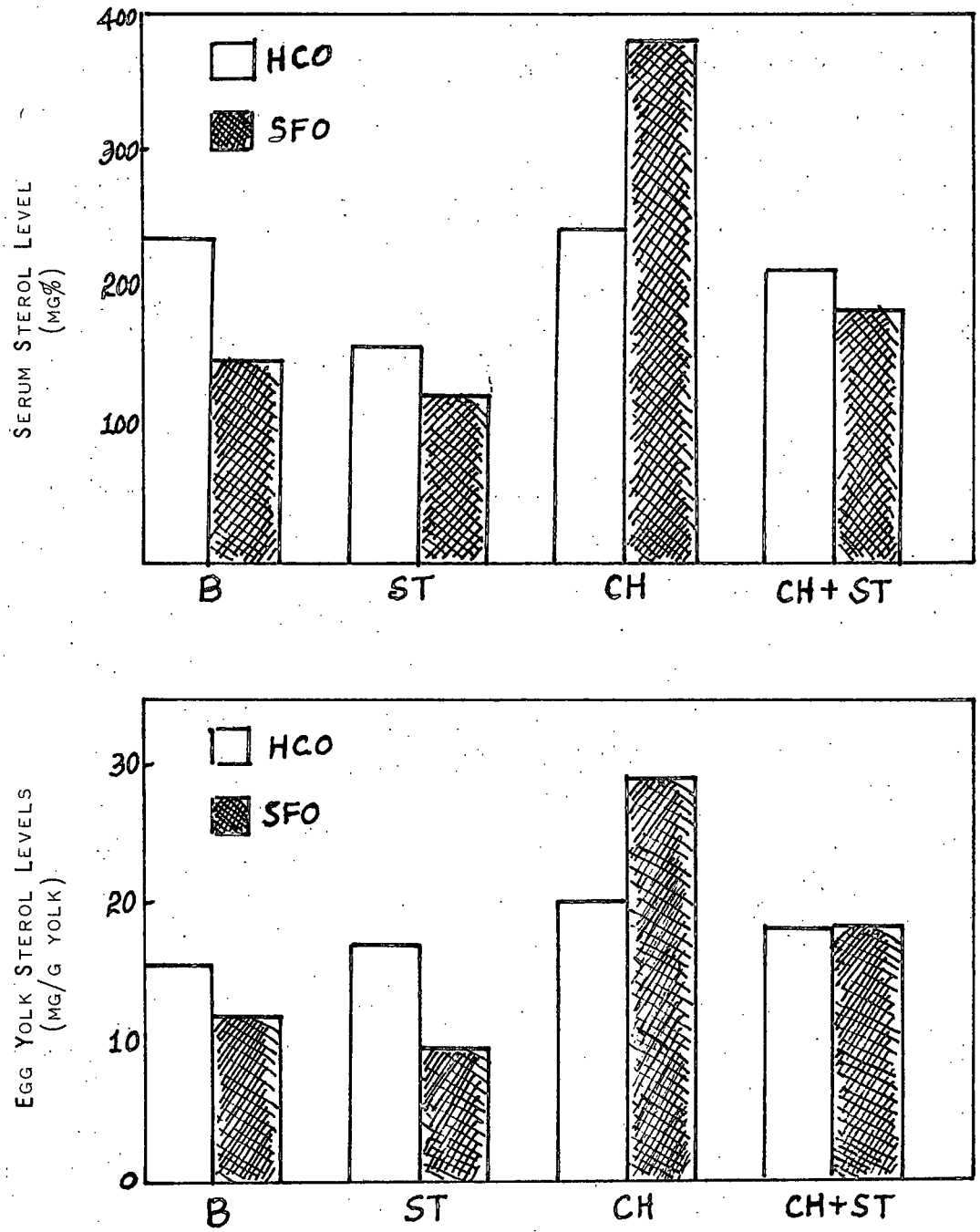

FIG. 6.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE SERUM AND EGG YOLK STEROL LEVELS (TRIAL 2).

TREATMENT. CHOLESTEROL ADDITION TO THE HCO DIET CAUSED NO INCREASE IN SERUM AND ONLY A 24% INCREASE WAS OBSERVED IN EGG YOLK. DIETARY CHOLESTEROL THAT CAN BE ACCOUNTED FOR IN SERUM AND EGG YOLK DEPENDS UPON THE NATURE OF DIETARY FAT AND/OR OTHER COMPONENTS WHICH AID CHOLESTEROL ABSORPTION OR TRANSPORT.

A SIGNIFICANT REDUCTION IN STEROL LEVELS OF BOTH SERUM AND EGG YOLK WAS OBTAINED WHEN 2% SOYSTEROLS WERE ADDED TO DIETS CONTAINING OIL WITH OR WITHOUT CHOLESTEROL. HOWEVER, THE INFLUENCE OF THE DIETARY OIL TYPE WAS ALSO APPARENT. VALUES OBTAINED FROM TREATMENT GROUPS THAT WERE FED SAFFLOWER OIL WERE SIGNIFICANTLY LOWER THAN VALUES FROM HENS FED HYDROGENATED COCONUT OIL AFTER SOYSTEROLS WERE INCORPORATED INTO DIETS (TABLE 7, FIG. 6). THIS INDICATES THAT THE ANTI-HYPERSTEROGENIC FUNCTION OF PLANT STEROLS WAS NOT SUFFICIENT TO MASK THE PROPERTIES OF DIETARY OILS IN REGULATING STEROL METABOLISM.

IT HAS BEEN SUGGESTED THAT PLANT STEROLS REDUCE STEROL OR CHOLESTEROL LEVELS IN SERUM AND TISSUES, WHEN INCORPORATED SIMULTANEOUSLY WITH CHOLESTEROL IN THE DIET. THE STEROL LOWERING EFFECT OF PLANT STEROLS HAS BEEN ATTRIBUTED TO INHIBITION OF CHOLESTEROL ABSORPTION (DAVIS, 1955; BARTOVE ET AL., 1969; WRIGHT, 1966). HOWEVER, EVIDENCE SHOWN IN BOTH TRIAL 1 AND TRIAL 2 DOES NOT REVEAL THAT CLOSE CONTACT BETWEEN SOYSTEROL AND CHOLESTEROL IN THE DIET OR IN THE INTESTINE IS NECESSARY IN ORDER TO ACHIEVE ANTI-STEROGENIC EFFECTS IN LAYING HENS.

Effect of Dietary Oil, Cholesterol and Soysterols on the Fecal Output of Acidic and Neutral Sterols

Bile Acids: Birds fed diets supplemented with safflower oil excreted fecal bile acids at a greater rate than birds fed diets containing hydrogenated coconut oil irrespective of sterol treatments (Table 8 and Fig. 7). The amount of bile acids excreted by hens fed the **SFO** diet was almost four-fold that of those fed the **HCO** diet (241.15 and 64.55 mg/hen/day respectively).

These results are in accord with reports that dietary unsaturated fats increase and saturated fats decrease the fecal output of acidic sterols in human subjects (Gordon *et al.*, 1957) and in chickens (Lindsey *et al.*, 1969).

The rate of bile acid output in feces, however, was markedly altered by the presence of cholesterol and/or soysterols in diets. The addition of soysterols to either **SFO** or **HCO** diets increased fecal bile acid excretion (Fig. 7). The influence of dietary soysterols was greatest when these were fed simultaneously with cholesterol. Feeding soysterols in combination with cholesterol increased the fecal bile acids by 42% or more than by feeding soysterol alone without cholesterol. The excretion rates of bile acids were 134.61 and 301.10 mg/hen/day for **HCO+ST** and **SFO+ST** treatment respectively, whereas the excretion rates were 191.82 and 448.17 mg/hen/day for **HCO+CH+ST** and **SFO+CH+ST** treatments respectively (Table 8).

No changes occurred in the amount of bile acids excreted when cholesterol was fed without dietary soysterols, although the values were slightly higher with the **HCO+CH** and lower with the **SFO**

TABLE 8.--FECAL EXCRETION OF BILE ACIDS AND THE UNIDENTIFIABLE NEUTRAL STEROLS (DEGRADED STEROL PRODUCTS).

TREATMENTS	TOTAL BILE ACIDS	DEGRADED STEROLS[1]
	(MG/HEN/DAY)	(MG/HEN/DAY)
HCO	64.55	4.08
SFO	241.15	5.59
HCO+CH	75.18	11.15
SFO+CH	225.96	29.26
HCO+ST	134.61	108.76
SFO+ST	301.10	97.83
HCO+CH+ST	191.82	199.98
SFO+CH+ST	448.17	168.38

[1] TOTAL STEROLS RESOLVED FROM BAND 2 AND BAND 3 IN TLC OF FECAL STEROLS.

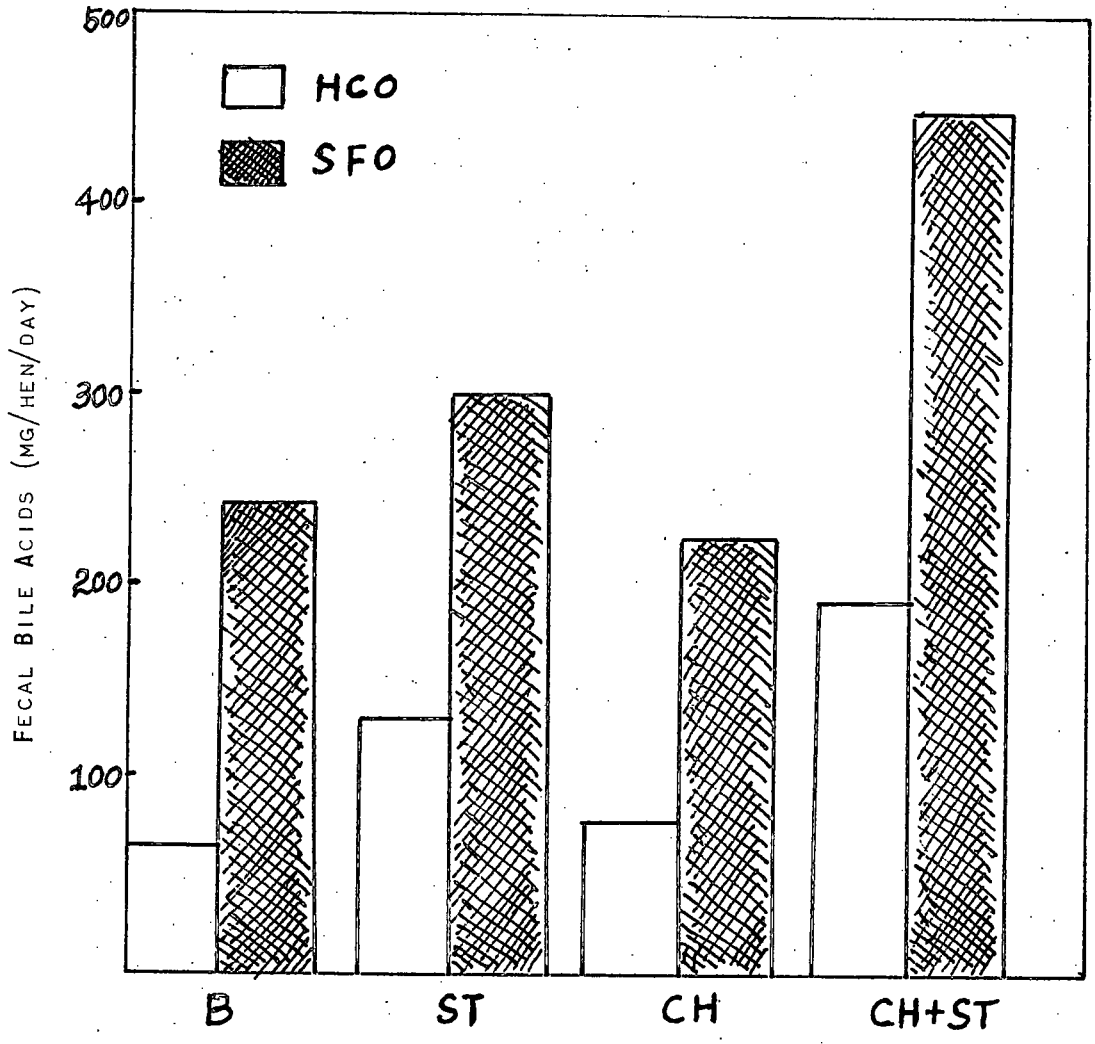

FIG. 7.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE FECAL BILE ACID OUTPUT.

DIET (TABLE 8, FIG. 7). THESE RESULTS INDICATED THAT PLANT STEROLS HAD A SPECIFIC PROPERTY TO STIMULATE FECAL BILE ACID OUTPUT, WHEREAS CHOLESTEROL DID NOT CONTRIBUTE ANY EFFECT, EVEN THOUGH THESE COMPOUNDS ARE STRUCTUALLY SIMILAR. THE EXCRETION OF BILE ACIDS DECREASED THE AMOUNT OF BILE ACIDS RECYCLED INTO THE SERUM WHICH RESULTED IN A DECREASE IN SERUM STEROL LEVELS. THIS EFFECT WAS OBSERVED IN HENS FED THE SOYSTEROL DIETS IN TRIALS 1 AND 2.

