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To the Graduate Council:

I am submitting herewith a dissertation written by Emmanuel D. Tebong entitled "Lipogenic and Lipotropic Factors in the Physiopathology of Fatty Liver Syndrome in Gallus Domesticus". I recommend that it be accepted for 36 quarter hours of credit in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Animal Science.

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LIPOGENIC AND LIPOTROPIC
FACTORS IN THE PHYSIOPATHOLOGY
OF FATTY LIVER SYNDROME IN
GALLUS DOMESTICUS

A Dissertation
Presented for the
Doctor of Philosophy
Degree
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Emmanuel D. Tebong

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Dedicated to my father, Marcus Tebong, whose untimely death deprived him from reaping the seeds he sowed, and to my children Burg, Ache, Njoh, Mbah, and Ndi, the most patient children in the world.

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ABSTRACT

Experiments were conducted to compare the lipogenic and lipotropic properties of selected treatments in laying hens.

"Lipogenic" factors studied were: high fat, rancid fat, rancid feed, orotic acid, aflatoxin, carbon tetrachloride and ethionine. "Lipotropic" factors included: vitamins E and C, choline, santoquin, biotin, linoleic acid and selenium. With a few exceptions, each "lipotropic" factor was tested in combination with each "lipogenic" factor.

Two-hundred and nineteen Leghorn type pullets, 30 weeks of age, were used in seven experiments. All hens were housed in individual laying cages and received standard management procedures.

Physiological parameters measured included the following: percent liver fat, liver weight, liver color, liver texture, liver haemorrhages, abdominal fat, comb condition, blood prothrombin time, sodium bromosulphthalein (BSP) clearance, egg production, egg weight, egg specific gravity and bone strength.

When compared with the standard laying ration, the rancid fat ration without additions significantly increased the percent liver fat, liver weight and the incidence of liver haemorrhages. The rancid feed ration also resulted in an increased in liver fat. BSP clearance rate was significantly reduced by the ethionine treatment. Egg weight was reduced by the high fat ration and egg specific gravity was reduced by the carbon tetrachloride treatment. Other differences were non-significant.

The effects of the "lipotropic" factors on most of the traits were highly variable and were influenced by the type of "lipogenic" treatment with which they were combined.

Choline exerted a lipogenic effect when fed in combination with a high fat ration - the percent of liver fat was increased. Choline reduced liver fat in the rancid feed and rancid fat treatments. The percent liver fat was decreased by vitamins C and E, santoquin and biotin in the rancid feed treatment and by choline in the rancid fat treatment.

Liver color was significantly influenced by "lipotropic" factors in the ethionine treatment only. Choline, santoquin and biotin resulted in livers with a greater degree of yellow color.

Choline in the ethionine treatment resulted in livers that were less fragile, while linolic acid combination with ethionine increased the fragility of the liver.

Choline reduced the incidence of haemorrhages in the rancid fat treatment. Liver haemorrhages were increased by dietary biotin and linoleic acid in the high fat treatment and santoquin in the rancid feed treatment.

The degree of abdominal fatness was reduced only by the feeding of biotin in the rancid feed treatment.

Prothrombin time was significantly increased by a wide variety of the "lipotropic" factors. Choline, santoquin, biotin, linoleic acid, vitamin C and vitamin E in the rancid feed treatment increased prothrombin time, as well as linoleic acid in the high

fat treatment. Biotin reduced the prothrombin time in the ethionine treatment.

Sodium bromosulphthalein clearance rate was lowered by choline but was raised by linoleic acid in the ethionine treatment.

Bone strength was reduced by choline, santoquin and biotin in rancid fat treatment but was increased by vitamin C in the rancid feed treatment.

These results suggest that rancidity in laying rations is a most likely cause of abnormally high fat deposition in the livers of hens and of other traits commonly associated with the "fatty liver syndrome". Of the substances tested in these experiments, the most effective were those that possess anti-oxidative properties or those subject to destruction by developing feed rancidity.

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CHAPTER I

INTRODUCTION

Today, twenty years after Couch observed "Fatty Liver Syndrome" in laying hens in Texas, the disease remains elusive, and continues to inflict significant losses to the poultry industry. These losses result from decreased egg production and to increased mortality in the laying flock.

Considering the numerous absorptive, metabolic, storage, secretory and excretory functions performed by the liver, it is not surprising that anything disturbing its normal functioning is reflected in the overall well being and performance of the animal.

The accumulation of excessive lipids in the liver has been referred to by a variety of terms including fatty livers, fatty liver disease, fatty liver syndrome (Couch, 1956), fatty liver haemorrhagic syndrome (Wolford and Polin, 1972), fatty infiltration, fatty metamorphosis and fatty degeneration (Isselbacher and Alpers, 1969).

Approximately five percent of the weight of a normal liver consists of fat, whereas in an animal with the fatty liver syndrome the level may be forty to sixty percent of the liver weight, with triglycerides (TG) forming the major component. A number of hypotheses have been advanced to explain this accumulation of TG

in the liver. These include increased mobilization of free fatty acids from peripheral depots; increased synthesis of TG in the liver; and decreased transport of TG from the liver to the plasma which could be the result of an impaired formation of lipoproteins.

Although the literature on "Fatty Liver Syndrome" in mammals is quite extensive, the major studies done with the avian species have centered mainly on the relationships of nutrient factors to its development.

The series of experiments reported herein were designed to study in laying hens the effects of selected agents alleged to possess lipogenic (production of fat by both fatty degeneration and infiltration) and lipotropic (prevention of fat from accumulation in the liver) properties for the development of fatty livers in various species, and the anatomical and physiological changes associated with these effects.

CHAPTER II

OBJECTIVES

The objectives of this study were to determine the effectiveness of selected chemicals, compounds and nutrients in the production and prevention of the "Fatty Liver Syndrome" in the domestic fowl, as measured by certain physical and physiological traits, and to categorize the mode of action of these substances on the basis of these traits.

CHAPTER III

REVIEW OF LITERATURE

Historical Background

Although the fatty liver syndrome has become a serious problem in only the last three decades, poultry producers have known about fatty livers for hundreds of years (Sunde, 1966). Aldrovandi in his book written in 1500 A.D. described in detail the fattening of hens by forced feeding or "stuffing" (Lind, 1963) to produce fatty livers and increased body fat.

The fatty liver syndrome in laying hens as presently known was first reported by Couch (1956) in Texas. Subsequently, the syndrome was reported in the North Central area and the Eastern seaboard of the U.S.A. (Woldford and Polin, 1972). In 1963, Ringer and Sheperd reported the incidence of fatty liver syndrome in Michigan.

Whitehead and Blair (1973), researchers at the Edinburg A.R.C. Poultry Center, reported that fatty liver syndrome has caused significant losses in the United Kingdom, Canada and Scandinavia. Particular heavy mortality has been reported from Australia. The amount of research into the fatty liver syndrome problem in England and Australia (Payne et al. 1974), and the several papers appearing in American literature are indicative of the magnitude of the problem.

Symptoms of "Fatty Liver Syndrome"

Victims of fatty liver syndrome are often excellent layers which on post-mortem examination contain excess abdominal and liver fat, as well as capillary hemorrhages and/or hematomas in their livers. Although there is no drop in production, mortality increases from 2-3% from fatty liver syndrome in the summer months. Paleness of the comb, wattles, and face are other characteristic symptoms observed on post-mortem examination in birds with hematomas or hemorrhaging of the liver. Woldford et al. (1971) reported that hens dying from fatty liver syndrome had numerous ova in the ovary, and there was usually an egg in the oviduct. Reedy (1968) reported fatty liver syndrome in laying hens to be characterised first by a decrease in egg production from 2 to 50 percent. Hens were usually heavier than anticipated for the age and stage of egg production which was due primarily to an excess of fat in the abdominal cavity and intestinal mesentery. The livers were enlarged, very fatty, extremely friable and vary in color from a tan to a pale yellow. Livers may show evidence of hemorrhages with some exhibiting multiple hematomas (Harms, et al., 1972; Abbott and Couch, 1971; and Nesheim and Ivy, 1970). A microscopic examination of the liver by Woldford et al. (1971) revealed an extremely disturbed cellular shape and configuration. The liver cells were distended due to fat infiltration and there appeared to be no organization of liver cells into the typical cord like structure observed in normal hens. Fat infiltration resulted in the cell nucleus being forced to one side of the cell rather than being in the center.

The fat content of the liver may go as high as 70% (Couch, 1956). The comb of affected birds have been described by Abbott and Couch (1971), Harms et al. (1972) as pale and sometimes exhibiting dark or cyanotic tips. A light dandruff or scaleness may be present on the combs and wattles and it may be excessively large and pale and relatively cool to the touch. Couch (1956) also observed that flocks affected by fatty liver syndrome were extremely more sensitive to stressors. Excessive heat, noise or handling of birds results in an increased mortality.

Factors Implicated in the Development of "Fatty Liver Syndrome" in Laying Hens

General

Numerous reports (Barton, 1967; Dean, 1968; and Bicknell, et al., 1969) indicate that the fatty liver syndrome is much more prevalent in birds in cages than in floor birds, suggesting that immobility may be a factor.

Several attempts were made to transmit the condition to normal layers through feed, transplantation of respiratory discharge, and injection of blood from affected birds without success (Abbott and Couch, 1971). However the syndrome tended to recur on the same premises year after year, yet did not occur on adjoining farms where the same strain of birds on the same feed source were maintained. Although several attempts have been made to reproduce the condition in chickens in the laboratory, Sunde (1966) and Barton (1967) were able to produce only a low incidence of fatty liver syndrome by

feeding diets high in energy. Duke et al. (1968); Bragg et al. (1972); Wolford and Murphy (1972); and Wolford and Polin (1972) were able to induce fatty liver syndrome in White Leghorn hens using a high energy diet in a controlled house temperature of 26.7°C.

The consumption of energy by a laying hen above that required for production and maintenance causes a positive energy balance and results in the syntheses and deposition of fat in the body. The liver is the organ which accumulates large amounts of fat when excess of energy is taken in by the bird.

Flock et al. (1938) produced very large, fatty livers in geese by stuffing them with a high carbohydrate diet; and in 1939, Flock et al. reported that the total lipids of the liver in geese, forced fed high carbohydrate diet, was due mainly to an increased level of neutral fats, while the total amount of liver phospholipids remained unchanged or was slightly increased.

Metabolic Imbalances

The accumulation of excessive lipid is due instead to an imbalance of those mechanisms which normally control the amount of fat in the organ (Jubb and Kennedy, 1963; Alper, Isselbacher, 1969). From this principle, it is deduced that a fatty liver may result from increased mobilization of depot fat, a decreased rate of oxidation of mobilized fat, and increased formation of fat, a reduced rate of removal from the liver, a decrease synthesis or release of lipoproteins from the liver cells, or a combination of those factors (Isselbacher, 1969).