DEGRADED STEROL PRODUCTS: FECAL OUTPUT OF DEGRADED STEROL METABOLITES ISOLATED FROM TOTAL FECAL STEROLS BY TLC WERE ALMOST NEGLIGIBLE WHEN HENS WERE FED LOW STEROL BASAL DIETS (4.08 AND 5.59 MG/HEN/DAY FOR HCO AND SFO DIETS RESPECTIVELY). ADDITION OF 1% CHOLESTEROL TO THE BASAL DIETS INCREASED METABOLITES BY MORE THAN TWO-FOLD WITH HCO+CH DIET, AND SIX-FOLD WITH SFO+CH DIET (11.15 AND 29.26 MG/HEN/DAY FOR HCO+CH AND SFO+CH RESPECTIVELY). THESE INCREASES, HOWEVER, WERE SMALL WHEN COMPARED TO THE CHANGES UPON FEEDING SOYSTEROLS.

SOYSTEROL INCORPORATION INTO DIETS PRODUCED A TREMENDOUS INCREASE IN THE FECAL OUTPUT OF STEROL CATABOLIC PRODUCTS AND THE INCREMENTS WERE APPROXIMATELY DOUBLED WHEN HENS WERE FED CHOLESTEROL AND SOYSTEROLS SIMULTANEOUSLY. HEN GROUPS FED CHOLESTEROL-FREE DIETS EXCRETED 108.76 MG/HEN/DAY WITH HCO+ST, AND 97.83 MG/HEN/DAY WITH SFO+ST DIETS, WHEREAS HEN GROUPS FED CHOLESTEROL DIETS EXCRETED 199.98 MG/HEN/DAY AND 168.38 MG/HEN/DAY WITH HCO+CH+ST AND SFO+CH+ST DIETS RESPECTIVELY (TABLE 8).

Cholesterol: The amounts of cholesterol detected in the feces from hens fed the basal diet were very small as observed by the amount of catabolic neutral sterols (less than 10 mg per day). The fecal cholesterol with the basal diet was considered of endogenous origin, since the diets fed were almost free of cholesterol. These results indicated that laying hens do not eliminate large amounts of endogenous cholesterol via the feces when no cholesterol is provided in the diet. Results also indicate that reabsorption of endogenous cholesterol at the enterohepatic circulation was efficient under these dietary conditions (Booreman and Fisher, 1966; Edwards et al., 1960).

It is shown in Figure 8 that soysterolsincorporation into the cholesterol-free diets caused a considerable increase in the fecal cholesterol excretion (6.26 to 19.20 mg with HCO diets, and 9.54 to 17.01 mg with SFO diets respectively). However, the amount of cholesterol excreted was small (less than 20%) as compared to the amounts of fecal catabolic sterol produced. These results suggested that soysterols included in the cholesterol free diets accelerated fecal excretion of endogenous cholesterol to a lesser extent, and catabolic sterol products were excreted to a greater extent.

The type of dietary oil affected the amounts of fecal cholesterol excreted when cholesterol was incorporated into diets. Birds fed diets containing hydrogenated coconut oil excreted 422.65 and 375.64 mg/hen/day with HCO+CH and HCO+CH+ST respectively. However, hens fed diets containing safflower oil

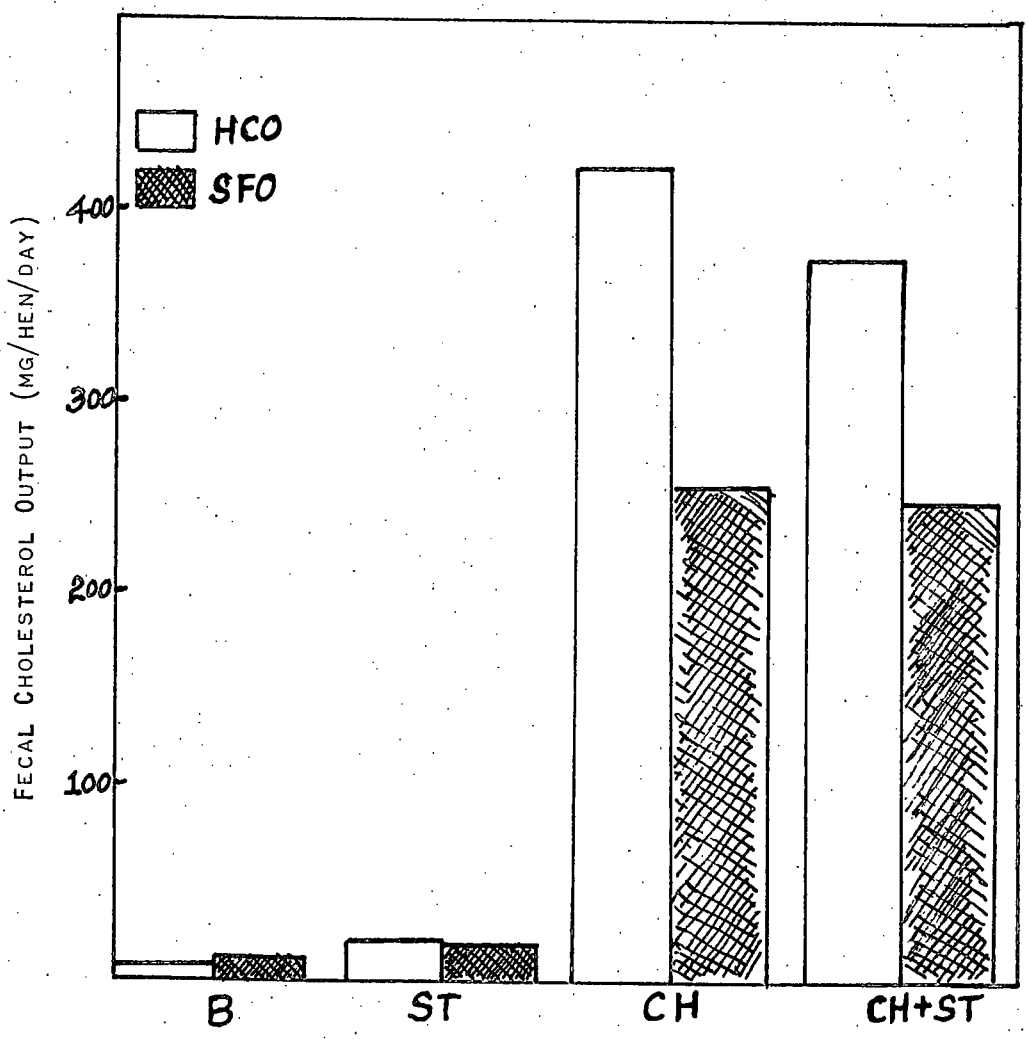

FIG. 8. EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE FECAL CHOLESTEROL OUTPUT.

EXCRETED ONLY 255.36 AND 243.00 MG/HEN/DAY WITH **SFO+CH** AND **SFO+CH+ST** RESPECTIVELY (TABLE 9 AND FIG.8).

THE AMOUNTS OF CHOLESTEROL DETECTED IN THE FECES FROM BIRDS FED EXOGENOUS CHOLESTEROL, WAS A POOL OF ENDOGENOUS AND DIETARY CHOLESTEROL, HOWEVER, THE CONTRIBUTION FROM ENDOGENOUS CHOLESTEROL IN THE FECES WAS SMALL ASSUMING CHOLESTEROL EXCRETED FROM BIRDS FED THE BASAL DIET WAS AN ESTIMATE OF ENDOGENOUS CHOLESTEROL. THE COMPARATIVELY LARGER QUANTITY OF FECAL CHOLESTEROL DETECTED IN THE CHOLESTEROL-TREATED GROUPS INDICATED THAT A LARGE PROPORTION OF THE DIETARY CHOLESTEROL WAS NOT ABSORBED. ON THE BASIS OF THIS ASSUMPTION, THE APPARENT ABSORPTION OF DIETARY CHOLESTEROL WAS CALCULATED BY THE DIFFERENCE BETWEEN THE AMOUNT INGESTED AND THE AMOUNT EXCRETED IN FECES (TABLE 9).

DATA CLEARLY INDICATE THAT LAYING HENS FED CHOLESTEROL WITH SAFFLOWER OIL RETAINED MORE CHOLESTEROL (75.68% FOR **SFO+CH**) THAN THOSE FED DIETARY CHOLESTEROL WITH HYDROGENATED COCONUT OIL (58.56% FOR **CHO+CH**). IT HAS BEEN DEMONSTRATED THAT UNSATURATED FATTY ACIDS IN THE DIET WERE SUPERIOR TO SATURATED FATTY ACIDS IN PROMOTING CHOLESTEROL ABSORPTION IN RAT STUDIES (KIM AND IVY, 1952; SWELL ET AL., 1955). THE SUPERIOR PROPERTY OF SAFFLOWER OIL IN FACILITATING CHOLESTEROL ABSORPTION MAY BE TWO-FOLD: (1) THE UNSATURATED FATTY ACIDS MAY BE NECESSARY FOR CHOLESTEROL ESTERIFICATION, (2) AN INCREASE IN THE AMOUNT OF BILE ACIDS SECRETED IN THE INTESTINE DUE TO THE PRESENCE OF SAFFLOWER OIL IN THE DIET MAY BE A CONTRIBUTING FACTOR (TABLE 8). SINCE BILE ACIDS HAVE BEEN SHOWN TO BE ESSENTIAL FOR CHOLESTEROL ABSORPTION

TABLE 9.--Fecal excretion of cholesterol and plant sterols and their apparent absorption rates.

Treatments	Fecal Sterol Excretion[1]		App. Absorption[2]	
	Cholesterol	Plant Sterols	Cholesterol	Plant Sterols
	(mg/hen/day)		(Percent)	
HCO	6.26	7.61	---	---
SFO	9.54	13.15	---	---
HCO+CH	422.65	19.01	58.56	---
SFO+CH	255.36	26.94	75.68	---
HCO+ST	19.20	404.76	---	78.10
SFO+ST	17.01	427.90	---	77.11
HCO+CH+ST	375.64	536.56	62.43	69.67
SFO+CH+ST	243.00	581.31	75.70	67.34

[1] The amounts of sterols resolved from Band 1 of TLC plate and computed to daily output per hen.

[2] Percent apparent absorption was computed by the difference between the amount fed and the amount excreted in feces.

(CHAIKOFF ET AL., 1952; SWELL ET AL., 1953; HERNANDEZ ET AL., 1953), IT APPEARS THAT BOTH UNSATURATION OF FATTY ACID AND STIMULATION OF BILE ACID SECRETION WERE INTERACTING FACTORS IN THE UTILIZATION OF CHOLESTEROL.

NEITHER THE AMOUNTS OF CHOLESTEROL EXCRETED IN FECES NOR THE RATE OF CHOLESTEROL RETENTION (%) SHOW ANY INDICATION THAT SOYSTEROL FEEDING INTERFERES WITH CHOLESTEROL ABSORPTION WHEN SOYSTEROLS WERE INCORPORATED INTO THE DIETS CONTAINING CHOLESTEROL (TABLE 9 AND FIG. 8). IT IS DIFFICULT TO RECONCILE THESE RESULTS WITH THE IDEA THAT PLANT STEROLS INHIBIT CHOLESTEROL ABSORPTION. THE MECHANISM OF INHIBITION WAS SUGGESTED BY DAVIS (1955) TO INVOLVE THE FORMATION OF A NON-ABSORBABLE COMPLEX OF PLANT STEROL AND CHOLESTEROL IN THE INTESTINE. HOWEVER, IT IS NOT CLEAR, WHY CHOLESTEROL AND PLANT STEROLS SHOULD FORM INSOLUBLE COMPLEXES WHILE CHOLESTEROL ALONE DOES NOT.