In the past, emphasis on specific lipotropic substances has tended to obscure the fact that fatty livers results from other nutritional imbalances such as diets low in proteins (Sunde, 1966) or high in fats or carbohydrates (Palafox and Flegel, 1971; Bragg et al., 1972; Wolford and Murphy, 1972). Endogenous substances, especially those involving deficiencies of insulin, thyroxine, adenohipophyseal hormones, sex hormones, adrenal hormones may result in severe fatty infiltration (Jubb and Kennedy, 1967). Among the toxic factors known to cause fatty degeneration of the liver are bacterial toxins, phytotoxins (Hamilton and Garlich, 1970; Hamilton et al., 1971; Smith and Hamilton, 1970) and chemical poisons (Isselbacher and Alpers, 1969).

Because the average hen egg contains 7.5 gms of fat (Abbott and Couch, 1971), the laying hen must, of necessity, synthesize large amounts of fat. This means that the lipid within the organs and tissues of laying hens is in a constant state of turnover. Abbott and Couch (1971) have determined the fat content of the liver of a hen in normal state of health, general well being, and egg production to range from 20 to 40%, whereas the fat content of the liver of a non-layer ranges from 9 to 11%.

While reports of attempts to produce the fatty liver syndrome experimentally in laying hens have been conflicting (Abbott and Couch, 1971) fatty degeneration of the liver has been produced in other species by using several factors.

Dietary Energy Level and Fatty Acids

Feigenbaum and Fisher (1963) starved 2-week-old chicks for

five days and when re-fed a diet devoid of supplemental fat, a very high liver fat content and an increased liver size were observed within three days. Sunde (1966) studied the effect of dietary energy level on liver fat accumulation in laying hens, and reported that as the energy level of the diet increased, liver weight and liver fat values tended to increase. In the same experiment, Sunde (1966) put birds with high liver fat content on a low energy (930 calories productive energy per pound) diet in a cold environment and observed that the liver weight and fat content were decreased.

Barton (1967) studied the effect of dietary energy levels on the livers of laying hens. The fat content of the livers was estimated by a visual scoring method based on liver color and the presence or absence of liver hemorrhage. Sunde (1966) and Jensen et al. (1970) argued that visual liver scores were not satisfactory for measuring liver fat levels. The effect of calorie protein ratio was also studied by Barton (1967), and reported inconclusive results.

Barton, (1967), also studied the effect of several factors including energy levels for lowering liver fat content of laying hens. He found that hens switched from high to low energy diets had lower liver fat content after three weeks, and reached the same level as birds fed low energy diets continuously. Choline, Vitamin B₁₂ and E supplements showed some lowering of liver fat after five weeks. Although Sunde (1966), Barton (1967) and Duke (1968) claimed that the high energy mainly from carbohydrates and animal tallow were

the main factors responsible for fatty liver syndrome, the reports of Bragg et al (1972) showed that the nature of dietary fatty acid composition were more important than energy, per se. Dietary linoleic acid from soybean oil meal and sunflower oil was inversely related to liver size and lipid content. This demonstrated that linoleic acid in the diet prevented fat accumulation in the liver of laying chickens. Animal tallow is low in linoleic acid. Hopkins and Nesheim (1967) and Edward (1967) showed that linolic acid deficiency in chick diets resulted in reduced growth rate, enlarged livers and increased liver fat content, compared to chicks receiving adequate levels of linoleic acid.

Vitamins

Experiments conducted to demonstrate the mode of action of Vitamin E, showed that the liver mitochondria usually undergo peroxidation when they are not protected by either Vitamin E or B. Thus, Vitamin E appears to prevent exudative diathesis by its presence in the lipid membrane of the capillary wall where, even in the presence of plasma peroxides, the Vitamin E was capable of protecting the polyunsaturated fatty acids of the phospholipid structures of the membrane from undergoing destructive peroxidation (Scott, 1963).

Scherhailder and Griffith, (1973) showed that choline supplementation, or a combination of choline and Vitamin B₁₂ decreased liver fat, while a combination of methionine and Vitamin B₁₂ did not decrease as much, and liver fat was significantly higher in hens kept at higher temperatures than those kept under cool conditions.

Liver fat content appeared to be more closely related to environmental temperatures than to age of the hens, since liver fat content tended to rise during the summer warm months with all treatments. Wolford (1971) reported that laying chickens placed in a 1.7°C room for 28 days significantly reduced total liver lipids, in comparison to values observed when layers were housed at 26.7°C.

Fluect (1954) demonstrated that the corn used to induce fatty livers was correlated with a low choline content. Best and Huntsman (1932) and Salcedo (1944), observed that choline deficiency in rats was characterized by increased liver fat deposition. These symptoms become visible two weeks after putting rats on a choline deficient diet which was also low in protein and methionine but high in fat. But Fritz et al. (1967) found that liver fat content was not affected by feeding choline deficiency diet to growing chicks. McCollum et al. (1912) and Ringrose and Cavie (1946) observed that laying hens could synthesize substantial quantities of choline for egg production, and Leach et al. (1966) also reported that hens were able to synthesize substantial quantities of choline if there was sufficient methyl groups present in the diet.

Fisher and Weiss (1956) observed fatty livers in laying hens when fed a diet high in animal fats fortified with choline, and Barton (1967) reported erratic results by feeding a high energy diet supplemented with choline, Vitamin B₁₂ and methionine. Sutton et al. (1956) reported that a diet with 1% cholesterol caused an increase in blood cholesterol level and total liver fat content, and the supplementation of this diet with 1% choline exerted a lipotropic

action and reduced both the blood cholesterol level and the total liver fat, whereas an addition of 1% methionine lowered only blood cholesterol level but not total liver fat.

Norvell (1970) reported a lower per cent liver fat in laying hens fed purified diets supplemented with 0.2% choline when compared to per cent liver fat values of hens fed purified diets unsupplemented with choline. The effect of choline on liver fat content has yet to be resolved.

Griffith et al. (1969) recently investigated the effect of various nutrients on liver fat levels of laying hens. Supplementation of a low methionine diet with choline reduced liver fat content, whereas methionine or B₁₂ supplementation had no effect on liver fat content when added alone.

A deficiency of inositol in the diet of rats and mice caused lipemia (Wolley, 1950; Pavcek and Baum, 1941; Nelson and Black, 1944). Inositol was also reported to be a lipotropic factor in the rat by McHenry and Gavin, 1940; Engel, 1942; Best et al., 1946.

Chen and Chanalampous (1960) used yeast to demonstrate at least two enzymatic steps in the biosynthesis of inositol from glucose-6-phosphoric. Glucose-6-phosphate cyclase, catalyse the formation of D-inositol-1-phosphate; and Mg ions were required in this process. Quissenbery (1966) showed that the same biosynthetic pathway for inositol formation from glucose occurred in rat tissues. Rapoport (1940) states that the chick erythrocyte contains large amounts of phytic acid, which indicates the possible requirement for high levels of dietary inositol if biosynthesis was inadequate.

Considerable amounts of inositol are synthesized by the intestinal microbial flora (Nielson and Black, 1941; and Wolley, 1940).

Daughaday et al. (1954), showed the ^{14}C labelled glucose incorporated into inositol in the chick embryo indicating that synthesis occurs even prior to hatching.

Reed et al. (1968) reported that supplementation (with inositol) of diets fed to hens, diagnosed with fatty liver syndrome alleviated the problem. Bull (1968) also reported that inositol exerted a lipotropic effect when supplemented to diets. But Bossard and Combs (1970) showed that supplementation of broiler breeders' diet with inositol did not affect liver fat content. When fed to White Leghorn hens, inositol supplementation by Leveille and Bray (1970) and Rangland et al. (1970), Nesheim and Ivy (1970) observed no effect on liver fat content, but these results were at variance with field observation.

Effect of Antioxidant Substances

Very frequently the role of the antioxidant substances in ameliorating fatty livers has been cited. Di Luzio (1973) states that there is clear evidence which indicates that antioxidants are profound modifiers of a variety of chemical-induced hepatic lesion. Studies of the protective action of antioxidants on carbon tetrachloride induced liver injury indicated that the oral, intravenous, or intraperitoneal administration of a variety of lipid antioxidants inhibited liver triglyceride accumulation, hepatic necrosis and modified the carbon tetrachloride-induced lethality (Di Luzio, 1960).

The administration of lipid antioxidants by DiLuzio (1973), as mixed tocopherols to have normal subjects was associated with a significant reduction in conjugated diseases and a significant enhancement in lipid-soluble antioxidant activity in plasma. Removal of vitamin E was associated with a fall in plasma lipid antioxidant activity and a rise in conjugated diene levels, suggesting that the presence of the abnormal conjugated dienes in plasma lipids might be due to a relative antioxidant deficient state. In contrast to the effective reduction in liver triglycerides following lipid-soluble antioxidants administration to carbon tetrachloride-treated rats, (Hartman, et al., 1968) the water-soluble antioxidants, glutathione, and uric acid, did not significantly modify the CCL_4 induced fatty liver.

As recently as 1970, Jensen et al. reported a new lipotropic factor for laying hens. The addition of selenium to a corn-soybean meal diet significantly reduced liver weight and total lipids per liver. Jensen and his co-workers have suggested that selenium may be involved in the etiology of the fatty liver syndrome. Lavender et al. (1973) gave alcohol as the sole drinking fluid, and caused a delay in the onset of liver necrosis in rats fed a vitamin E and selenium deficient diet for 5 to 6 weeks. Those fed the basal vitamin E and selenium deficient diet tended to have less fat in their livers than animals fed the diet supplemented with vitamin E and selenium. These results conflict with the expected effect of the antioxidant properties of vitamin E and selenium.

Khairallah and Wolf (1965) observed a lipotropic action in

carnitine, when fed to rats in a diet deficient in choline and methionine and low in protein; although Adams et al. (1960) were unable to demonstrate any lipotropic action with the use of carnitine. Because carnitine is necessary for the transport of fatty acids across the mitochondria membrane where the fatty acids are oxidized, many researchers maintain that carnitine is a lipotropic agent (Khairallah and Wolf, 1965).

Effect of Age, Sex and Hormone Levels

Farenbacher et al. (1963) observed that forced feeding of geese resulted in fatty liver production, and the response was greater in females than in males.

Lorenz et al. (1938) studied the effect of age, sex and ovarian activity on blood lipids of chickens. They found no differences in the levels of cholesterol, phospholipids or total fatty acids in the blood of male birds from 71 to 276 days of age. Blood lipid values of immature females from 17 to 135 days of age were similar to those found in male chickens, whereas an increase in blood lipid levels occurred in females shortly before the start of egg laying. The lipemia became more pronounced during the laying cycle. The rise in blood lipid was confined to triglycerides, although there was a slight rise in phospholipids and cholesterol. Values of up to 4000 milligrams (percent) total blood lipids were observed in mature laying hens. In another experiment, Lorenz (1938) reported that a low concentration of free and esterified cholesterol, phospholipids and triglycerides was present in the liver of mature and immature males and in the immature female chicken. However, at

the onset of production, there was a pronounced increase in triglyceride level in the liver of the female chicken, whereas no notable change was found in the phospholipids or cholesterol level of the liver. The triglyceride content of the laying hen liver was decreased by feeding a diet containing 18% fat, whereas hens fed a diet of less than one percent fat had a more variable triglyceride content.