PLANT STEROL: THE FECAL EXCRETION OF PLANT STEROLS ARE SHOWN IN TABLE 9 AND FIGURE 9. BIRDS RECEIVING NO ADDED SOYSTEROLS IN THE DIET EXCRETED A SUBSTANTIAL AMOUNT OF PLANT STEROLS (7.61 AND 13.15 MG/HEN/DAY) WITH HCO AND SFO BASAL DIETS RESPECTIVELY. THE AMOUNTS OF PLANT STEROLS EXCRETED WAS GREATER FROM HENS FED SAFFLOWER OIL DIETS THAN THOSE FED DIETS CONTAINING HYDROGENATED COCONUT OIL. THEREFORE, PLANT STEROL EXCRETION INDICATES THAT DIETARY OIL SOURCE CONTRIBUTED TO THE PLANT STEROLS EXCRETION. THE HIGHER CONTENTS OF PLANT STEROLS IN SAFFLOWER OIL (0.65%) THAN HYDROGENATED COCONUT OIL (0.35%) WOULD ACCOUNT FOR THE

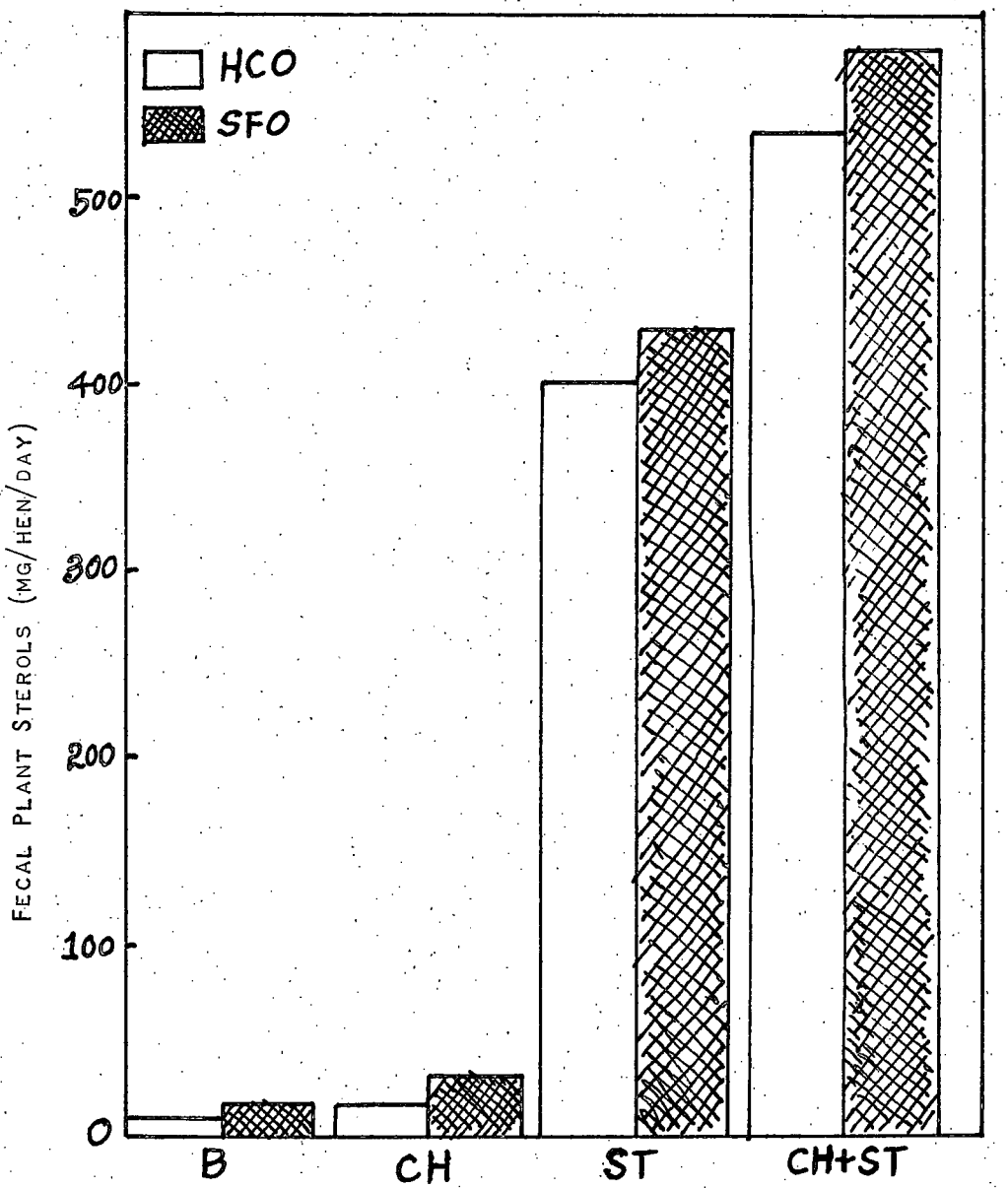

FIG. 9.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE FECAL PLANT STEROL OUTPUT.

DIFFERENCE BETWEEN THE TWO DIETARY OIL TREATMENTS (TABLE 3).

WHEN BIRDS WERE FED DIETS SUPPLEMENTED WITH 2% OF SOYSTEROLS, THE FECAL PLANT STEROLS INCREASED TO MORE THAN 400 MG/HEN/DAY. HOWEVER, THE TYPE OF DIETARY OIL AND THE PRESENCE OF DIETARY CHOLESTEROL HAD A DIRECT INFLUENCE ON FECAL PLANT STEROL LEVELS. SOYSTEROL FEEDING WITH THE SAFFLOWER OIL DIET RESULTED IN A GREATER FECAL OUTPUT OF PLANT STEROLS COMPARED TO EXCRETION DUE TO THE HYDROGENATED COCONUT OIL DIET. THE DIETARY RRESENCE OF CHOLESTEROL FURTHER ACCELERATED THE FECAL OUTPUT OF PLANT STEROLS (TABLE 9, FIG. 9). THE INFLUENCE OF DIETARY CHOLESTEROL ON FECAL OUTPUT OF PLANT STEROLS WAS SHOWN IN BIRDS THAT WERE FED EITHER SOYSTEROL SUPPLEMENTED OR UNSUPPLEMENTED DIETS (FIG. 9).

WHEN THE RETENTION RATE (%) OF DIETARY SOYSTEROLS WAS CALCULATED BY THE DIFFERENCE BETWEEN THE AMOUNT FED AND THE AMOUNT RECOVERED IN FECES, THE APPARENT ABSORPTION OF SOYSTEROL IN LAYING HENS FED **HCO+ST** AND **SFO+ST** DIETS WERE 78.10 AND 77.11% RESPECTIVELY. RESULTS REVEALED THAT DIETARY CHOLESTEROL INGESTION SLIGHTLY SUPPRESSED THE PLANT STEROL ABSORPTION.

DATA OBTAINED CLEARLY DEMONSTRATED THAT PLANT STEROLS USED IN THIS STUDY WERE ABSORBED BY LAYING HENS IN RELATIVELY LARGE AMOUNTS AND THE ABSORBABILITY WAS COMPARABLE TO THAT OF CHOLESTEROL. THESE RESULTS ARE IN CONTRAST TO THE ORIGINAL REPORT THAT PLANT STEROLS ARE VIRTUALLY UNABSORBABLE (SCHOENHEIMER, 1931). CRYSTALLINE SITOSTEROL BLENDED IN THE DIET OF MAN (SCHON, 1959) OR RAT (SWELL ET AL., 1956) WERE ALSO REPORTED AS NON-ABSORBABLE. HOWEVER, THE ADDITION OF LARGE AMOUNTS OF DIETARY FATTY ACIDS

(OLEIC ACID) OR UNSATURATED OIL HAVE BEEN FOUND TO IMPROVE STEROL ABSORPTION IN RATS (IVY ET AL., 1955). FURTHERMORE, SCHON AND ENGELHARDT (1957) REPORTED THAT MORE THAN 50% OF STEROL FROM DIETS CONTAINING 2 OR 4% SITOSTEROL WAS ABSORBED BY THE RAT.

CLARENBURG ET AL. (1971) ALSO OBSERVED THAT LAYING HENS RETAINED MORE THAN 60% OF THE DIETARY PLANT STEROLS WHEN 2% OR 4% B-SITOSTEROL EMULSION WAS FED. THEREFORE, THE RELATIVELY HIGH RETENTION OF PLANT STEROL IN THE PRESENT STUDY WAS PROBABLY THE RESULT OF EITHER THE PHYSICAL MODIFICATION OF SOYSTEROL MIXTURES DUE TO THE HIGH LEVEL OF DIETARY OIL, OR THAT BIRDS USED POSSESSED A HIGH CAPACITY FOR STEROL ABSORPTION.

EVIDENCES DESCRIBED ABOVE INDICATED THAT THE ANTI-STEROGENIC FUNCTION OF PLANT STEROLS IN LAYING HENS IS DUE TO AN INFLUENCE ON CHOLESTEROL CATABOLISM RATHER THAN CHOLESTEROL ABSORPTION. THIS FUNCTION APPEARS TO INCREASE THE DEGRADATION AND EXCRETION OF CHOLESTEROL AS BILE ACIDS AND STEROL METABOLITES.

PLANT STEROL DEPOSITION IN EGG YOLK, LIVER AND HEART TISSUES

PLANT STEROLS AND CHOLESTEROL OBSERVED IN THE EGG YOLK AND LIVER AND HEART TISSUES BY THE GLC TECHNIQUE ARE PRESENTED IN TABLES 10 AND 11 RESPECTIVELY.

ALL THE EGG YOLK SAMPLES FROM THE BIRDS FED DIETS CONTAINING SOYSTEROLS CONSISTANTLY CONTAINED PLANT STEROLS, WHEREAS NO DETECTABLE QUANTITIES WERE DEPOSITED IN THE EGG YOLK FROM BIRDS FED THE NON-SOYSTEROL SUPPLEMENTED DIET (TABLE 10). THE LACK OF DETECTION OF PLANT STEROLS IN THE EGG YOLK FROM THE NON-SOYSTEROL

TABLE 10.--Effect of dietary oil, cholesterol, and soysterols on the cholesterol and plant sterol deposition into egg yolk of laying hens (Trial 2).

Treatments	Cholesterol	Plant Sterols
	(mg)	(mg)
HCO	18.89	--
SFO	15.83	--
HCO+CH	20.76	--
SFO+CH	23.36	--
HCO+ST	13.73	0.17
SFO+ST	12.17	0.39
HCO+CH+ST	14.85	0.18
SFO+CH+ST	17.02	0.48

TABLE 11.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE CHOLESTEROL AND PLANT STEROL DEPOSITION IN LIVER AND HEART TISSUES OF LAYING HENS (TRIAL 2).

TREATMENTS	LIVER	HEART	
	CHOLESTEROL	CHOLESTEROL	PLANT STEROLS
		(MG/G. OF DRY TISSUE)	
HCO	12.94	7.40	--
SFO	16.63	3.38	--
HCO+CH	17.90	5.59	--
SFO+CH	22.92	3.84	--
HCO+ST	13.40	2.82	5.54
SFO+ST	15.95	2.90	3.75
HCO+CH+ST	16.85	4.71	3.72
SFO+CH+ST	24.55	3.35	5.37

TREATMENT REVEALED THAT THE ABSORBED PLANT STEROLS DERIVED FROM DIETARY SOYSTEROLS WERE TRANSFERRED TO EGG YOLK.

THE LEVELS OF PLANT STEROLS DEPOSITED IN EGG YOLK, HOWEVER, WERE SMALL COMPARED TO THAT OF CHOLESTEROL. THESE EXTREMELY LOW LEVELS OF PLANT STEROLS IN RELATION TO CHOLESTEROL IN EGG YOLK STEROLS MAY EXPLAIN WHY SOME INVESTIGATORS HAVE NOT OBSERVED THE PLANT STEROL IN THE EGG YOLKS FROM LAYING HENS FED CONSIDERABLE AMOUNTS OF PLANT STEROLS EITHER AS NATURAL OIL (CORN OIL OR SOYBEAN OIL) OR PURIFIED BETA-SITOSTEROL (WOOD ET AL., 1961; WEISS ET AL., 1967A). IT IS POSSIBLE, HOWEVER, THAT A VERY LOW CONCENTRATION OF PLANT STEROL COULD HAVE ESCAPED DETECTION IN THE PRESENCE OF A LARGE CONCENTRATION OF CHOLESTEROL (WEISS ET AL., 1967A) OR SOME STEROL-LIKE IMPURITY OF PIGMENTS WHICH MIGHT OVERLAP THE RETENTION IN GLC ANALYSIS MASK THE APPEARANCE OF PLANT STEROLS (MIETTINEN ET AL., 1965).

THE AMOUNT OF PLANT STEROLS DEPOSITED IN EGG YOLK WAS MARKEDLY GREATER WHEN SOYSTEROLS WERE FED WITH SAFFLOWER OIL THAN WITH HYDROGENATED COCONUT OIL (ABOUT TWO-FOLD). VALUES FOR PLANT STEROL CONCENTRATIONS WERE ONLY 0.17 AND 0.18 MG/G OF WET EGG YOLK WITH HCO+ST AND HCO+CH+ST DIETS RESPECTIVELY, WHEREAS THERE WERE MARKEDLY HIGHER VALUES OF 0.39 AND 0.48 MG/G WITH SFO+ST AND SFO+CH+ST DIETS RESPECTIVELY (TABLE 10). THIS INDICATES THAT HIGHLY UNSATURATED FATTY ACIDS FACILITATE THE PLANT STEROL TRANSPORT INTO EGG YOLK. IF IT IS ASSUMED THAT THE METABOLISM OF PLANT STEROLS IS SIMILAR TO THAT OF CHOLESTEROL, THEN IT IS POSSIBLE THAT UNSATURATED FATTY ACIDS ARE ESSENTIAL TO ESTERIFY

THE PLANT STEROLS WHICH ARE INCORPORATED PREFERABLY INTO LIPOPROTEIN AND TRANSPORTED TO EGG YOLK (SINCLAIR, 1935; GOULD, 1955).

IT IS INTERESTING TO NOTE THAT THE DEPOSITION OF PLANT STEROLS IN THE EGG YOLK WAS ALSO ACCOMPANIED WITH A REDUCTION IN THE EGG CHOLESTEROL CONTENT, WHEN SOYSTEROLS WERE FED TO LAYING HENS IRRESPECTIVE OF THE TYPE OF DIETARY OIL OR AMOUNT OF DIETARY CHOLESTEROL IN THE DIET (TABLE 10). THESE RESULTS INDICATED THE POSSIBILITY THAT YOLK CHOLESTEROL LEVELS WERE REDUCED CONCOMITTANTLY WITH THE DEPOSITION OF PLANT STEROLS.

THE PHARMACEUTICAL USE OF PLANT STEROLS HAS BEEN PROPOSED (GERSON ET AL., 1965), DUE TO THEIR POTENCY IN LOWERING BLOOD CHOLESTEROL LEVELS WITHOUT CAUSING HARMFUL SIDE EFFECTS EVEN WHEN ADMINISTERED IN LARGE DOSES. HENCE, THE INCORPORATION OF PLANT STEROL IN EGG YOLK MAY BE BENEFICIAL TO THE CONSUMER.

NO DETECTABLE PLANT STEROLS WERE OBSERVED IN THE LIVER TISSUE, WHEREAS CONSIDERABLE CONCENTRATIONS WERE DEPOSITED IN THE HEART TISSUE WHEN SOYSTEROLS WERE ADDED TO THE LAYING HEN DIETS (TABLE 11).

DATA ON STEROL LEVELS IN THE LIVER TISSUES DID NOT EXHIBIT ANY CHOLESTEROL REDUCING EFFECT DUE TO DIETARY SOYSTEROLS AS SHOWN IN THE EGG YOLK. IRRESPECTIVE OF SOYSTEROL LEVEL IN THE DIET, CHOLESTEROL ACCUMULATED IN THE LIVER TISSUE WHEN HENS WERE FED DIETS CONTAINING CHOLESTEROL. THE CHOLESTEROL ACCUMULATION INCREASED TO A GREATER EXTENT WITH SAFFLOWER OIL THAN HYDROGENATED COCONUT OIL TREATMENT (TABLE 11). THESE RESULTS ARE IN ACCORD WITH OBSERVATIONS IN RAT EXPERIMENTS THAT FEEDING CHOLESTEROL LED

TO AN INCREASE IN LIVER STEROL, AND SITOSTEROL HAD NO EFFECT ON THE LIVER STEROL LEVEL (GOULD, 1954; GOULD ET AL., 1955; SWELL ET AL., 1956).

THE ABSENCE OF PLANT STEROLS IN THE LIVER TISSUES OF LAYING HENS WITH A HIGH LEVEL OF PLANT STEROLS ABSORPTION (TABLE 9) INDICATE THAT PLANT STEROLS WERE RAPIDLY REMOVED FROM THE SERUM AND DEGRADED BY THE LIVER (PROBABLY TO BILE ACIDS AS INDICATED BY SUBBIAH ET AL., 1969) AND/OR TRANSFERRED TO OTHER TISSUE COMPARTMENTS.

IT HAS BEEN REPORTED THAT THE RATE OF DISAPPEARENCE FROM BLOOD OF LABELLED PLANT STEROLS AFTER ABSORPTION OR INJECTION WAS MUCH FASTER THAN THAT OF CHOLESTEROL, SUGGESTING THAT PLANT STEROLS ARE NOT ACCUMULATED IN THE LIVER BECAUSE OF RAPID EXCRETION OR DEGRADATION (GOULD, 1954; GOULD ET AL., 1955; SWELL ET AL., 1956).

THE PREDOMINANT PROPORTIONS OF PLANT STEROLS DETECTED IN THE HEART TISSUES INDICATE THAT PLANT STEROLS MAY BE SELECTIVELY TRANSFERRED TO THE HEART. HOWEVER, NO DEFINITE TREND RELATIVE TO DIETARY TREATMENT WAS OBSERVED FOR PLANT STEROL OR CHOLESTEROL DEPOSITION IN THE HEART (TABLE 11).

EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE LIPID CONCENTRATIONS IN SERUM, EGG YOLK AND LIVER

SERUM AND EGG YOLK: TOTAL LIPIDS EXTRACTED FROM SERUM AND EGG YOLK SAMPLES WERE EXPRESSED AS G OF LIPIDS PER 100 ML OF SERUM AND MG/G OF WET EGG YOLK (TABLE 12).

THE PERCENTAGE LIPID CONTENT OF EGG YOLK WAS NOT

TABLE 12.--Effect of dietary oil, cholesterol, and soysterols on the lipid concentrations of serum and egg yolk in laying hens (Trial 2).

Treatments	Serum Lipids	Egg Yolk Lipids
	(g/100 ml)	(mg/g)
HCO	3.15^{A}[1]	284.52^{A}[1]
SFO	2.93^{A}	286.88^{A}
HCO+CH	3.43^{A}	285.54^{A}
SFO+CH	3.67^{A}	276.54^{A}
HCO+ST	2.61^{A}	284.20^{A}
SFO+ST	2.27^{A}	295.05^{A}
HCO+CH+ST	2.46^{A}	284.48^{A}
SFO+CH+ST	1.63^{A}	288.13^{A}

[1] Means within a column followed by the same superscript are not significantly different at 5% level of probability.

SIGNIFICANTLY AFFECTED BY THE DIETARY OIL AND/OR STEROL TREATMENT. RESULTS AGREE WITH REPORTS THAT VARIOUS OILS OR FATS EITHER WITH OR WITHOUT CHOLESTEROL IN THE HEN'S DIET, DID NOT INFLUENCE THE TOTAL LIPID CONTENT OF EGG YOLK (REISER, 1950; WHEELER ET AL., 1959; CHUNG ET AL., 1965). UNLIKE THE EGG YOLK, SERUM LIPIDS WERE INCREASED BY DIETARY CHOLESTEROL, AND DECREASED BY DIETARY SOYSTEROLS IRRESPECTIVE OF DIETARY OIL TYPE (FIG. 10), ALTHOUGH DIFFERENCES WERE NOT STATISTICALLY SIGNIFICANT ($P < 0.05$). LAYING HENS FED SAFFLOWER OIL HAD LOWER SERUM LIPID LEVELS THAN THOSE FED HYDROGENATED COCONUT OIL WITH THE EXCEPTION OF HENS FED CHOLESTEROL WITHOUT SOYSTEROLS.

CHOLESTEROL INGESTION MARKEDLY ELEVATED THE SERUM LIPID LEVELS, HOWEVER, THIS EFFECT ON SERUM LIPIDS DUE TO CHOLESTEROL FEEDING WAS PREVENTED WHEN SOYSTEROLS WERE FED IN THE DIET. IN FACT, THE SERUM LIPID LEVELS WERE DEPRESSED WHEN SOYSTEROLS WERE FED (FIG. 10).

THE LIPID LOWERING EFFECT OF SOYSTEROLS WAS ALSO OBSERVED WHEN HENS WERE FED DIETS CONTAINING SOYSTEROLS ALONE WITHOUT CHOLESTEROL.

THE TREND OF SERUM TOTAL LIPID LEVELS INFLUENCED BY DIFFERENT TREATMENTS APPEARED TO BE CLOSELY REFLECTED BY THAT OF SERUM STEROL LEVELS, ALTHOUGH THE ABSOLUTE CHANGE DUE TO TREATMENT WAS NOT IDENTICAL (TABLE 12, FIG. 10 AND TABLE 7, FIG. 6). HOWEVER, IT SHOULD BE NOTED THAT THE SERUM STEROL LEVEL WAS ONLY A SMALL FRACTION (LESS THAN 10%) OF TOTAL LIPIDS (TABLE 7).

THE SERUM LIPID LEVELS AFTER STEROLS WERE SUBSTRACTED FOR

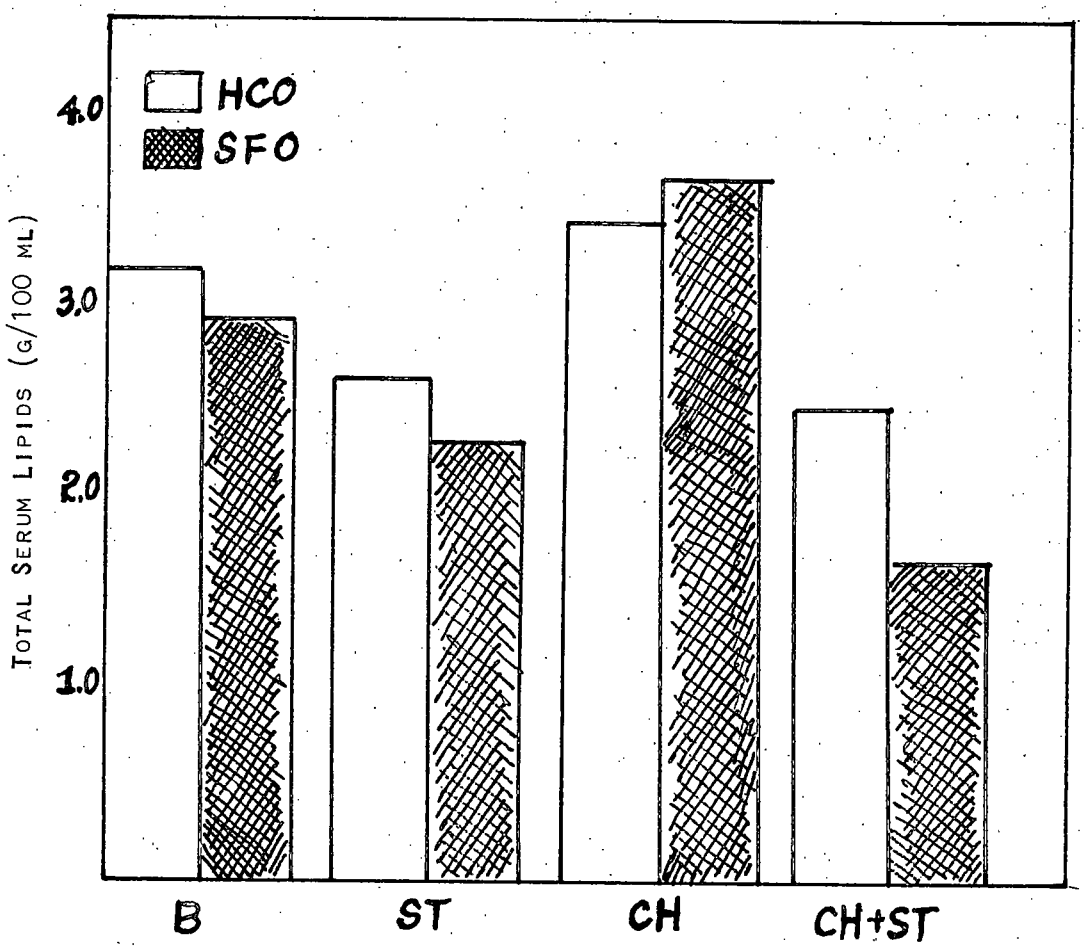

FIG. 10.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE TOTAL SERUM LIPID LEVELS.

EACH TREATMENT ARE SHOWN IN FIGURE 11. THE SERUM STEROL-FREE LIPID CONCENTRATION FROM EACH TREATMENT GROUP CLEARLY SHOWED MARKED DIFFERENCES. THIS INDICATED THAT THE HYPERSTEROLEMIC EFFECT OF DIETARY CHOLESTEROL AND THE HYPOSTEROLEMIC EFFECT OF DIETARY PLANT STEROLS WAS A RESULT OF CHANGES IN TOTAL LIPID CONCENTRATION RATHER THAN ALTERATION IN SERUM STEROL LEVELS *PER SE*.

AN EARLIER REPORT HAS SHOWN THAT EITHER EXOGENOUS CHOLESTEROL OR DIETHYLSTILBESTEROL ADMINISTRATION TO CHICKENS LED TO A SIGNIFICANT INCREASE IN BLOOD LIPIDS (LINDSEY *ET AL.*, 1946). IN ANY EVENT, IT WOULD BE INTERESTING TO KNOW WHAT ROLE PLANT STEROLS PLAY IN DECREASING SERUM LIPIDS AND WHAT ROLE CHOLESTEROL PLAYS IN INCREASING THE SERUM LIPIDS.

LIVER WEIGHT AND LIPID ACCUMULATION: LIVER WEIGHT (G OF FRESH LIVER PER 100 G OF BODY WEIGHT) AND THE LIPID CONTENT (MG OF LIPID EXTRACT PER G DRIED TISSUE) WERE USED TO ASSESS THE DIETARY EFFECT ON LIPID ACCUMULATION IN THE LAYING HEN'S LIVER (TABLE 13).