Lepkovsky and Yasude (1966) made hypothalamic lesions which resulted in hyperphagia in male Leghorn adult chickens. These chickens ate more feed and gained more weight over a seven-week period, compared to normal and sham-operated chickens, although feed efficiency was about the same. The effect of increased food intake upon body composition was studied. Normal males showed little abdominal fat and there was no fat deposition in the gizzard. However, males with functional castration superimposed upon the hyperphagic lesion became very obese with large quantities of abdominal, as well as visceral fat and fatty livers. Male chickens with only a hyperphagic lesion became obese, but had less body fat than the hyperphagic chickens that were functionally caponized, and the liver fat values were similar to those of normal male chickens.

Marion and Edwards (1962) reported newly hatched chicks to contain a high liver fat content, which declines rapidly in the first week. Day-old chicks have a liver fat value of up to 8% on a day weight basis. Chicks fed a low-protein diet required four weeks for liver fat values to return to normal. In contrast to the rat, blood and liver lipids of chickens are exceedingly high at the time of

hatching and lipid levels begin to decline within 4 days (Enterman, et al., 1940). Debb and Chakrabarti (1900) observed that the liver lipid content of different breeds of chickens were more or less the same. Quissenberry (1966) fed diets dupplemented with iodinated casein, and obtained lowered fatty liver scores with 100 gm per ton added; but with 200 gm per ton, the liver fat content rose. The addition of methionine or thyroprotein to layer diets caused an increased in the per cent of liver fat content (Roberson, 1970).

Hormone balance is a factor that has long been associated with the fatty liver syndrome. Only White Leghorn laying hens, both on the floor and in cages, have exhibited the fatty liver syndrome, whereas male and immature chickens have not (Ivy, 1971).

Endogenous imbalances involving deficiencies of insulin, thyroxine, adenohipophyseal hormones, the sex steriods, and the adrenal steroids may result in severe fatty infiltration of the liver (Jubb and Kennedy, 1963). Corticosteroid, catecholamines and sympathetic stimulation will also increase the rate at which fatty acids are released from adipose tissue (Raben, 1964). Feigelson et al. (1961) reported increased lipid deposition in the liver after peritoneal administration of epinephrine and norepinephrine. Brodie and Maikel, (1963) suspected that the catecholamines were indirectly implicated in fatty liver infiltration, particularly when CCl_4 was administered. Although the mechanism of fatty acids uptake by the liver is not well documented, Isselbacher and Alpers (1969) have noted that agents such as norepinephrine have been shown to diminish fatty acid uptake

by the isolated perfused liver. Fatty livers have also been reported in patients receiving high doses of exogenous steroids over a period of weeks. In rats, plasma free fatty acids increased when cortisol was given (Hill, 1965). Corticosteroids, catecholamines and sympathetic stimulation likewise increased the rate at which fatty acids were released from adipose tissue (Raben, 1965). Thus, peritoneal administration of epinephrine and norepinephrine can lead to an increased lipid deposition in the liver (Feigelson et al., 1966). Striking changes in blood and tissue composition may take place during the period of ovarian activity or following estrogen administration in birds. Dickson (1970) noted that these changes include lipemia and a significant deviation in blood calcium concentration; these responses are contrary to those seen in mammals in which estrogen appears to depress both blood calcium and blood lipid concentrations.

Accumulation of lipids in the liver is usually seen in the depancreatized dog and this lipid increase has been attributed to the fact that in the absence of the external secretion of the pancreas, there exists a subnormal capacity for the utilization of dietary lipids. Best et al. (1932) demonstrated that fatty livers in rats could be prevented, and purified lecithin was equally effective in preventing fatty livers in rats on a high energy diet.

The involvement of the sex hormones in the production of the fatty livers has been suggested by MacBryde et al. (1942). Estrogenically equivalent doses of estrone, estradiol, benzoate and diethyl-stilbestrol

in dogs produced fatty livers although estrogens administered orally to rats showed lipotropic activity. No changes have been reported with androgen and progesterone.

Epinephrine plays a major role in full fatty acid immobilization in mammals (Carlson, 1966) but the effect in the avian species have not been well documented. Recent investigations (Langslow et al., 1970) show that glucagon alleviates plasma FFA from chicken adipose tissue in vitro, thereby making glucagon an important lipolytic hormone in chickens.

Blood lipid levels in mature laying hens are elevated when compared to young females or males, thus demonstrating a hormonal change in the laying hen at the beginning of oviposition (Lorenz et al., 1937). In 1966, Lepkovsky and Yasuda, reported that adult male chickens with induced hyperphagia caponized exhibited very fatty livers whereas normal roosters had a very low liver fat content. Heald and Rookledge (1964) demonstrated that estrogen treatment caused the free fatty acid level of plasma, total lipids and lipophosphoprotein level in mature fowls to rise. Thyroxine and testosterone decreased levels of plasma lipids and lipoproteins in laying hens, whereas no effects were observed on plasma free fatty acids in both laying and young chickens.

Aflatoxins

Stout and Adair (1970) observed the incidence of a hemorrhagic liver disease in the mink which appeared to be similar to aflatoxicosis. Herring meal was a common feed ingredient in the diet of mink dying

from hemorrhagic liver, and an analysis revealed the presents of dimethylnitrosamine, a potent carcinogen (Magee and Swann, 1969). Butler (1964) reported that the administration of aflatoxin or dimethylnitrosamine to ducklings caused extensive biliary proliferation in the liver with fatty degeneration of the peripheral parenchyma cells. Extensive bile duct hyperplasia, proliferation of ductular cells and parenchymal necrosis were observed by Vles (1967) when ducklings were dosed with either aflatoxin 15, or lithocholic acid or dimethylnitrosamine. Aflatoxin contamination of feed or litter has been suspected as an agent involved in the development of fatty liver syndrome in laying hens. *Aspergillus flavus*, a fungus common in feedstuffs is capable of producing aflatoxins. Aflatoxins posses carcinogenic properties (Lancaster et al., 1961).

Carnaghan et al. (1963) working with two strains of aflatoxin found that aflatoxin B₁ to be twice as toxic as G₁, when administered orally to day-old ducklings. Sarith and Hamilton (1970) fed several levels of aflatoxin-up to 10 ppm to broiler chicks to 3 weeks. Growth rate was depressed with levels above 1.25 ppm, and with higher levels of aflatoxin, chick livers were pale and enlarged. The increased liver size resulted from high lipid content.

In 1970, Hamilton and Garlich (were reported to have) successfully induced fatty liver syndrome in laying hens with aflatoxin. Graded levels of aflatoxin were fed to laying chickens and enlarged fatty livers, and a drop in egg production were obtained in 3 weeks. Sims et al. (1970) reported that hens fed 8 ppm of aflatoxin B₁ in the diet

had reduced body weight and egg production, and the liver size varied from normal to enlarged. Liver colors were brown to pale yellow and hemorrhages were seen in some livers. The mode of action of aflatoxin has been studied in ducks by Sarcsim and Monle (1973) who observed that injection of aflatoxin B₁ strongly inhibited in vivo protein synthesis in the liver by impairing RNA metabolism in the liver. The action of aflatoxin in the chicken, a species similar to the duck, has not been investigated thoroughly, but Carneghan et al. (1966) reported a reduced RNA: DNA ratio in chicken livers during aflatoxicosis.

Chemical Compounds

A. Carbon Tetrachloride: Many chemicals notably ethionine, CCL₄, and orotic acid have been used to induce fatty livers. Jubb and Kennedy (1963) have suggested that these poisonous chemicals produce fatty liver syndrome by causing fatty degeneration of the liver by injuring the mitochondria. Carbon tetrachloride, they maintain, alters the permeability of the mitochondria and leads to disorganization of the enzymes, thereby allowing the accumulation of tricyclerides. Smuckler and Benditt, (1965), and Smuckler et al. (1962) have observed that the administration of CCL₄ interferred with protein synthesis by preventing the breakdown of the polyribosome to simple ribosomes needed for protein synthesis. Peroxidation of lipids, including microsomal lipids is increased following CCL₄ and it is possible that microsomal membrane function is affected by this process (Recknagel, et al., 1966).

B. Ethionine: The chemical and physical properties of ethionine resemble closely those of methionine (Greenstein and Winitz, 1961). This close chemical similarity, Farber (1967) maintains, is responsible no doubt for the many similarities in the metabolic handling of ethionine and methionine in a wide variety of biological organisms and systems. Although originally considered not to occur in nature, ethionine or derivatives have been shown more recently to occur in several species of microorganisms (Shlenk, 1956; Fisher and Mallatte, 1961; and Loerch and Mallette, 1963).

The accumulation of lipid in the liver of 160 to 200 gm female rats increases with increasing dose of ethionine from about 25 mg to 150 to 200 mg (Farber et al., 1963). Following the administration of a large dose of ethionine, there is no significant rise in total liver lipid or in liver triglycerides at 3 hours and barely detectable increases at 4 hours. However, the liver triglycerides accumulated rapidly thereafter so that it was almost four times the control level at 6 hours, ten times at 12 hours, and fifteen to twenty times at 24 hours and 48 hours (Farber et al., 1963). The peak liver lipid concentration was reached at 24 hours or at 48 hours with a return to normal values at 72 to 120 hours.

Ethionine interferes with protein synthesis and leads to fatty livers. Ethionine lowers ATP levels, leads to a decrease in polyribosomes, diminished protein and phospholipid synthesis (Farber et al., 1964). Protein synthesis is affected two hours after ethionine is given but fat accumulation begins in one or two hours thereafter.

C. Orotic Acid: The administration of orotic acid to animals leads to fatty livers. Orotic acid interferes with the interaction of apoprotein and triglycerides (Rohein et al., 1966), thereby inhibiting the formation of lipoproteins which are vehicles for triglyceride removal from the liver. These results are confirmed by Windmueller et al. (1969) who reported that orotic acid selectively blocks the hepatic secretion of very low density lipoproteins and consequently the major part of the triglyceride secretions from the liver. Jatlow et al. (1965) produced fatty livers in rats in 3 to 5 days by feeding a diet containing 1% orotic acid. The most striking ultrastructural alterations seen in the fatty livers involved the endoplasmic reticulum which underwent marked dilation and depletion of ribosomes. Large lipid droplets accumulated within the vesicles of the endoplasmic reticulum. However, Hernell and Johnson (1973) made a slightly different observation. Ethionine and orotic acid, they reported, induce fatty livers mainly by a common mechanism, i.e. an almost complete blockage of the triglyceride secretion from the liver to the plasma. Hernell and Johnson also maintained that in rats there were large increases in the liver triglyceride in female rats compared to male rats. Given the same conditions, male rats tended to secrete more triglycerides from the liver than did female rats.

CHAPTER IV

MATERIALS AND METHODS

Experimental Animals and Management

Two-hundred and nineteen white Leghorn (B-300) type laying hens, 30 weeks of age at the beginning of the study were used in these series of experiments. The laying hens weighed an average of 1750 gm at the start of the experiments, and were selected for uniformity in body weight and for egg production. The birds were placed in individual cages and permitted fourteen hours of light. Feed and water was available ad libitum. The management of each experimental group is discussed below.

Experimental Design

The hens were divided into seven experimental groups (Table I). In each of these groups a standard laying ration (Table II) and one of seven lipogenic rations were fed. Because of differences in length of treatment periods, each group was considered a separate experiment and was statistically analysed as such.

In each experiment the hens were subdivided into groups of four hens each. One group was fed the standard ration while other groups were given the lipogenic treatment, with and without supplementation with a lipotropic factor.

TABLE I

OVERALL EXPERIMENTAL DESIGN

Lipogenic Factors Exp. No.	II No. Additions	Treatment Groups							IX Selenium
		Lipotropic Factors					VIII Vitamin C		
		III Vitamin E	IV Choline Chloride	V Santo- quin	VI Biotin	VII Linoleic Acid			
I	8% Animal Tallow	X	X	X	X	X	X	X	X
II	Rancid Feed	X	X	X	X	X	X	X	X
III	Rancid Fat	X	X	X	X	X			
IV	Orotic Acid	X	X	X	X	X			
V	Aflatoxin	X	X	X	X	X			
VI	CCL ₄	X	X	X	X	X			
VII	Dl-ethionine	X	X	X	X	X			
	Standard Ration	X							

TABLE II
STANDARD RATION¹

Feedstuffs	Percent
Yellow Corn	56.00
Fishmeal, 65 percent	2.50
Alfalfa meal, 17 percent	5.00
Soybean oil meal, 50 percent	19.76
Vitamin mix	0.50
Salt	0.50
Manganese sulfate, 75 percent	0.03
Animal Fat	4.71
Defluorinated rock phosphate	1.00
Limestone	6.00
Sand	4.00
Total	100.00

¹Crude Protein-17.23%; Kilo-calories 1989 P.E. per Kg; Ca .- 4.25%; P.-0.6%. Vitamin mix supplied the following amounts per kilogram of diet; 2463 I.U. of vitamin A; 2956 I.C.U. of vitamin D; 4.43 mg of ribolfavin; 6.5 mg of vitamin B₁₂; 440 mg of 3 choline; 24.2 mg of niacin; and 4.76 mg of pantothenic acid.

Treatments

Lipogenic Factors: The following seven "lipogenic" substances were used - animal tallow, rancid feed, rancid fat, orotic acid, aflatoxin, carbon tetrachloride and D1-ethionine.

The high fat ration treatments contained 8% animal tallow. The feed was mixed on a weekly basis to ensure its freshness and freedom from rancidity.

The rancid feed ration consisted of the standard ration (Table II, p. 26) which was placed in 20 gallon galvanized cans with lids and subjected to heat from infra-red lamps for a period of three weeks.

The rancid fat ration consisted of the standard ration except that its animal tallow fraction was rendered rancid by heating it at 70°C while air was bubbled through it for 72 hours. A fat sample was tested by the method of Hartman and Glevind, (1949) for rancidity at the end of the 72 hours.

The orotic acid treatment consisted of the standard ration plus one percent of orotic acid added to the feed.

Aflatoxin was fed in the standard ration at a rate of 10 mg per gram of feed.

The carbon tetrachloride was mixed by volume (1:1) with olive oil and injected intraperitoneally three times a week at the rate of 3 ml per kilogram of live-weight.

D1-ethionine was dissolved in hot avian saline solution (25 mg/ml) and injected intraperitoneally at the rate of 80 mg/100g bird live-weight.

Lipotropic Factors: Vitamin E in wheat germ oil was purchased from ICN Nutritional Biochem. Corp., Cleveland, Ohio, and fed at the rate of 5.5 i.u. per kg of feed. Choline chloride was obtained locally and fed at the rate of 0.5 gm per kg of feed. Santoquin, a commonly used antioxidant for animal rations, was fed at the rate of 60 mg per 100 gm of feed. Biotin was obtained from Cal. Biochem., La Jolla, California, and fed at the rate of 320 mg per kg of feed. Linoleic acid, purchased from ICN, was fed at the rate of 1% of the diet. Vitamin C tablets were purchased locally and fed at the rate of 250 mg per kg of feed. Selenium fed at the rate of 0.1 ppm and was obtained as a pre-mix from Calcium Carbonate Co., Quincy, Illinois.

Experiment I: High Fat Diet

Thirty-six birds were divided into nine groups of four birds each. Group I was fed the standard laying ration. Group II received a ration containing 8% animal tallow while Groups III through IX each received 8% animal tallow in their diet plus vitamin E (5.5 i.u. per kg feed); choline chloride (0.5 gm per kg feed); Santoquin (60 mg per 100 gm feed); biotin (320 mg per kg feed); linoleic acid (1% of diet); vitamin C (250 mg per kg); and selenium (0.1 ppm) respectively. This experiment was begun on March 8, 1974 and ended May 22, 1974.

Experiment II: Rancid Feed

Thirty-six birds were divided into nine treatment groups of four birds each. Group I received the standard diet while Groups II through IX received the rancid feed plus the other additions as in

Experiment I. This experiment lasted from March 8, 1974, through May 22, 1974.

Experiment III: Rancid Fat Diet

Twenty-eight laying hens were divided into seven treatment groups of four birds each. Group I received the standard diet while Groups II through VII received a diet containing rancid fat. The additions in treatments III through VII were vitamin E, choline chloride, Santoquin, biotin, and linoleic acid respectively. This experiment lasted 8 weeks, from March 8th to May 22, 1974.

Experiment IV: Orotic Acid

Twenty-eight hens were divided into seven treatment groups of four birds each. Group I was placed on the standard diet while Groups II through VII received one percent orotic acid in their diet. In addition vitamin E, choline chloride, Santoquin, biotin, and linoleic acid was added to the diets of treatments III through VII, respectively. This experiment lasted one week, from May 15 to May 22, 1974.

Experiment V: Aflatoxin

Twenty-eight birds were divided into seven groups of four birds each. Groups II through VII received 10 mg of aflatoxin per gram of feed for four weeks. Groups III through VII received additions in the diet as in Experiment IV. Group I was fed the standard diet.

Experiment VI: Carbon Tetrachloride Administration

Twenty-eight laying hens were divided into seven treatment groups

of four birds each. Two ml of CCl_4 per kg of body weight mixed with an equal volume of olive oil was administered intraperitoneally three times a week. Group I, the control group, received an equivalent amount of olive oil. The diets of Groups III through VII received vitamin E, choline chloride, Santoquin, biotin, and linoleic acid, respectively. The experiment lasted 9 weeks from March 13th through May 22, 1974.

Experiment VII: Ethionine Administration

Twenty-eight birds were divided into seven Groups. D1-ethionine was injected intraperitoneally at the rate of 80 mg/100 gm bird live-weight in birds in Groups II through VII. In addition, Group III through VII received the same supplements in their diets as in Experiment VI, both before and after the ethionine administration. The control group, Group I received an equivalent dose of avian saline solution. This experiment lasted for 48 hours, from May 20th to May 22, 1974.

Reproductive Performance:

During the experimental period, daily egg records were kept. Weights and specific gravity were measured on one day's eggs of each week.

Physical Examination:

At the end of the experimental period the birds were weighed. A numerical scoring system of 1 through 5 was used for comb color. A score of one was designated for combs with the normal bright red color, whereas those with extreme paleness received a score of 5.

Liver Physical Features:

All hens were sacrificed by decapitation at the end of each experimental period. The livers were examined for haemorrhages, then removed and weighed. The liver was scored for firmness and color, using the numerical scoring system as shown in Table III and described previously by Wolford and Polin (1972), and then placed in polyethylene bags and stored at -15°C .

Liver Function:

A basic liver function test was conducted at the end of each experimental period. Sodium bromosulphthalein (BSP) in distilled water at a concentration of 20 mg per milliliter of water was injected intravenously at a dosage of 20 mg/kg body weight. Five ml of blood was withdrawn with a syringe by heart puncture 10 minutes after BSP administration and transferred to a heparinized centrifuge tube and spun at 1200 rpm for 3 minutes. One ml of the plasma from each sample was pipetted into a 3 ml glass cuvette. Color was developed by adding .7 ml of N-NaOH to each ml plasma, and readings were made with a Coleman Jr. II spectrophotometer set at 575 m using a 19 mm cuvette (Campbell, 1957).

Prothrombin Time:

Prothrombin time was determined by a modification of the method of Faulkner and King (1970). The one-stage prothrombin time was used. A 3 ml blood sample was withdrawn into a vacutainer from each bird by heart puncture, stored at zero degree C for not more than 3 hours before the determination. The blood was centrifuged

TABLE III
SCORING SYSTEM FOR LIVER TEXTURE AND COLOR

Score	Liver	
	Texture	Color
1	Firm	Mahogany
2	Less firm	Slight yellow in mahogany
3	Fairly firm	Yellow, little mahogany
4	Ruptured less easily	Yellow, more mahogany
5	Ruptured easily	Very yellow, no mahogany

at 1200 rpm for 5 minutes just before the determination.

A thromboplastin preparation was made by crushing the brains of 3-week old chickens, dissolving in chloroform and filtering (Bell and Alton, 1954). The extract was stored at zero degree C. A mixture of the thromboplastin preparation and calcium chloride (1:1) was made just before the determination.

A fibrometer, an electro-mechanical instrument for determination of the critical end point of the coagulation time of the plasma, was used.

Determination of Liver Lipid Levels:

Lipid extraction was carried out by a modification of the methods of Folch et al. (1951). The frozen livers were thawed, and approximately 10 gm of each was weighed and dried at 105°C for 24 hours, then crushed in a mortar. A crushed sample of approximately 2 gm was weighed and extracted with chloroform-methanol, (2:1) by volume. After 12 hours of extraction, the extracted fat was subsequently dried in an oven at 100°C for 2 hours, cooled in a desiccator and weighed. The beaker plus the fat weight was compared to the original weight of the beaker. The difference was expressed as a percent fat of the wet liver.

Bone Breaking Strength:

The right wing of each bird was severed and the humerus defleshed after cooking for approximately five minutes in boiling water.

The force required to break each humerus was measured by the use of an Alo-Kramer shear press. An attachment was designed for

use with the shear press which allowed the bone to be supported at both ends and the pressure to be applied to the mid-point of the humerus. The attachment provided a span of 3 cm.

A 100 pound (45.45 Kg) ring was used and the speed of the shear press was 2 cm per second. The maximum force used to break each bone was read from the dial.

Analyses of Data:

Data were statistically analyzed by analysis of variance (Snedecor, 1956), and differences between the treatments were determined by Duncan's Multiple Range Test (1955).

CHAPTER V

RESULTS AND DISCUSSIONS

Per Cent Liver Fat

The analyses of variance for the per cent liver fat for the various lipogenic and lipotropic substances are shown in Table IV. Significant differences were exhibited for the high fat ration, rancid feed and rancid fat experiments ($P < 0.01$). Significant differences for per cent liver fat were not obtained from the orotic acid, aflatoxin, carbon tetrachloride and D1-ethionine experiments.

Table V presents the means for the per cent liver fat for the various combinations of lipogenic and lipotropic factors. According to Duncan's Multiple Range Test, the rancid feed and the rancid fat experiments resulted in highly significant increases in the per cent fat in the livers of hens compared to those fed the standard laying ration. Although no significant increases were indicated in the orotic acid, carbon tetrachloride and D1-ethionine experiments, there was a consistent trend for those birds receiving these lipogenic substances without lipotropic supplementation to have higher mean values for per cent of liver fat.