LIVERS FROM HENS FED THE SAFFLOWER OIL SOURCE WERE GENERALLY SMALLER WITH A LOWER LIPID CONTENT THAN LIVERS FROM HENS FED HYDROGENATED COCONUT OIL IRRESPECTIVE OF DIETARY CHOLESTEROL AND/OR SOYSTEROL SUPPLEMENTATION (TABLE 13 AND FIG. 12). WHEN **HCO** BASAL DIET WAS FED, BOTH LIVER WEIGHT AND LIPID CONTENT WERE SIGNIFICANTLY ($P < 0.01$) INCREASED AS COMPARED TO HENS FED THE **SFO** BASAL DIET.

ADDITION OF CHOLESTEROL TO THE DIETS PRODUCED A SIGNIFICANT ($P < 0.01$) INCREASE IN LIVER WEIGHT AND LIPID CONTENT. SOYSTEROLS

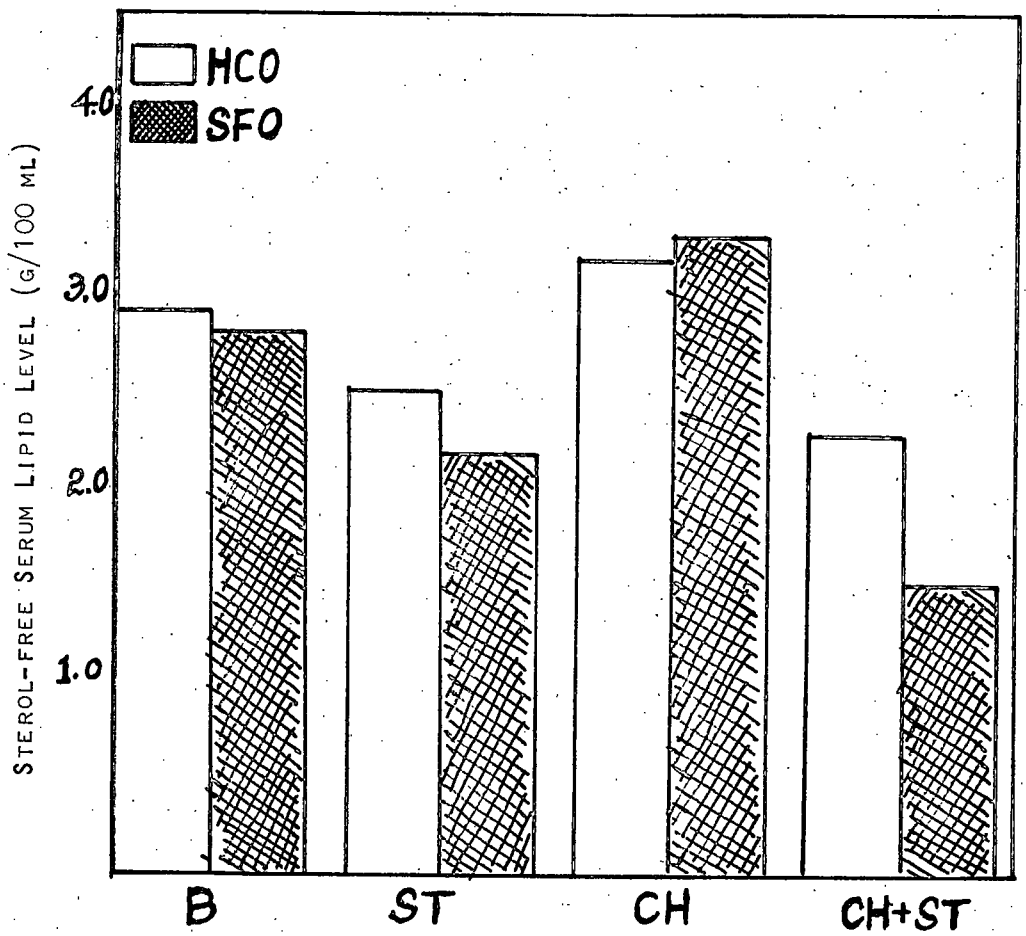

FIG. 11.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE STEROL-FREE SERUM LIPID LEVELS. (TOTAL LIPIDS MINUS TOTAL STEROLS.)

TABLE 13.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE WEIGHT AND LIPID CONTENT IN LIVER OF LAYING HENS (TRIAL 2).

TREATMENTS	LIVER WEIGHT[1]	LIPID CONTENT[2]
	(G/100G B.W.)	(MG/G DRY TISSUE)
HCO	2.72^{BC}[3]	484.2^{D}[3]
SFO	1.67^{A}	145.7^{A}
HCO+CH	2.91^{C}	568.2^{E}
SFO+CH	2.50^{BC}	397.5^{C}
HCO+ST	2.50^{BC}	457.5^{D}
SFO+ST	1.80^{A}	184.4^{AB}
HCO+CH+ST	2.67^{BC}	488.8^{D}
SFO+CH+ST	2.31^{B}	217.8^{B}

[1] RATIO OF FRESH LIVER WEIGHT TO BODY WEIGHT.

[2] LIPID CONCENTRATION IN DRIED TISSUE.

[3] MEANS WITHIN A COLUMN FOLLOWED BY THE SAME SUPERSCRIPT ARE NOT SIGNIFICANTLY DIFFERENT AT 1% LEVEL OF PROBABILITY.

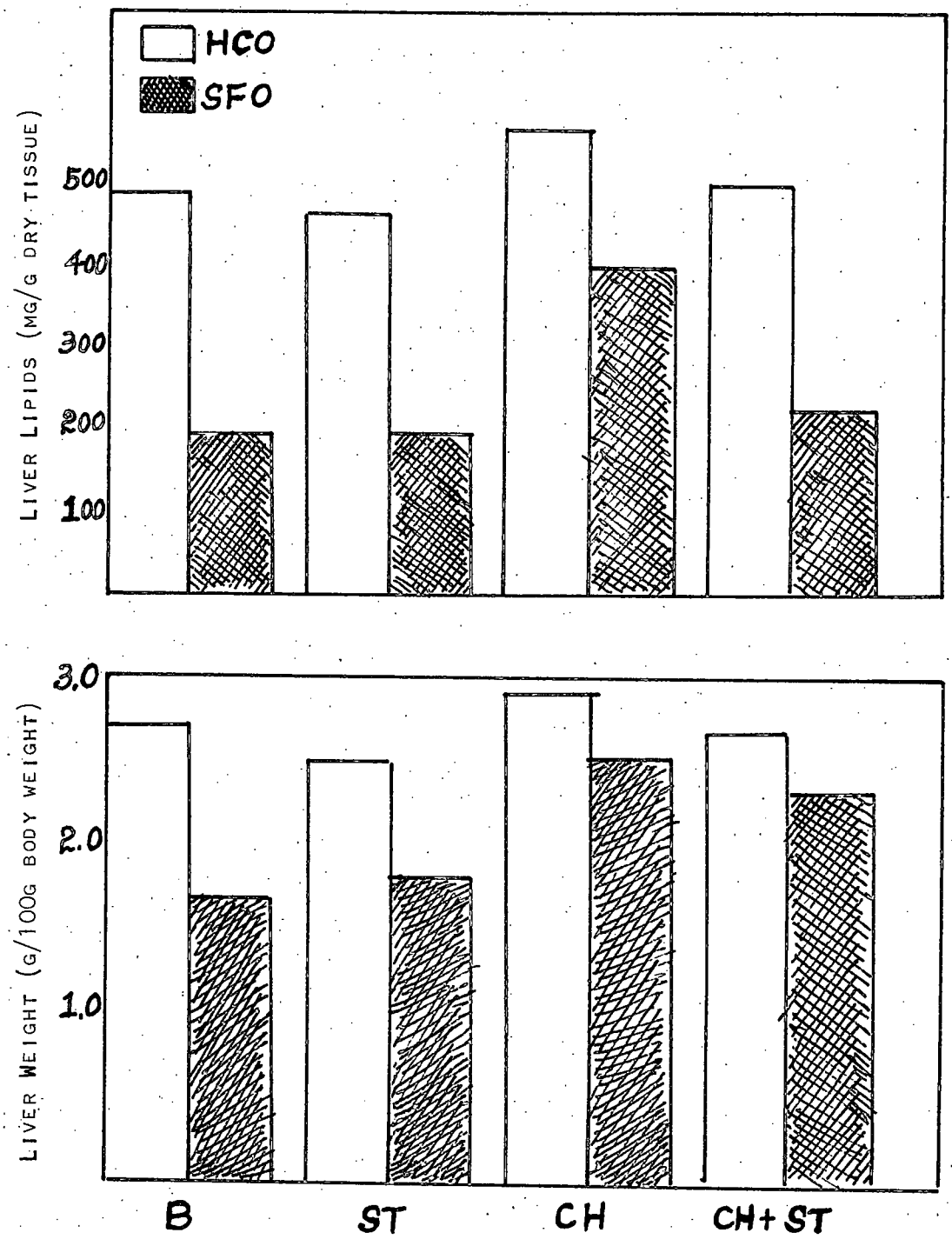

FIG. 12.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE LIVER WEIGHT AND LIPID CONTENTS.

ADDITION TO THESE DIETS HAD NO EFFECT IN THE ABSENCE OF DIETARY CHOLESTEROL, BUT IN THE PRESENCE OF CHOLESTEROL THE SOYSTEROL COUNTERACTED THE EFFECT OF DIETARY CHOLESTEROL ON LIPID ACCUMULATION AND RESULTED IN A NET DECREASE OF THE LIVER LIPID CONCENTRATION.

THE DEGREE OF LIPID ACCUMULATION APPEARS IN GENERAL TO BE CLOSELY RELATED TO LIVER WEIGHT (FIG. 12). THIS RELATIONSHIP (IN THE CASE OF FATTY LIVER SYNDROME) HAS BEEN DEMONSTRATED IN THE GROWING CHICK (HOPKINS AND NESHEIM, 1967), THE LAYING HEN (BRAGG ET AL., 1973) AND THE RAT (OSTWALD AND LYMAN, 1968).

THE DIFFERENTIAL EFFECT OF DIETARY OIL ON THE LIPID ACCUMULATION OBSERVED IN THE PRESENT STUDY ARE ATTRIBUTED TO THE DIFFERENT FATTY ACID MAKE-UP OF THE OIL PER SE. THE NATURE OF DIETARY OILS ARE CHARACTERIZED WITH HIGHLY UNSATURATED FATTY ACIDS IN SAFFLOWER OIL AND SATURATED MEDIUM CHAIN FATTY ACIDS IN THE HYDROGENATED COCONUT OIL (TABLE 3). THE RESULTS ARE IN AGREEMENT WITH PREVIOUS REPORTS THAT FAT ACCUMULATION IN THE LIVER OF GROWING AND LAYING CHICKENS CAN BE PRODUCED BY FEEDING SATURATED DIETARY FAT OR ESSENTIAL FATTY ACID DEFICIENT DIETS (DONALDSON AND GORDON, 1960; MENGE, 1967; BRAGG ET AL., 1973).

ALTHOUGH THE EXACT ROLE OF POLYUNSATURATED FATTY ACIDS ON THE HEPATIC LIPID METABOLISM IS NOT COMPLETELY UNDERSTOOD, EXPERIMENTAL EVIDENCE INDICATES THAT SATURATED FATTY ACIDS DERIVED FROM DIETS ACCELERATE HEPATIC LIPOGENESIS AND UNSATURATED FATTY ACIDS SUPPRESS LIPOGENESIS IN LIVER (BORTZ ET AL., 1963; SABINE ET AL., 1969; SIM ET AL., 1973). HOWEVER, LIPID SECRETION FROM

LIVER IS RETARDED BY SATURATED OR POLYUNSATURATED FATTY ACID DEFICIENCY (SINCLAIR AND COLLINS, 1968; MADSEN, 1969; FUKAMZAWA ET AL., 1970).

ESSENTIAL FATTY ACIDS NECESSARY FOR THE FORMATION OF THE PHYSICOCHEMICAL STRUCTURE OF LIPOPROTEIN ARE ESSENTIAL FOR THE TRANSPORT OF LIPIDS INTO THE CIRCULATION (ALFIN-SLATER AND AFTERGOOD, 1968; RUDERMAN ET AL., 1968). THEREFORE, LIPID ACCUMULATION IN THE LIVER FROM HENS FED HYDROGENATED COCONUT OIL OBSERVED IN THIS STUDY MAY BE THE RESULT OF AN IMBALANCE BETWEEN LIPOGENESIS AND THE RATE OF HEPATIC LIPID SECRETION. IF A SUFFICIENT AMOUNT OF LIPOPROTEIN WERE FORMED, THERE WOULD BE MINIMAL LIPID ACCUMULATION IN THE LIVER EVEN THOUGH LIPOGENESIS WAS PROCEEDING AT A RAPID RATE. HOWEVER, IF THE LIVER CAN ONLY PRODUCE A MINIMUM OR LIMITED AMOUNT OF LIPOPROTEIN (BUT NOT ENOUGH IN RESPONSE TO THE HIGHER RATE OF LIPOGENESIS), LIPIDS WILL ACCUMULATE.

IT HAS OFTEN BEEN DEMONSTRATED THAT FEEDING CHOLESTEROL RESULTS IN FAT ACCUMULATION IN LIVER AND EVENTUALLY LEAD TO A SEVERE FATTY LIVER SYNDROME IN RATS (KLEIN, 1958; MORIN ET AL., 1962) AND IN CHICKENS (MARCH, 1973). FURTHERMORE, IT HAS BEEN FOUND THAT THE COMPOSITION OF HEPATIC LIPIDS ACCUMULATED BY CHOLESTEROL FEEDING DIFFERS FROM THAT CAUSED BY THE ORDINARY DIETARY FATTY LIVER SYNDROME. THE LATTER CAUSES ACCUMULATION OF MAINLY TRIGLYCERIDES, WHEREAS THE FATTY LIVER CAUSED BY FEEDING CHOLESTEROL IS CHARACTERIZED BY AN UNUSUAL PROPORTION OF TRIGLYCERIDES AND CHOLESTEROL ESTERS (LUCAS AND RIDOUT, 1967).

CHUNG ET AL. (1970) USING GROWING CHICKS AND WEISS ET AL. (1967B) USING LAYING HENS OBSERVED THAT CHOLESTEROL FEEDING STIMULATED LIPOGENESIS IN THE LIVER TISSUES AND THAT THE FATTY ACID COMPOSITION OF TISSUE LIPIDS WERE GREATLY ALTERED DUE TO DIETARY CHOLESTEROL BY INCREASING OLEIC ACID (CHUNG ET AL., 1967, 1966). IT MIGHT BE THE RESULT OF A HOMEOSTATIC MECHANISM IN LIVER RESPONDING TO AN UNUSUAL AMOUNT OF EXOGENOUS CHOLESTEROL BY INCREASING FATTY ACID SYNTHESIS IN ORDER TO ELIMINATE THE OVERLOAD OF CHOLESTEROL FROM LIVER. FURTHERMORE, IT IS KNOWN THAT LONG CHAIN FATTY ACIDS, PARTICULARLY OLEIC ACID, ARE IMPORTANT FOR THE FORMATION OF CHOLESTEROL ESTERS AND THEIR TRANSPORT (SINCLAIR AND COLLINS, 1968; STEINBERG, 1963).

THE ROLE OF SOYSTEROLS COUNTERACTING THE CHOLESTEROL EFFECT ON HEPATIC LIPID ACCUMULATION IS NOT CLEAR. HOWEVER, THERE IS SOME INDIRECT EXPERIMENTAL EVIDENCE THAT INTRAPERITONEAL INJECTION OF PLANT STEROL LEADS TO DECREASED LIPID CONTENTS IN THE LIVER AND AORTA DUE TO AN ACCELERATION OF OXIDATIVE DEGRADATION OF LIPIDS (GERSON, ET AL., 1964, 1965).

FATTY ACID COMPOSITION: FATTY ACID COMPOSITIONS OF LIVER, SERUM AND EGG YOLK LIPIDS (PERCENT OF TOTAL METHYL ESTERS) ARE PRESENTED IN TABLES 14, 15 AND 16 RESPECTIVELY. BIRDS ON DIETARY HYDROGENATED COCONUT OIL (HCO) CONSUMED A LARGE PROPORTION OF SATURATED MEDIUM-CHAIN FATTY ACIDS, WHILE THE BIRDS ON THE SAFFLOWER OIL (SFO) CONSUMED MAINLY UNSATURATED LONG-CHAIN FATTY ACIDS, LINOLEIC ACID AND OLEIC ACID (TABLE 3).

TABLE 14.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE FATTY ACID COMPOSITION OF LIVER LIPIDS IN LAYING HENS (TRIAL 2).

TREATMENTS	FATTY ACIDS[1]							
	$C_{12:0}$	$C_{14:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
	% OF TOTAL METHYLESTERS[2]							
HCO	2.5	5.6	1.4	26.7^{D}	6.3	9.6^{B}	41.3^{D}	6.3^{A}
HCO+CH	1.5	4.0	0.9	22.0^{BC}	5.4	7.2^{A}	51.3^{F}	7.5^{AB}
HCO+ST	2.0	6.7	1.6	20.1^{B}	6.1	8.5^{AB}	47.3^{E}	8.4^{B}
HCO+CH+ST	2.3	7.7	1.5	27.7^{D}	5.2	9.2^{B}	39.2^{D}	7.0^{A}
SFO	---	0.4	TR	19.7^{B}	1.8	20.2^{D}	22.6^{A}	27.0^{DE}
SFO+CH	---	0.4	TR	15.8^{A}	2.7	10.0^{B}	37.8^{D}	27.8^{E}
SFO+ST	---	0.4	TR	23.0^{C}	1.9	14.9^{C}	32.9^{C}	26.5^{CD}
SFO+CH+ST	---	0.6	TR	21.9^{BC}	2.2	13.3^{C}	27.0^{B}	25.7^{C}

[1] CARBON CHAIN LENGTH:NUMBER OF DOUBLE BOND.

[2] MEANS WITHIN COLUMN FOLLOWED BY SAME SUPERSCRIPTS ARE NOT SIGNIFICANTLY DIFFERENT AT 1% LEVEL OF PROBABILITY.

TABLE 15.--EFFECT OF DIETARY OIL, CHOLESTEROL, AND SOYSTEROLS ON THE FATTY ACID COMPOSITION OF SERUM LIPIDS IN LAYING HENS (TRIAL 2).

	PERCENT OF TOTAL METHYLESTERS[1]							
	FATTY ACIDS							
TREATMENTS	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$	$C_{20:4}$
	% OF TOTAL METHYLESTERS[2]							
HCO	1.7	3.9	25.4^{F**}	4.5^{C**}	13.4^{AB**}	37.8^{D**}	11.5^{B**}	1.2^{A*}
HCO+CH	1.3	3.3	23.5^{DE}	4.6^{C}	12.9^{A}	41.0^{E}	11.4^{B}	1.6^{A}
HCO+ST	2.1	2.6	22.7^{CD}	3.0^{B}	15.9^{C}	31.8^{B}	17.2^{D}	3.8^{C}
HCO+CH+ST	3.4	4.1	25.1^{EF}	4.4^{C}	13.7^{AB}	35.3^{C}	10.1^{A}	2.8^{B}
SFO	---	0.5	23.3^{DE}	2.5^{B}	14.1^{B}	33.3^{BC}	22.7^{E}	2.6^{B}
SFO+CH	---	0.4	18.9^{A}	2.4^{B}	12.8^{A}	40.1^{DE}	23.2^{E}	1.8^{A}
SFO+ST	---	1.5	22.0^{C}	1.4^{A}	22.3^{D}	24.9^{A}	23.4^{E}	2.7^{B}
SFO+CH+ST	---	1.0	20.6^{AB}	1.7^{A}	22.0^{D}	23.0^{A}	26.9^{F}	3.7^{C}

[1] CARBON CHAIN LENGTH:NUMBER OF DOUBLE BOND.

[2] MEANS WITHIN COLUMN FOLLOWED BY SAME SUPERSCRIPTS ARE NOT SIGNIFICANTLY DIFFERENT AT 1% (**) OR 5% (*) LEVEL OF PROBABILITY.

TABLE 16.--EFFECT OF DIETARY OIL, CHOLESTEROL, AND SOYSTEROLS ON THE FATTY ACID COMPOSITION OF EGG YOLK LIPIDS (TRIAL 2).

TREATMENTS	FATTY ACIDS[1]						
	$C_{12:0}$	$C_{14:0}$	$C_{16:0}$	$C_{16:1}$	$C_{18:0}$	$C_{18:1}$	$C_{18:2}$
	% OF TOTAL METHYLESTERS[2]						
HCO	0.9	4.2	22.8^{AB}	3.9^{C}	9.6^{BC}	49.5^{C}	7.9^{A}
HCO+CH	0.8	3.6	22.9^{AB}	4.2^{CD}	8.0^{AB}	50.1^{C}	9.3^{B}
HCO+ST	1.1	4.0	22.5^{AB}	5.0^{E}	9.6^{A}	49.7^{C}	9.0^{B}
HCO+CH+ST	1.4	4.0	24.3^{B}	4.8^{DE}	7.2^{A}	42.0^{B}	11.2^{C}
SFO	---	0.3	33.0^{C}	2.1^{A}	9.9^{C}	32.8^{A}	20.8^{D}
SFO+CH	---	0.6	19.9^{A}	2.8^{B}	9.5^{BC}	42.5^{B}	24.1^{E}
SFO+ST	---	0.5	24.5^{B}	2.6^{A}	9.9^{C}	35.0^{A}	27.9^{G}
SFO+CH+ST	---	0.5	21.3^{AB}	3.0^{B}	9.2^{BC}	39.2^{B}	26.3^{F}

[1] CARBON CHAIN LENGTH: NUMBER OF DOUBLE BOND.

[2] MEANS WITHIN COLUMN FOLLOWED BY SAME SUPERSCRIPTS ARE NOT SIGNIFICANT AT 1% LEVEL OF PROBABILITY.

When HCO was fed, however, no fatty acids with a chain-length shorter than 12-carbon atoms was detected in the liver, serum and egg yolk lipids. Only low levels of lauric acid ($C_{12:0}$) were deposited in liver, serum and egg yolk lipids, even though the HCO diet contained a high level of this fatty acid. Large amounts of oleic (about 50%), palmitic and stearic acids were observed in tissue and egg yolk (Tables 14, 15 and 16). These results indicate that short chain fatty acids (caprylic, capric and lauric acids) supplied by the diet are rapidly catabolized or converted in laying hens to longer chain fatty acids, probably to palmitic, stearic and oleic acids (Kirschner and Harris, 1961; Guenter et al., 1971).

Although the level of linoleic acid in HCO was minimal (4.55%), a substantial proportion was found in liver, egg yolk and serum lipids. Furthermore, arachidonic acid ($C_{20:4}$), the metabolite of linoleic acid, was also detected in serum. The consistent appearence of this dienoic acid ($C_{18:2}$) in the liver, egg yolk and serum is due to either a very low level in the basal feed ingredients and/or from the existing body reserves, since the laying hen can not synthesize this dienoic acid (Reiser, 1951; Murty et al., 1960).

There was no detection of eicosatrienoic acid ($C_{20:3}$), the metabolite of oleic acid which has been known as a biochemical indicator of essential fatty acid-deficiency symptoms (Machlin and Gordon, 1961; Hill et al., 1961). Therefore, laying hens are not sensitive to essential fatty acid deficiency even when a saturated oil (HCO) was fed for long period of time. Machlin and

GORDON (1962) OBSERVED THAT EGG YOLK CONTAINED RELATIVELY HIGH LEVELS (6%) OF LINOLEIC ACID, EVEN AFTER A 12-WEEK DEPLETION AND GUENTER ET AL. (1971) OBSERVED 3.09% AFTER 16 WEEKS OF DEPLETION IN WHICH ESSENTIAL FATTY ACID DEPLETION DIETS WERE FED. THESE WORKERS SUGGESTED THAT ADULT HENS MOBILIZED THIS FATTY ACID FROM THE DEPOT FAT, WHERE A LARGE AMOUNT HAD BEEN STORED BEFORE THE ONSET OF LAYING.

WHEN BIRDS WERE FED SAFFLOWER OIL (SFO) CONTAINING A HIGH LEVEL OF LINOLEIC ACID AND A RELATIVELY LOW LEVEL OF OLEIC ACID, THE FATTY ACID COMPOSITION IN LIVER, SERUM AND EGG YOLK LIPIDS SHOWED A CLOSER RELATIONSHIP TO THAT OF DIETARY OIL FED THAN WAS OBSERVED FROM BIRDS FED HCO (TABLES 14, 15 AND 16). THE SIMILARITY IN FATTY ACID COMPOSITION OF LIVER, SERUM AND EGG YOLK LIPIDS WITH THAT OF THE DIETARY FATTY ACIDS SUGGESTS THAT THEY WERE EITHER DEPOSITED DIRECTLY OR WITH LITTLE MODIFICATION DUE TO DE NOVO BIOSYNTHESIS OR CATABOLISM IN THE LIVER TISSUE.

IT HAS BEEN SHOWN THAT POLYUNSATURATED FATTY ACIDS IN THE DIET HAVE A SUPPRESSING EFFECT ON FATTY ACID SYNTHESIS IN LIVER (ALLMAN AND GIBSON, 1965; MUTO AND GIBSON, 1970; GUENTER ET AL., 1971), AND ARE OXIDIZED TO CO_2 AT A MUCH SLOWER RATE (KIRSCHNER AND HARRIS, 1961) COMPARED TO THE DIETARY SHORT CHAIN FATTY ACIDS.

CHUNG ET AL. (1970) USING GROWING CHICKS, AND WEISS ET AL. (1967B) USING LAYING HENS HAVE DEMONSTRATED THAT LIPOGENESIS WAS GREATLY DIMINISHED IN THE LIVER WHEN SAFFLOWER OIL WAS FED, WHEREAS LIPOGENESIS WAS STIMULATED WHEN HYDROGENATED COCONUT OIL WAS FED.