In the high fat experiment, choline chloride significantly increased the percent liver fat over that of the high fat ration without choline chloride supplementation, thus behaving as a lipogenic

TABLE IV
ANALYSES OF VARIANCE FOR PER CENT LIVER FAT
OF HENS RECEIVING LIPOGENIC AND LIPTROPIC FACTORS

Lipogenic Factors	Mean Square				
	Source of Variance		Residual	F Value	Prob > F
	Total	Lipotropic Factors			
High Fat	(35)*111.90	(8) 231.66	(27) 76.42	3.03	0.01
Rancid Feed	(35) 43.87	(8) 95.03	(27) 28.71	3.31	0.009
Rancid Fat	(27) 65.63	(6) 146.42	(21) 42.55	3.44	0.001
Orotic Acid	(27) 50.61	(6) 71.99	(21) 44.50	1.62	0.19
Aflatoxin	(27) 26.19	(6) 24.87	(21) 26.57	0.94	0.51
Carbon Tetrachloride	(27) 136.29	(6) 90.38	(21) 149.41	0.61	0.73
D1-ethionine	(27) 33.60	(6) 18.32	(21) 37.97	0.48	0.82

*Degrees of freedom are given within parentheses.

TABLE V
EFFECTS OF LIPOGENIC AND LIPOPOTROPIC FACTORS ON PERCENT
LIVER FAT

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride
	Mean Percent Liver Fat					
Standard Ration ¹	8.23*	8.62 ^c	8.62 ^d	25.04 ^a	15.33	16.13
Without Lipotropic Factor	18.81 ^{bc}	24.45 ^a	22.66 ^{ab}	23.25 ^{ab}	14.80	27.72
Vitamin E	25.81 ^{ab}	12.90 ^{bc}	19.47 ^{abc}	13.17 ^b	14.04	18.61
Choline Chloride	35.61 ^a	13.02 ^{bc}	9.80 ^{cd}	20.61 ^{ab}	14.23	20.39
Santoquin	19.15 ^{bc}	13.27 ^{bc}	12.33 ^{bcd}	15.02 ^{ab}	21.25	22.99
Biotin	25.24 ^{ab}	10.20 ^{bc}	14.27 ^{abcd}	20.38 ^{ab}	14.89	15.83
Linoleic Acid	16.87 ^{bc}	18.61 ^{ab}	23.36 ^a	20.72 ^{ab}	15.94	14.07
Vitamin C	16.47 ^{bc}	11.85 ^{bc}				
Selenium	21.48 ^{bc}	17.30 ^{abc}				

¹The standard ration was fed without supplementation with lyotropic factor to one group of four hens each in each of the lipogenic experiments.

*Mean values in the same column which are followed by different superscripts are significantly different.

factor. In the rancid fat and rancid feed experiments, choline chloride exerted a significant lipotropic effect. Vitamin E, Santoquin and vitamin C were significantly lipotropic in the rancid feed experiment.

A point of great significance can be made regarding the relative effectiveness of the anti-oxidative lipotrophic factors in the rancid feed and rancid fat experiments. In the rancid feed experiment vitamins E and C, and Santoquin significantly reduced the per cent liver fat, whereas in the rancid fat experiment these compounds did not lower liver fat. In the latter case the oxidative rancidity of the fat occurred before the fat was combined with the other feed components, including the supplements. This would suggest that these compounds protect against fatty infiltration of the liver by preventing oxidative fat rancidity of the feed and are less effective once rancidity has developed.

Biotin supplementation in the rancid feed experiment exerted a lipotropic effect, apparently by replacing the biotin that was lost through oxidation. Biotin, reportedly, is rapidly destroyed by developing fat rancidity.

The failure of vitamin E, selenium and biotin to prevent liver lipid accumulation in birds fed the high fat ration is not inconsistent with results obtained by numerous researchers, particularly Wolford and Murphy (1972) who failed to prevent or treat fatty liver syndrome with these substances.

It should be pointed out that the variation within treatments was high and thus large differences between many of the mean values

were significant. Also as previously indicated, several of the lipogenic treatments did not result in significant lipogenic effects. Obviously the lipotropic value of a substance would be more difficult to demonstrate when used under conditions not conducive to fatty liver development.

The erratic results on the liver lipid levels obtained in these experiments may be partially explained by the fact that the fatty liver haemorrhagic syndrome is a disease that plagues the poultry industry mostly in the hot weather months and with older hens (Wolford, 1971, and Griffith et al., 1970). This assertion is shared by Shirley (1974) based upon the high incidence of mortality caused by the fatty liver haemorrhagic syndrome at the University of Tennessee Poultry Farm; although Ivy (1971) at Cornell, sacrificed some hens at the end of summer and found liver fat values essentially the same as for those hens sacrificed during the winter. The present experiments were conducted under conditions of relatively low environmental temperatures and with relatively young hens.

In Experiments II and III where rancid feed and rancid fat were fed, excessive accumulation of lipids in the liver were obtained. This fat accumulation probably resulted from lipid peroxidation *in vivo* or from the consumption of oxidised feed whose lipotropic substances had been destroyed by peroxidation and thus rendering no protection to the chicken. Lipid peroxidation is particularly damaging to membranes and organelles (Tappel, 1973), because the mitochondrial and microsomal membranes become relatively large, disrupted and dissolved.

This no doubt interferes with normal metabolism, hence the importance of the protection given by the antioxidant substances, such as vitamin E, selenium, and Santoquin. DiLuzio (1973) noted that antioxidants are profound modifiers of a variety of chemically induced hepatic lesions, and inhibitors of liver lipid accumulation. Santoquin exerted an antioxidant influence on the level of fats in both rancid feed and rancid fat experiments. Vitamin E decreased lipid levels in the rancid feed experiment only. Scott (1967) showed that vitamin E was capable of protecting the polyunsaturated fatty acids of the phospholipid structures of the membrane from undergoing destructive peroxidation, while Jensen et al. (1970) demonstrated that rats fed vitamin E and selenium were protected from liver lipid accumulation.

Aflatoxin is known to be highly dose-related in causing fatty livers (Hamilton and Garlich, 1970) and this may partially explain the failure to induce fatty liver in this experiment. The method of mixing the aflatoxin, as minute as it was, could have rendered the toxin unavailable to most of the hens. If the aflatoxin was not thoroughly mixed up in the feed for continuous consumption, but was eaten up as a single dose, the hen could metabolise and excrete it rapidly as compared with continual dosing through the feed (Tung, 1970). Aflatoxin is known to inhibit protein and nucleic acid synthesis in the liver (Tung, 1970), hence the interference with production of lipoproteins which act as a vehicle for lipids from the liver.

Although there was an indication that the chemical compounds, carbon tetrachloride, orotic acid and ethionine increased the liver lipid levels, their increases were non-significant. The compounds are well known for elevating liver lipids in mammals. Friedman et al. (1969) noted that these agents have been shown to induce fat in livers of such animals as rats, mice, rabbits, and guinea pigs. The livers of chickens, unlike those of mammals, are quite tolerant to toxins. Friedman and His associates (1969) found that it takes about 20 times the toxin that may cause injury to livers in rats to induce injury to chicken livers. The chicken liver has also been noted to have an immense capacity for detoxification (Nordstrom and Smith, 1967). Friedman (1969) did not succeed in inducing fatty liver syndrome in chickens with carbon tetrachloride. Orotic acid and ethionine have been used to induce fatty livers in rats (Hamuro, 1972, and Bassan et al., 1971), but not in chickens. Jatlow (1965) has emphasized the participation of the endoplasmic reticulum in the hepatic lipid accumulation which occurs in rats fed a diet containing orotic acid. Dilatation and disorganization of the endoplasmic reticulum are said to be non-specific, and is commonly seen in the livers of animals with choline deficiency, or in those injected with carbon tetrachloride or ethionine. Jordan (1964) observed large lipid droplets in the liver cells of orotic acid fed rats, and suggested that these might be the lipoproteins. Dislocation of ribosomes from the endoplasmic reticulum is an effect common to hepatoxin which inhibit protein synthesis.

Although the precise mechanism of fatty change may vary with the inducing agent (Jatlow, 1965) a common denominator is thought to be an impairment of lipid transport from the hepatic cells. This concept is consistent with the depressed levels of plasma lipids which have been found in animals with fatty livers induced by carbon tetrachloride, ethionine, choline deficiency, and orotic acid (Creasey et al., 1961). This defect in lipid mobilization from the liver may result from impaired protein synthesis, and more particularly defective lipoprotein synthesis.

In all the experiments except with the carbon tetrachloride one, the abdominal fat score was uniformly high, and indication that lipid transport from the liver site of synthesis to the adipose tissue was adequate. Therefore the question of inadequate lipoprotein synthesis may not arise except in the carbon tetrachloride experiment.

Liver Weights:

The analyses of variance for wet liver weights (Table VI) shows a significant ($P < 0.001$) difference between the treatments of the high fat ration experiment, while the other experiments showed no differences among treatments. Average liver weights shown in Table VII indicate that in the high fat experiments, only linoleic acid effectively reduced wet liver weights, although differences are not significant. The highest average liver weights were recorded in the choline chloride treatment where mean weight was twice as high as the weights of livers from the control birds. Although no significant differences were observed in the other experiments, liver weights of

TABLE VI

ANALYSES OF VARIANCE FOR LIVER WEIGHT OF HENS
RECEIVING LIPOGENIC AND LIPO TROPIC FACTORS

Lipogenic Factors	Mean Square		Residual	F Value	Prob > F
	Source of Variance				
	Total	Lipotropic Factors			
High Fat	(35)*134.53	(8) 448.07	(27) 41.63	10.76	0.0001
Rancid Feed	(35) 116.08	(8) 129.04	(27) 112.24	1.15	0.36
Rancid Fat	(27) 312.16	(6) 479.21	(21) 264.44	1.81	0.15
Orotic Acid	(27) 187.90	(6) 196.23	(21) 185.52	1.06	0.42
Aflatoxin	(27) 211.28	(6) 374.03	(21) 164.78	2.27	0.08
Carbon Tetrachloride	(27) 175.48	(6) 144.21	(21) 184.41	0.78	0.60
Dl-ethionine	(27) 253.51	(6) 332.42	(21) 230.97	1.44	0.25

*Degrees of Freedom are given within the parentheses.

TABLE VII
EFFECT OF LIPOGENIC AND LIPOTROPIC FACTORS ON
LIVER WEIGHTS

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride
	Mean Liver Weights (Grams)					
Standard Ration	33.10 ^c	32.43	32.43 ^{b*}	47.96	31.49	39.73
Without Lipotropic Factor	38.99 ^{bc}	44.28	64.10 ^a	54.12	59.36	64.23
Vitamin E	52.65 ^{ab}	45.36	58.75 ^{ab}	42.85	34.00	53.98
Choline Chloride	61.40 ^a	34.54	41.15 ^{ab}	40.89	41.13	52.89
Santoquin	43.95 ^b	40.30	44.98 ^{ab}	37.59	46.68	41.64
Biotin	56.94 ^{ab}	32.85	46.86 ^{ab}	42.82	33.78	45.21
Linoleic Acid	33.27 ^c	38.54	40.88 ^{ab}	56.39	40.95	45.81
Vitamin C	42.99 ^{bc}	44.23				
Selenium	56.36 ^{ab}	48.11				

*Mean values in the same column which are followed by different superscripts are significantly different.

those birds receiving lipogenic factors without the additions of lipotropic factors were consistently higher than in the other treatments. In the rancid fat, aflatoxin, and Dl-ethionine experiments, average liver weights of birds receiving only lipogenic factors (64.10 gm, 59.36 gm and 64.23 gm, respectively) were twice as large as the weights of livers from birds fed the standard diet (32.43 gm, 31.49 gm, and 36.79 gm, respectively).