THE PATTERNS OF FATTY ACIDS IN LIVER, SERUM AND EGG YOLK

WERE GREATLY ALTERED BY DIETARY TREATMENTS OF CHOLESTEROL AND/OR SOYSTEROLS. CHOLESTEROL FEEDING INCREASED OLEIC ACID AND DECREASED THE LEVELS OF PALMITIC AND/OR STEARIC ACIDS IN TISSUE AND EGG YOLK. SOYSTEROLS EXERTED A SIMILAR EFFECT TO THAT OF DIETARY CHOLESTEROL, ALTHOUGH THE DEGREE OF CHANGE WAS NOT AS PRONOUNCED AS WITH CHOLESTEROL. WHEN SOYSTEROL WAS FED IN COMBINATION WITH CHOLESTEROL, THE CHOLESTEROL EFFECT (INCREASE IN OLEIC ACID) WAS COMPLETELY DIMINISHED, IN THE LIVER, AND EGG YOLK (HCO+CH+ST) OR SIGNIFICANTLY REDUCED (SFO+CH+ST) AS SHOWN IN FIGURES 13 AND 15 FOR LIVER AND EGG YOLK. IN CONTRAST, SOYSTEROL EITHER WITH OR WITHOUT CHOLESTEROL DEPRESSED OLEIC ACID LEVEL IN SERUM LIPIDS, AND APPEARED TO INCREASE SERUM ARACHIDONIC ACID LEVELS (FIG. 14). THESE RESULTS ARE IN ACCORD WITH THE REPORTS BY CHUNG ET AL. (1966, 1967) THAT THE MONOENOIC ACID IN THE CHICKEN LIVER AND BODY FAT WAS INCREASED, AND SATURATED ACIDS SUCH AS PALMITIC AND STEARIC ACIDS WERE DECREASED WHEN CHOLESTEROL WAS FED TO THE GROWING CHICK OR TURKEY. THIS SUPPORTS THE OBSERVATIONS WITH RABBITS (EVANS ET AL., 1959) AND CHICKS (LEVEILLE ET AL., 1963). HOWEVER, A SEARCH OF THE LITERATURE DID NOT REVEAL ANY REPORTS ON THE EFFECTS OF PLANT STEROLS ON THE FATTY ACID COMPOSITION. IF THE METABOLIC FATE OF PLANT STEROLS RESEMBLES THAT OF CHOLESTEROL (SWELL ET AL., 1959; WERBIN ET AL., 1960), IT IS POSSIBLE THAT PLANT STEROLS AFFECT THE FATTY ACID METABOLISM AS DOES CHOLESTEROL.

THE INCREASE IN OLEIC ACID, AND DECREASE IN PALMITIC OR STEARIC ACIDS IN LIVER INDICATE THAT LAYING HENS WERE STIMULATED TO INCREASE FATTY ACID SYNTHESIS, MAINLY FROM PALMITIC TO OLEIC

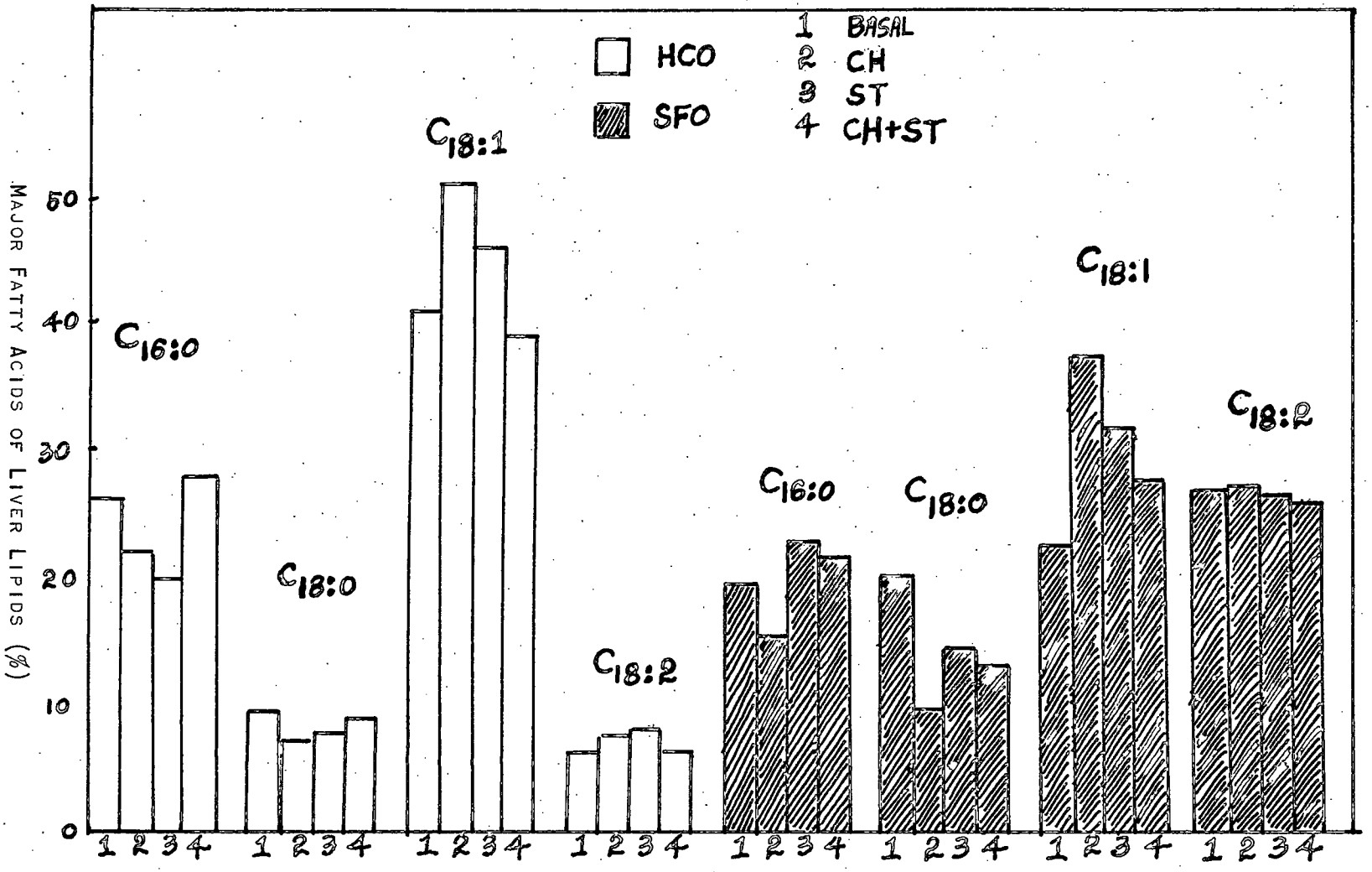

FIG. 13.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE FATTY ACID COMPOSITION OF LIVER LIPIDS.

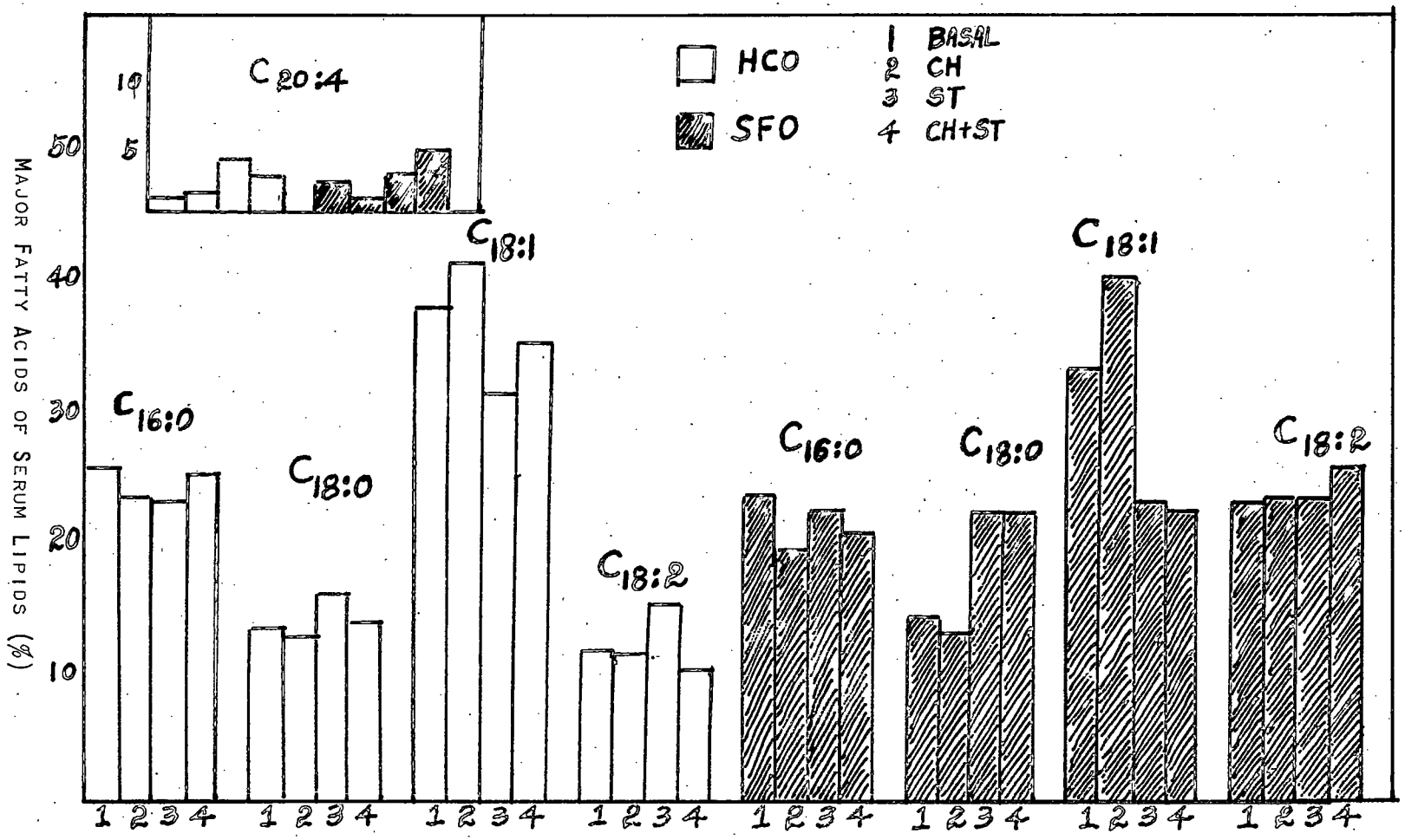

FIG. 14.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE FATTY ACID COMPOSITION OF SERUM LIPIDS.

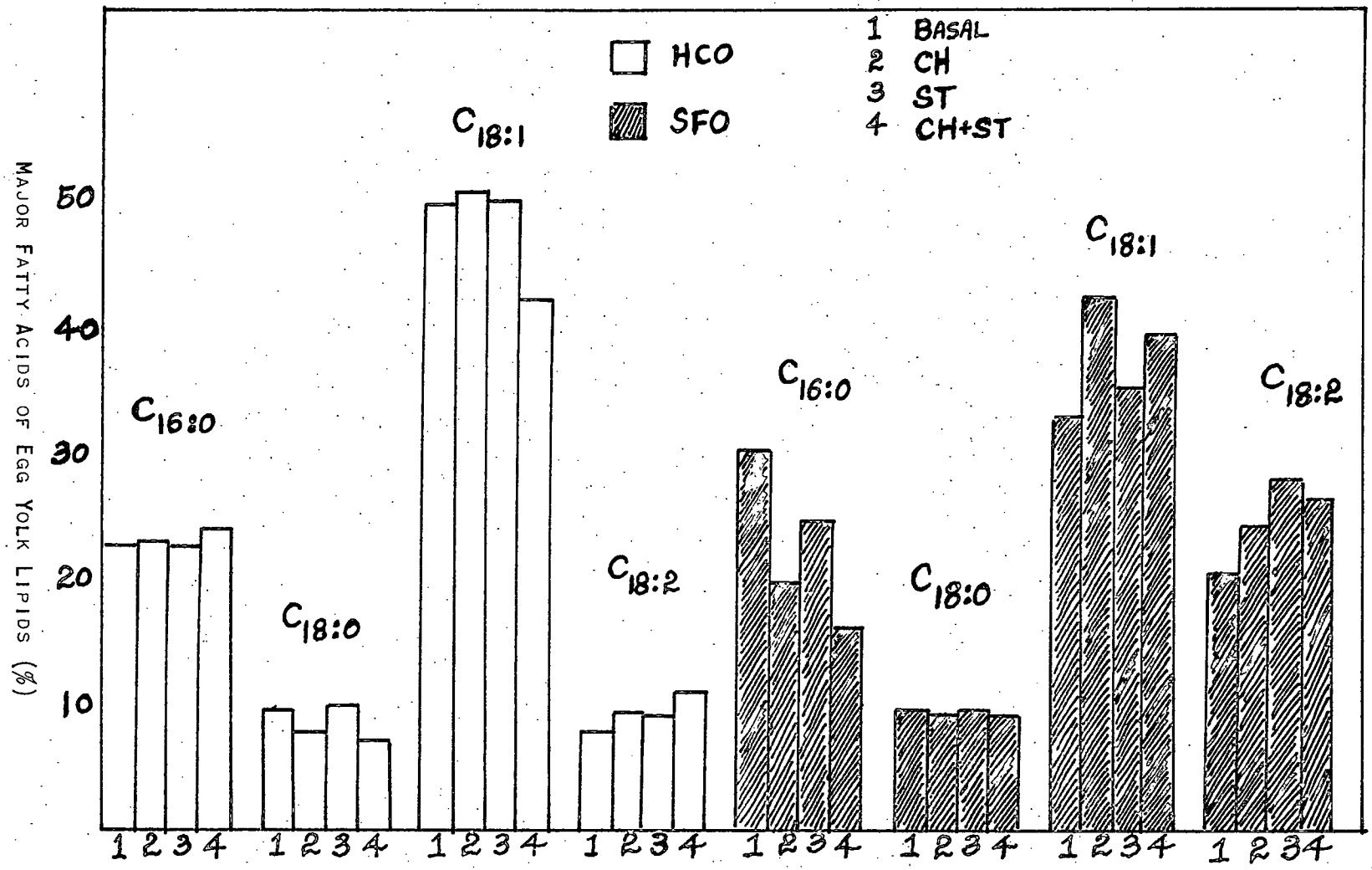

FIG. 15.--EFFECT OF DIETARY OIL, CHOLESTEROL AND SOYSTEROLS ON THE FATTY ACID COMPOSITION OF EGG YOLK LIPIDS.

ACID BY TWO CARBON-ELONGATION OF DESATURATION OF STEARIC ACID IN THE CASE OF LARGE AMOUNTS OF EXOGENOUS CHOLESTEROL. IT IS BELIEVED THAT THE HIGHER CHOLESTEROL POOL IN THE LIVER OR THE INTESTINAL WALL CREATES A DEMAND FOR MORE OLEIC ACID NECESSARY TO SATISFY THE HIGHER AFFINITY OF THE CHOLESTEROL-ESTERIFYING ENZYMES (VAHOUNY, 1958; GOODMAN, 1965; SWELL AND TREADWELL, 1955).

THERE IS EXPERIMENTAL EVIDENCE IN RATS THAT PLANT STEROL STIMULATES FATTY ACID SYNTHESIS IN LIVER, BUT THE TURNOVER RATE IS FURTHER ACCELERATED BY OXIDATIVE DEGRADATION (GERSON ET AL., 1965). HOWEVER, INSUFFICIENT INFORMATION IS AVAILABLE AT PRESENT TO COMPLETELY DELINEATE THE ROLE OF SOYSTEROL ON THE FATTY ACID METABOLISM.

SUMMARY AND CONCLUSIONS

EFFECTS OF DIETARY LIPID FACTORS (SATURATED AND UNSATURATED OIL, CHOLESTEROL AND PLANT STEROLS) ON THE SERUM AND EGG YOLK STEROL LEVELS, AND INFLUENCE ON CHOLESTEROL AND FATTY ACID METABOLISM IN THE LAYING HEN WERE INVESTIGATED.

SINGLE COMB WHITE LEGHORN LAYING HENS AT THIRTY-WEEK OF AGE WERE USED IN TWO TRIALS BY FEEDING TWO BASAL DIETS CONTAINING 8% HYDROGENATED COCONUT OIL OR SAFFLOWER OIL. THESE BASAL DIETS WERE FED WITH OR WITHOUT SUPPLEMENTAL CHOLESTEROL (1%), SOYSTEROLS (2%) OR COMBINATION.

LAYING HENS RESPONDED TO THE DIETARY LIPID FACTORS BY INCREASING TOTAL CHROMOGENIC STEROL LEVELS IN SERUM AND EGG YOLK UPON CHOLESTEROL INGESTION AND RAPIDLY DECREASING UPON SOYSTEROL FEEDING. THE CHANGES IN EGG YOLK STEROL LEVELS CAUSED BY DIETARY FACTORS WERE GENERALLY PARALLEL TO THE SERUM STEROL LEVELS, AND INCREASED OR DECREASED STEROL LEVELS OF EGG YOLK WERE PRECEDED BY SERUM CHANGES INDICATING THAT EGG YOLK STEROLS ARE DERIVED FROM SERUM. IT ALSO INDICATED THAT EGG STEROL DEPOSITION IS AN IMPORTANT PATHWAY IN LAYING HENS TO ELIMINATE CIRCULATING STEROLS.

SAFFLOWER OIL *PER SE* HAD A SUPERIOR PROPERTY TO HYDROGENATED COCONUT OIL IN SUPPRESSING STEROL LEVELS IN BOTH SERUM AND EGG YOLK. THE STEROL-LOWERING EFFECT OF SAFFLOWER OIL WAS ACCOMPANIED BY A GREATER FECAL OUTPUT OF BILE ACIDS AND, TO

A LESS EXTENT, CATABOLIC NEUTRAL STEROLS AS COMPARED TO HYDROGENATED COCONUT OIL.

CHOLESTEROL SUPPLEMENTATION TO THE SAFFLOWER OIL BASAL DIET RESULTED IN A SIGNIFICANT ($P < 0.01$) ELEVATION OF SERUM AND EGG YOLK STEROL LEVELS, WHEREAS FEEDING CHOLESTEROL IN COMBINATION WITH HYDROGENATED COCONUT OIL DID NOT CHANGE THE SERUM LEVEL. THIS SYNERGISTIC EFFECT OF SAFFLOWER OIL ON THE INCREASE IN SERUM AND EGG YOLK STEROL LEVELS DUE TO DIETARY CHOLESTEROL WAS CAUSED BY AN INCREASE IN ABSORPTION OF DIETARY CHOLESTEROL.

STEROL-LOWERING EFFECT OF SOYSTEROLS WAS CLEARLY DEMONSTRATED IN BOTH SERUM AND EGG YOLK BY FEEDING SOYSTEROL ALONE AS WELL AS BY FEEDING SOYSTEROLS IN COMBINATION WITH CHOLESTEROL. DEPRESSION IN THE STEROL LEVELS OF SERUM AND EGG YOLK CAUSED BY DIETARY SOYSTEROLS WAS ACCOMPANIED BY AN INCREASE IN FECAL OUTPUT OF BILE ACIDS AND CATABOLIC NEUTRAL STEROLS. THE FECAL OUTPUT OF CATABOLIC STEROLS AS BILE ACIDS AND NEUTRAL STEROLS, WERE FURTHER ENHANCED WHEN SOYSTEROLS AND CHOLESTEROL WERE FED SIMULTANEOUSLY. WHEN SOYSTEROLS WERE FED ALONE, ENDOGENOUS CHOLESTEROL EXCRETION IN FECES APPEARED TO INCREASE, BUT SOYSTEROL FEEDING WITH CHOLESTEROL DID NOT RETARD THE APPARENT ABSORPTION RATE OF CHOLESTEROL. THEREFORE, IT IS SUGGESTED THAT THE STEROL-LOWERING FUNCTION OF PLANT STEROLS WAS AT THE SITE OF CHOLESTEROL CATABOLISM RATHER THAN AT THE SITE OF CHOLESTEROL ABSORPTION.

THE APPARENT ABSORPTION OF PLANT STEROLS DERIVED FROM THE DIETARY SOYSTEROLS WAS 78% AND 77% WITH HCO+ST AND SFO+ST DIETS RESPECTIVELY AND WAS SLIGHTLY LOWERED BY SIMULTANEOUS INGESTION

OF SOYSTEROLS WITH CHOLESTEROL RESULTING IN APPARENT ABSORPTION OF 69% AND 67% WITH **HCO+CH+ST** AND **SFO+CH+ST** DIETS RESPECTIVELY. PLANT STEROL ABSORPTION BY LAYING HENS WAS FURTHER SUPPORTED BY DETECTING PLANT STEROL DEPOSITION IN THE HEART TISSUE AND EGG YOLK. HOWEVER, NO PLANT STEROLS WERE DETECTED IN THE LIVER TISSUE. THE RESULTS SUGGESTED THAT ABSORBED PLANT STEROLS DISAPPEARED RAPIDLY FROM THE LIVER BY CATABOLISM AND EXCRETION VIA THE FECES.

RESULTS SHOW THAT DEPOSITION OF PLANT STEROLS IN EGG YOLK WAS ACCOMPANIED BY A SIMULTANOUS REDUCTION (MORE THAN 20%) IN EGG CHOLESTEROL. THEREFORE, DIETARY PLANT STEROL CAN BE USED EFFECTIVELY TO REDUCE THE CHOLESTEROL CONTENT OF EGGS.

TOTAL LIPID LEVELS IN SERUM AND LIVER TISSUE WERE SIGNIFICANTLY HIGHER WITH DIETARY HYDROGENATED COCONUT OIL THAN WITH SAFFLOWER OIL. CHOLESTEROL INGESTION RESULTED IN LIPID ACCUMULATION AND SOYSTEROL INGESTION APPEARED TO DECREASE THE LIPID CONCENTRATION IN SERUM AND LIVER TISSUE. THE LIVER WEIGHT AND LIPID CONTENT WERE CLOSELY RELATED.

FATTY ACID COMPOSITIONS OF LIVER, SERUM AND EGG YOLK WERE ALTERED BY CHOLESTEROL AND SOYSTEROL INGESTION. THE PROPORTION OF OLEIC ACID WAS INCREASED AND SATURATED FATTY ACIDS (PALMITIC AND STEARIC ACID) WERE DECREASED BY CHOLESTEROL AND, TO A LESSER EXTENT, BY SOYSTEROL FEEDING. HOWEVER, SIMULTANEOUS INGESTION OF CHOLESTEROL AND SOYSTEROLS DIMINISHED THE OLEIC ACID ACCUMULATION IN TISSUE LIPIDS.

THE OBSERVATIONS OBTAINED FROM THE PRESENT INVESTIGATION LED TO A CONCLUSION THAT THE ANTI-HYPERSTEROGENIC LIPID FACTORS

SUCH AS DIETARY UNSATURATED FATTY ACIDS AND PLANT STEROLS

REGULATE LIPOGENESIS AND STEROL CATABOLISM IN THE LAYING HEN.

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APPENDIX

APPENDIX TABLE 1.--Analysis of variance for total sterol levels (chromogenic) in serum and in egg yolk (Trial 2).

Source of Variation	D.F.	Mean Square	
		Serum	Egg Yolk
Treatment	7	50325.194**	247.294**
Replication	7	88.729	2.963
Error	49	30.791	23.587
Total	63		

**Indicates significance at the 1.0 percent level of probability.

APPENDIX TABLE 2.--Analysis of variance for liver weight (g/100g body weight), liver lipid content (mg/g dry tissue), serum lipid level (g/100ml) and egg yolk lipid concentration (percent of fresh yolk).

Source of Variation	D.F.	Mean Square			D.F.	
		Liver Weight	Serum Lipid	Egg Yolk Lipid		Liver Lipid
Treatment	7	0.772**	1.778	11.098	7	78174.594**
Replicate	3	0.041	0.268	19.685	2	136.670
Error	21	0.056	4.349	53.583	14	75.673
Total	31				23	

**Indicates significance at the 1.0 percent level of probability.

APPENDIX TABLE 3.--Analysis of variance for major fatty acids of liver lipids (linoleic, oleic, stearic palmitic, and palmitoleic acid).

Source of Variation	D.F.	Mean Square			
		Linoleate	Oleate	Stearate	Palmitate
Treatment	7	325.665**	270.521**	55.593**	43.783**
Replicate	2	0.419	0.367	1.172	0.284
Error	14	0.226	1.107	0.448	0.825
Total	23				

**Indicates significance at the 1.0 percent level of probability.

APPENDIX TABLE 4.--Analysis of variance for the major fatty acids of serum lipids (arachidonate, linoleate, oleate, stearate, palmitoleate, and palmitate).

Source of Variation	D.F.	Mean Square					
		Arachidonate	Linoleate	Oleate	Stearate	Palmitoleate	Palmitate
Treatment	7	2.616*	103.720**	124.120**	47.766**	5.040**	14.078**
Replicate	2	0.078	1.702	1.741	0.105	0.156	1.347
Error	14	0.086	0.247	0.948	0.197	0.106	0.488
Total	23						

**Indicates significance at the level of 1.0 percent, * 5.0 percent level of probability.

APPENDIX TABLE 5.--Analysis of variance for the major fatty acids of egg yolk lipids (linoleate, oleate, stearate, palmitoleate and palmitate).

Source of Variation	D.F.	Mean Square				
		Linoleate	Oleate	Stearate	Palmitoleate	Palmitate
Treatment	7	205.007**	137.049**	4.0598**	3.445**	47.054**
Replicate	2	0.385	0.028	1.195	0.049	2.163
Error	14	0.156	1.867	0.453	0.128	1.826
Total	23					

**Indicates significance at the 1.0 percent level of probability.