The failure of the lipotropic substances to influence liver weights is consistent with the results obtained by Wolford and Murphy (1972) and Wolford and Polin (1973) who demonstrated that lipotropic vitamins and selenium failed to alter liver weights.

Ivy (1971), among other investigators, has found that liver size is highly correlated with fatty liver haemorrhagic syndrome, the increased liver weight resulting from increased fat infiltration. However, not all increased liver size results from excessive fat accumulation, nor does the increase automatically lead to liver haemorrhages. Wolford and Polin (1974) forced-fed chickens at the rate of 150 percent and found that the livers underwent a "tremendous hypertrophy". On examination of the constituent data in such livers they found that on a relative basis, water and the non-lipid dry matter increased at an equivalent rate as daily feed intake exceeded control intake.

Liver Haemorrhages:

The analyses of variance for liver haemorrhages are found in Table VIII. There existed a significant difference between the

TABLE VIII

ANALYSES OF VARIANCE FOR LIVER HAEMORRHAGES OF
HENS RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square					
	Source of Variance					
	Total	Lipotropic Factors		Residual	F Value	Prob > F
High Fat	(35)*0.22	(8)	0.674	(27) 0.83	8.08	0.0001
Rancid Feed	(35) 0.22	(8)	0.174	(27) 0.23	0.75	0.65
Rancid Fat	(27) 0.25	(6)	0.726	(21) 0.12	6.10	0.001
Orotic Acid	(27) 0.25	(6)	0.23	(21) 0.26	0.86	0.54
Aflatoxin	(27) 0.23	(6)	0.50	(21) 0.15	3.33	0.01
Carbon Tetrachloride	(27) 0.24	(6)	0.155	(21) 0.26	0.60	0.74
DL-ethionine	(27) 0.21	(6)	0.119	(21) 0.24	0.50	0.80

*Degrees of freedom are given within the parentheses.

treatments in three of the experiments: high fat ration ($P < 0.001$), rancid fat ($P < 0.001$), and aflatoxin ($P < 0.01$). There were no significant differences between the treatments in the rancid feed, orotic acid, carbon tetrachloride, and Dl-ethionine experiments. The mean liver haemorrhage scores for all treatments are tabulated in Table IX. The rancid fat ration resulted in a significant increase in the mean liver haemorrhage score whereas the remaining six lipogenic treatments had no effect when compared to the standard ration. Choline chloride supplementation reduced significantly the haemorrhage score in the rancid fat experiment. Santoquin increased the incidence of haemorrhages in the aflatoxin experiment. Biotin increased the haemorrhage score in both the high fat and aflatoxin experiments. Linoleic acid and selenium also increased the score in the high fat treatment.

In this study, choline chloride significantly decreased the incidence of liver haemorrhage induced by rancid fat. The mode of liver haemorrhage development in hens is not yet quite understood. In the case of aflatoxin Tung, *et al.* (1970) speculated that aflatoxin causes a general inhibition of protein and nucleic acid synthesis, which results in a rapid loss of tissue strength and further leads to a degradation of preformed structural elements. As the activity of the "lysosomal" enzyme activity rises so does the increase in capillary fragility. If this explanation holds true for aflatoxins, it may apply equally to the other compounds -- carbon tetrachloride, orotic acid and ethionine which induce fatty livers by

TABLE IX

EFFECTS OF LIPOGENIC AND LIPOPOTROPIC FACTORS ON THE LIVER
HAEMORRHAGES SCORE

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin Haemorrhages Score	Carbon Tetrachloride
				Mean Liver		Dl-ethionine
Standard Ration	0 ^{b*}	0	0 ^b	0.75	0.25 ^{bc}	0.75
Without Lipotropic Factor	0 ^b	0.50	1.00 ^a	0.25	0 ^c	0.50
Vitamin E	0 ^b	0.50	0.50 ^{ab}	0.75	0.25 ^{bc}	0.75
Choline Chloride	0 ^b	0.50	0 ^b	0.25	0 ^c	0.50
Santoquin	0 ^b	0.25	0.75 ^a	0.50	1.00 ^a	0.75
Biotin	0.75 ^a	0.25	0.75 ^a	0.75	0.75 ^{ab}	0.75
Linoleic Acid	0.75 ^a	0.25	1.00 ^a	0.75	0.25 ^{bc}	1.00
Vitamin C	0.25 ^b	0				
Selenium	1.00 ^a	0.50				

*Mean values in the same column which are followed by different superscripts are significantly different.

causing tissue degeneration. In the case of oxidative rancidity where peroxidation takes place, resulting in mitochondrial displacement, enlarged and disrupted or dissolved membranes (Tappel, 1973) it is easy to see how liver haemorrhage can result. But what happens in the case of liver haemorrhage induced by non-toxic substances such as nutritional, metabolic or hormonal imbalances? Wolford and Murphy (1972) increased the dietary level of protein, vitamin B₁₂, vitamin E, choline, inositol, selenium and/or cobalt and failed to prevent the occurrence of liver haemorrhage. Tudor (1967) reported that excessive accumulation of fat within the liver cord cells resulted in a friable and soft breakdown, yielding haemorrhages within the liver capsule. According to him, the haemorrhages as well as the reduced blood flow result in parenchymatous degeneration and fibrosis i.e., formation of scars tissue. In addition, he observed fatty liver syndrome haemorrhages into the body cavity from rupture of the liver capsule only when there had been a definite necrosis of liver tissue.

Wolford et al., (1971) has provided a detailed photographic description of fatty liver haemorrhagic syndrome. Livers photographed were extremely soft and fragile, and contained multiple antemortem haemorrhages within the liver tissue. Mortality from the fatty liver syndrome was concluded to occur when the liver capsule (capsule of Glisson) ruptured resulting in the fatal haemorrhages into the abdominal cavity.

A microscopic view (Wolford et al., 1971) of liver cells

containing haemorrhages revealed extremely distorted cellular shape and configuration. Many cells were distended due to fat infiltration and there appeared to be no organization of the liver cells into the cord-like structure seen in normal livers. Fat infiltration resulted in the cell nucleus being forced to one side of the cell rather than being in the center. These workers then concluded that fatty liver syndrome becomes a problem where fat infiltration into the liver is associated with liver hematomas or massive liver haemorrhages.

Wolford and Murphy (1972) have suggested that increased liver size may be a possible mechanism by which haemorrhages occur. As the liver size increases in size, tensions on the relatively stationary liver vascular system may cause a vessel to rupture, resulting in haemorrhages.

While current research did not result in the excessive liver haemorrhages described by the Michigan workers, the postmortem results of birds dying of the fatty liver syndrome at the University of Tennessee Poultry Farm have tended to confirm these findings.

Liver Color:

Analyses of variance for liver color for the various lipogenic and lipotropic factors are shown in Table X. Significant differences are shown for the High Fat ($P < 0.002$), Rancid Feed ($P < 0.06$), Rancid Fat ($P < 0.08$), Orotic Acid ($P < 0.05$), and D1-ethionine ($P < 0.01$) experiments. Significant differences for this characteristic were not obtained for the aflatoxin, and carbon tetrachloride experiments.

TABLE X

ANALYSES OF VARIANCE FOR LIVER COLOR FOR HENS
RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square				
	Source of Variance				
	Total	Lipotropic Factors	Residual	F Value	Prob > F
High Fat	(35)*0.91	(8) 2.236	(27) 0.52	4.31	0.002
Rancid Feed	(35) 1.06	(8) 1.813	(27) 0.83	2.18	0.06
Rancid Fat	(27) 0.89	(6) 1.560	(21) 0.70	2.22	0.08
Orotic Acid	(27) 1.01	(6) 2.00	(21) 0.73	2.75	0.04
Aflatoxin	(27) 0.63	(6) 0.50	(21) 0.67	0.75	0.62
Carbon Tetrachloride	(27) 0.66	(6) 0.226	(21) 0.79	0.29	0.94
Dl-ethionine	(27) 1.43	(6) 3.155	(21) 0.94	3.35	0.01

*Degrees of Freedom are given within the parentheses.

The mean liver color for the lipogenic and lipotropic factors are given in Table XI. DL-ethionine was the only lipogenic factor that significantly influenced liver color. Regarding the effect of lipotropic factors, vitamin E resulted in a lower score (darker color) in the Rancid Feed and Orotic Acid Experiments. Choline chloride reduced the score in the Orotic Acid Experiment. In the DL-ethionine Experiment, vitamin E, choline chloride, Santoquin, biotin, and linoleic acid all resulted in livers that were more yellow in color as compared to the control birds.

Results obtained in these experiments indicate that visual liver color scores were fairly indicative of the percentage of liver lipids. Although this system has been used to estimate liver lipid levels, Wolford and Polin (1972) do not consider it reliable. Sunde (1966), Baton (1967), Nesheim and Ivy (1970) have used a visual score to evaluate liver fattiness, but they included the incidence of haemorrhages as part of the highest score. These investigators assumed that yellowish color and haemorrhages occurred only in livers with the greatest lipid content, an evaluation found invalid by Wolford and Polin (1972). However our findings tend to support the contention that liver lipid levels can be fairly ascertained by visual liver color. Tudor (1967) observed that excessive accumulation of fat within the liver cord cells resulted in a uniformly pale yellow color, and in 1971, Wolford et al. found during necropsy that birds having died from fatty liver syndrome revealed light yellowish-brown livers. How the abnormal yellow color increases with fat levels is still little understood.

TABLE XI

EFFECTS OF LIPOGENIC AND LIPOTROPIC FACTORS ON
LIVER COLOR SCORE

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride
	Mean Liver Score					
Standard Ration	3.75 ^{abc*}	2.75 ^{ab}	2.75 ^b	4.50 ^a	3.00	3.25
Without Lipotropic Factor	4.00 ^{abc}	4.25 ^a	4.00 ^{ab}	4.50 ^a	3.75	2.75
Vitamin E	3.50 ^{bc}	2.25 ^b	4.00 ^{ab}	3.00 ^{bc}	3.75	3.25
Choline Chloride	3.50 ^{bc}	4.00 ^a	3.00 ^b	2.75 ^c	3.50	3.25
Santoquin	4.75 ^{ab}	3.00 ^{ab}	4.00 ^{ab}	3.75 ^{abc}	3.75	3.25
Biotin	5.00 ^a	2.75 ^{ab}	3.50 ^{ab}	4.25 ^{ab}	3.75	3.00
Linoleic Acid	2.75 ^c	3.00 ^{ab}	4.50 ^a	3.50 ^{abc}	3.00	2.75
Vitamin C	4.75 ^{ab}	2.75 ^{ab}				
Selenium	4.50 ^{ab}	3.75 ^{ab}				

*Mean values in the same column which are followed by different superscripts are significantly different.

Liver Texture:

The analyses of variance for liver texture for various lipogenic and lipotropic factors are shown in Table XII. Significant differences were shown in the high fat ($P < 0.05$) and the Dl-ethionine ($P < 0.0009$) experiments.

The mean liver texture scores for all lipogenic and lipotropic treatments are presented in Table XIII. The high fat ration was the only lipogenic treatment that increased the fragility of the liver. Choline chloride in the Dl-ethionine experiment reduced liver fragility. All other comparisons were non-significant.

Liver texture is known to be highly correlated with fatty liver syndrome. Tudor, (1967), found the livers of birds dying of fatty liver syndrome to be extremely soft and fragile. Wolford et al. (1971) obtained the same results in Michigan. Hepatorrhaxis seems to be very evident in almost all cases of fatty liver syndrome.

Prothrombin Time:

The analyses of variance for prothrombin time for all factors studied are shown in Table XIV. Significant differences between treatments were observed in two experiments: Rancid Feed ($P < 0.0001$) and Dl-ethionine ($P < 0.05$).

The mean values for effect of lipogenic and lipotropic factors on prothrombin time are reported in Table XV. In the rancid feed experiment, all the lipotropic substances increased rather than decreased prothrombin time, when the mean values are compared to the mean value of those hens fed only rancid feed. In the ethionine

TABLE XII

ANALYSES OF VARIANCE FOR LIVER TEXTURE FOR HENS
RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square				F Value	Prob > F
	Source of Variance					
	Total	Lipotropic Factors	Residual			
High Fat	(35)*1.54	(8) 2.736	(27) 1.89	2.31	0.04	
Rancid Feed	(35) 0.94	(8) 1.153	(27) 0.88	1.31	0.28	
Rancid Fat	(27) 1.37	(6) 1.32	(21) 1.36	1.03	0.44	
Orotic Acid	(27) 1.59	(6) 2.25	(21) 1.40	1.60	0.20	
Aflatoxin	(27) 1.06	(6) 1.32	(21) 0.99	1.34	0.28	
Carbon Tetrachloride	(27) 1.21	(6) 0.405	(21) 1.44	0.28	0.94	
Dl-ethionine	(27) 1.22	(6) 3.536	(21) 0.56	6.32	0.0009	

*Degrees of Freedom are given within the parentheses.

TABLE XIII

EFFECT OF LIPOGENIC AND LIPOTROPIC FACTORS ON
LIVER TEXTURE

Lipotropic Factors	Lipogenic Factors						
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride	DL-ethionine
	Mean Liver Texture Score						
Standard Ration	1.50 ^{c*}	2.25	2.25	4.25	2.75	2.75	3.50 ^a
Without Lipotropic Factor	3.25 ^{ab}	2.75	3.75	3.75	3.25	3.25	3.25 ^a
Vitamin E	2.75 ^{ab}	1.75	3.75	2.25	2.25	3.00	3.25 ^a
Choline Chloride	3.00 ^{ab}	3.50	3.00	2.75	3.50	2.50	1.25 ^b
Santoquin	2.75 ^{ab}	3.00	3.75	3.75	2.75	3.00	3.25 ^a
Biotin	4.00 ^a	2.00	3.75	4.25	4.00	3.25	2.50 ^{ab}
Linoleic Acid	2.50 ^{ab}	2.25	3.75	3.50	3.25	2.50	4.25 ^a
Vitamin C	3.50 ^a	2.50					
Selenium	4.25 ^a	2.25					

*Mean values in the same column which are followed by different superscripts are significantly different.

TABLE XIV
ANALYSES OF VARIANCE FOR PROTHROMBIN TIME
FOR HENS RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square					F Value	Prob > F
	Source of Variance			Residual			
	Total	Lipotropic Factors					
High Fat	(35)* 3.45	(8) 7.258	(27) 2.61	2.79	0.11		
Rancid Feed	(35) 1.58	(8) 0.64	(27) 4.77	7.45	0.0001		
Rancid Fat	(27) 1.80	(6) 2.952	(21) 1.47	2.01	0.11		
Orotic Acid	(27) 1.17	(6) 0.98	(21) 1.23	0.79	0.59		
Aflatoxin	(27) 1.32	(6) 1.07	(21) 2.23	2.09	0.10		
Carbon Tetrachloride	(27) 37.30	(6) 34.48	(21) 38.11	0.91	0.51		
Dl-ethionine	(27) 19.68	(6) 2.476	(21) 0.94	2.64	0.05		

*Degrees of Freedom are given within the parentheses.

TABLE XV

EFFECTS OF LIPOGENIC AND LIPTROPIC FACTORS ON PROTHROMBIN TIME

Lipotropic Factors	Lipogenic Factors						
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride	Dl-ethionine
Standard Ration	12.93	12.93 ^d	12.93	13.74	13.93	15.68	13.80 ^a
Without Lipotropic Factor	13.75	14.10 ^d	12.41	13.44	13.76	13.49	13.24 ^{ab}
Vitamin E	--	15.31 ^{bc}	12.64	13.69	14.36	12.28	13.35 ^a
Choline Chloride	--	15.05 ^{bc}	14.49	13.93	14.34	19.88	12.74 ^{bc}
Santoquin	--	16.19 ^a	12.06	13.24	15.23	14.61	12.55 ^c
Biotin	--	16.05 ^a	12.41	12.49	14.80	12.35	11.93 ^d
Linoleic Acid	15.56	15.53 ^b	13.73	13.03	12.93	18.31	11.61 ^d
Vitamin C	--	16.29 ^a					
Selenium	--	14.80 ^c					

experiment, biotin, linoleic acid, and Santoquin significantly reduced prothrombin time when compared to those hens injected with ethionine.

Sodium Bromosulphthalein Clearance Test (BSP):

Analyses of variance for hens receiving lipogenic and lipotropic factors are reported in Table XVI. Significant ($P < 0.01$) differences between the treatments were obtained only in the D1-ethionine experiments. Table XVII shows the mean values for the effect of lipogenic and lipotropic substances on the rate of BSP clearance. There was a highly significant increase in BSP retention between hens injected with D1-ethionine and those fed the standard ration signifying an alteration in liver function. When the diet of birds injected with ethionine were supplemented with choline chloride, there was a highly significant increase in BSP clearance rate. Linoleic acid supplementation had an opposite effect, significantly decreasing the rate of clearance. Although no significant differences were obtained in the other experiments, mean values for BSP retention, with the exception of the aflatoxin experiment, showed a trend that hens receiving the lipogenic substances had higher retention values than birds fed the standard diet.

The failure of the BSP clearance test to prove the presence of high fat levels in the rancid feed and rancid fat tests can partially be explained by the fact that some kinds of fatty liver syndromes, particularly that induced by nutritive imbalances does not result in hepatism.

TABLE XVI

ANALYSES OF VARIANCE FOR BSP FOR HENS
RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square				
	Source of Variance		Lipotropic		
	Total		Factors	Residual	F Value Prob > F
High Fat	(35)* 5.57	(8)	6.58	(27) 5.34	1.23 0.34
Rancid Feed	(35) 9.65	(8)	15.25	(27) 7.99	1.91 0.10
Rancid Fat	(27) 6.52	(6)	7.577	(21) 6.22	1.22 0.34
Orotic Acid	(27) 3.29	(6)	3.26	(21) 3.29	0.99 0.54
Aflatoxin	(27) 27.10	(6)	14.25	(21) 30.77	0.46 0.83
Carbon Tetrachloride	(27) 49.78	(6)	50.21	(21) 48.24	0.96 0.52
Dl-ethionine	(27) 19.87	(6)	45.155	(21) 12.64	3.57 0.01

*Degrees of Freedom are given within the parentheses.

TABLE XVII

EFFECTS OF LIPOGENIC AND LIPOTROPIC FACTORS
ON SODIUM BROMOSULPHTHALEIN

Lipotropic Factors	High Fat Ration	Lipogenic Factors					Carbon Tetrachloride	DL-ethionine
		Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin			
Standard Ration	4.88	3.88	3.88	3.88	6.75		3.88	3.88 ^d
Without Lipotropic Factor	3.38	6.00	5.75	4.63	4.88		4.63	9.75 ^{bc}
Vitamin E	--	4.38	6.00	3.88	5.00		12.13	10.63 ^b
Choline Chloride	--	4.50	6.25	4.13	5.38		4.00	4.13 ^d
Santoquin	--	3.25	3.38	6.13	3.88		8.38	9.25 ^c
Biotin	--	7.88	6.88	5.00	9.63		8.25	10.13 ^{bc}
Linoleic Acid	4.13	8.50	4.00	5.75	5.13		11.50	12.75 ^a
Vitamin C	--	4.25						
Selenium	--	3.13						

Secondly, it has been suggested that the liver of the chicken has an immense capacity for performance even when there is some interference. For example, up to 85 percent of the chicken liver can be removed and the remaining 15 per cent will take over the normal functions of the liver. Thirdly, in birds as opposed to mammals, as the level of fat in the liver increases so does the rate of BSP clearance (Nordstrom and Smith, 1967).

Abdominal Fat:

The analyses of variance for the abdominal fat score for the various lipogenic and lipotropic factors are found in Table XVIII. Significant ($P < 0.05$) differences were found in the rancid fat experiment. No significant differences were shown in the remainder of the comparisons (Table XIX). In the rancid fat experiment, the rancid fat did not significantly affect the abdominal fat score when compared to the standard ration. None of the lipotropic factors significantly influenced this trait.

Age, nutrition and productive status, temperature and metabolic rate play a role in abdominal fat accumulation. As the ovaries of pullets develop there is a corresponding increase in estrogen output. This estrogen output increases with production and soon reaches a level that influences the rate of abdominal fat deposition (Campbell, 1957). Abdominal fat levels are only important because they reflect the liver lipid levels. In chickens, the liver is the site of most of the lipid synthesized which is then transported via the blood stream to the adipose tissue. Wolford (1971) postulates that excessive

TABLE XVIII

ANALYSES OF VARIANCE FOR ABDOMINAL FAT FOR HENS
RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square				
	Source of Variance		Lipotropic		
	Total	Factors	Residual	F Value	Prob > F
High Fat	(35)*0.34	(8) 0.500	(27) 0.10	1.69	0.15
Rancid Feed	(35) 1.24	(8) 2.382	(27) 0.91	2.63	0.23
Rancid Fat	(27) 0.56	(6) 1.083	(21) 0.41	2.68	0.04
Orotic Acid	(27) 0.80	(6) 0.79	(21) 0.81	0.97	0.53
Aflatoxin	(27) 0.71	(6) 0.33	(21) 0.82	0.41	0.87
Carbon Tetrachloride	(27) 1.50	(6) 1.953	(21) 1.24	1.58	0.20
Dl-ethionine	(27) 0.84	(6) 0.905	(21) 0.82	1.10	0.40

*Degrees of Freedom are given within the parentheses.

TABLE XIX

EFFECTS OF LIPOGENIC AND LIPOTROPIC FACTORS ON
ABDOMINAL FAT

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride
	Mean Abdominal Fat Score					
Standard Ration	5.00	5.00 ^{a*}	5.00 ^a	4.75	4.50	4.00 ^a
Without Lipotropic Factor	4.25	4.50 ^a	4.25 ^{ab}	4.75	4.50	2.50 ^a
Vitamin E	4.25	3.00 ^b	4.50 ^{ab}	4.25	4.50	2.50 ^a
Choline Chloride	4.75	4.25 ^a	3.50 ^b	4.25	3.75	3.00 ^a
Santoquin	5.00	4.00 ^a	5.00 ^a	4.00	4.25	3.00 ^a
Biotin	5.00	2.50 ^c	4.50 ^{ab}	4.50	4.25	2.00 ^b
Linoleic Acid	4.25	3.50 ^a	4.75 ^a	3.50	4.00	2.00 ^b
Vitamin C	4.25	4.25 ^a				
Selenium	5.00	4.00 ^a				

*Mean values in the same column which are followed by different superscripts are significantly different.

caloric intake, as well as high temperatures leads to increase fat synthesis which ultimately ends up being stored in the adipose tissue as abdominal fat. Kubena (1974) also found that as the rearing temperature increased, the percent of abdominal fat increased as well, but while they were unable to significantly lower the per cent abdominal fat by the use of dietary choline chloride and inositol, choline chloride in this report significantly influenced the abdominal fat content in the rancid fat experiment.

Comb Condition:

Analyses of comb score are shown in Table XX. Significant differences between the treatments were observed in the rancid fat and DL-ethionine experiments. The mean scores for this trait are shown in Table XXI. The comb condition was neither affected by the lipogenic nor the lipotropic factors in all experiments except in the DL-ethionine experiment where ethionine and the lipotropic substances all helped to reduce the mean comb score. The "light dandruff or scaliness" (Abbott and Couch, 1971) and "the dandruff appearance" (Harms, et al., 1972), which are indicative of the fatty liver haemorrhagic syndrome was not observed in these experiments.

Egg Numbers:

The analyses of variance for egg numbers for lipogenic and lipotropic factors are shown in Table XXII. Significant differences were observed for the orotic acid ($P < 0.001$), rancid feed ($P < 0.06$), rancid fat and carbon tetrachloride ($P < 0.05$) experiments. Significant differences for this trait were not observed in the high fat

TABLE XX
ANALYSES OF VARIANCE FOR COMB SCORE
OF HENS RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square			Residual	F Value	P > F
	Source of Variance					
	Total	Lipogenic Factors				
High Fat	(35)* 7.64	(8) 2.39	(27) 5.25	1.54	0.19	
Rancid Feed	(35) 4.31	(8) 2.06	(27) 2.25	3.08	0.01	
Rancid Fat	(27) 3.43	(6) 0.93	(21) 2.50	1.30	0.30	
Orotic Acid	(27) 8.43	(6) 3.42	(21) 5.00	2.40	0.06	
Aflatoxin	(27) 5.43	(6) 0.93	(21) 4.50	0.72	0.64	
Carbon Tetrachloride	(27) 15.00	(6) 2.00	(21) 13.00	0.54	0.77	
Dl-ethionine	(27) 26.63	(6) 18.93	(21) 7.75	8.55	0.0002	

*Degrees of freedom are given within parentheses.

TABLE XXI

EFFECTS OF LIPOGENIC AND LIPOTROPIC FACTORS ON
COMB CONDITION

	Lipogenic Factors							Carbon Tetrachloride	DL-ethionine
	High Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin				
	Mean Comb Scores								
Standard Ration	1.00	1.00 ^a	1.00	1.00	1.50		1.00	4.50 ^c	
Without Lipotropic Factor	1.00	1.00 ^a	1.50	1.00	1.00		1.50	1.75 ^b	
Vitamin E	1.75	1.75 ^b	1.25	1.00	1.25		1.50	2.00 ^b	
Choline Chloride	1.25	1.00 ^a	1.00	2.00	1.00		1.50	1.75 ^b	
Santoquin	1.00	1.00 ^a	1.25	1.50	1.00		1.50	1.75 ^b	
Biotin	1.00	1.25 ^{ab}	1.00	1.50	1.25		1.50	1.25 ^{ab}	
Linoleic Acid	1.25	1.00 ^a	1.00	1.50	1.00		2.00	1.00 ^a	
Vitamin C	1.00	1.00 ^a							
Selenium	1.00	1.25 ^{ab}							

TABLE XXII
ANALYSES OF VARIANCE FOR EGG NUMBERS FOR HENS
RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square						F Value	Prob > F
	Source of Variance				Residual	F Value		
	Total	Lipotropic Factors						
High Fat	(35)*4.02	(8)	6.313	(27)	3.34	1.89	0.10	
Rancid Feed	(35) 8.59	(8)	14.94	(27)	6.70	2.23	0.06	
Rancid Fat	(27) 8.25	(6)	15.31	(21)	6.24	2.45	0.05	
Orotic Acid	(27) 0.13	(6)	0.57	(21)	0.00	9999.99	0.0001	
Aflatoxin	(27)16.45	(6)	19.60	(21)	15.56	1.26	0.32	
Carbon Tetrachloride	(27)32.17	(6)	63.45	(21)	23.24	2.73	0.04	
Dl-ethionine	--- ^a	---	---	---	---	--	--	

*Degrees of Freedom are given within the parentheses.

^aData not available.

and aflatoxin experiments.

The mean egg numbers are given in Table XXIII. The only significant difference in egg numbers among these treatments consisted of an increase in production by Santoquin in the rancid feed experiment, and increases in production by all lipotropic factors tested in the orotic acid experiment. Eggs were not collected for the Dl-ethionine experiment.

Egg Weight:

Analyses of variance for egg weight for the various lipogenic and lipotropic factors are found in Table XXIV. Significant differences were exhibited in the high fat and rancid fat experiments ($P < 0.001$); orotic acid experiment ($P < 0.0001$); and aflatoxin experiment ($P < 0.05$). There were no significant differences within the rancid feed and carbon tetrachloride experiments. On the whole, all the lipogenic treatments had a depressing effect on mean egg weight when these were compared to the standard ration except in the orotic acid experiment where orotic acid seemed to have increased the mean egg weight. Linoleic acid significantly increased egg weight in the Rancid Fat Experiment while choline, Santoquin and biotin significantly increased mean egg weight when compared to mean egg weight of birds fed only the high fat rations (Table XXV).

Egg Specific Gravity:

The analyses of variance (Table XXVI) showed no significant differences for egg specific gravity in all the treatments. Mean

TABLE XXIII

EFFECT OF LIPOGENIC AND LIPOTROPIC FACTORS ON
NUMBER OF EGGS

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride
(Hen-Days)	(45)	(45)	Total Number of Eggs (45)	(8)	(35)	(45)
Standard Ration	34.25*	34.00 ^{abc}	34.25 ^a	6.00 ^a	23.50	27.25 ^a
Without Lipotropic Factors	34.75	31.25 ^{bc}	33.00 ^{ab}	5.00 ^b	30.50	22.25 ^{abc}
Vitamin E	33.00	32.00 ^{bc}	34.50 ^a	6.00 ^a	27.50	24.50 ^{abc}
Choline Chloride	33.75	34.25 ^{abc}	34.75 ^a	6.00 ^a	27.75	24.75 ^{ab}
Santoquin	31.75	36.50 ^a	32.25 ^{ab}	6.00 ^a	27.00	17.50 ^{bc}
Biotin	33.75	35.00 ^{ab}	35.50 ^a	6.00 ^a	26.50	19.50 ^{abc}
Linoleic Acid	31.75	33.75 ^{abc}	29.75 ^b	6.00 ^a	25.00	16.75 ^c
Vitamin C	35.25	30.25 ^c				
Selenium	32.50	33.50 ^{abc}				

*Mean values in the same column which are followed by different superscripts are significantly different.

TABLE XXIV

ANALYSES OF VARIANCE FOR EGG WEIGHT FOR HENS
RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square					F Value	Prob > F
	Source of Variance						
	Total	Lipotropic Factors	Residual				
High Fat	(35)*0.04	(8) 0.105	(27) 0.02	4.69	0.001		
Rancid Feed	(35) 0.10	(8) 0.047	(27) 0.02	1.94	0.09		
Rancid Fat	(27) 0.04	(6) 0.125	(21) 0.02	8.41	0.0002		
Orotic Acid	(27) 0.48	(6) 0.15	(21) 0.00	9999.99	0.0001		
Aflatoxin	(27) 0.02	(6) 0.04	(21) 0.02	2.51	0.05		
Carbon Tetrachloride	(27) 0.08	(6) 0.086	(21) 0.07	1.16	0.37		

*Degrees of Freedom are given within the parentheses.

TABLE XXV
EFFECTS OF LIPOGENIC AND LIPOTROPIC FACTORS ON
EGG WEIGHTS

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride
	Mean Egg Weight (grams)					
Standard Ration	64.57 ^{a*}	62.54	64.87 ^a	61.44 ^b	61.79 ^{ab}	60.92
Without Lipotropic Factor	61.97 ^{bcd}	60.82	60.88 ^{cd}	65.10 ^a	60.53 ^{abc}	58.34
Vitamin E	62.59 ^{abc}	62.97	59.28 ^d	65.09 ^a	59.62 ^c	61.01
Choline Chloride	61.87 ^{bcd}	62.75	62.50 ^{bc}	63.52 ^b	62.29 ^a	58.04
Santoquin	63.43 ^{ab}	60.97	61.57 ^{bc}	60.65 ^b	62.04 ^{ab}	58.34
Biotin	64.84 ^a	62.60	62.16 ^{bc}	62.92 ^b	61.00 ^{abc}	57.17
Linoleic Acid	60.90 ^{cd}	63.38	63.21 ^{ab}	65.61 ^a	60.10 ^{bc}	59.19
Vitamin C	60.15 ^d	62.63				
Selenium	63.87 ^{ab}	64.30				

TABLE XXVI

ANALYSES OF VARIANCE FOR EGG SPECIFIC GRAVITY FOR HENS
RECEIVING LIPOGENIC AND LIPOGENIC FACTORS

Lipogenic Factors	Mean Square				
	Source of Variance				F Value Prob > F
	Total	Lipogenic Factors	Residual		
High Fat	(35)*0.80	(8) 0.532	(27) 0.88	0.60	0.77
Rancid Feed	(35) 1.16	(8) 0.701	(27) 1.30	0.54	0.82
Rancid Fat	(27) 0.01	(6) 1.219	(21) 0.95	1.28	0.31
Orotic Acid	(27) 0.48	(6) 2.17	(21)-0.00	0.100	1.00
Aflatoxin	(27) 0.78	(6) 0.33	(21) 0.91	0.36	0.90
Carbon Tetrachloride	(27) 1.67	(6) 2.683	(21) 1.38	1.94	0.12

*Degrees of Freedom are given within the parentheses.

values are presented in Table XXVII.

Bone Strength:

Analyses of variance for bone strength for hens receiving lipogenic and lipotropic factors are shown in Table XXVIII. Significant differences among treatment groups were obtained in the Rancid Fat ($P < 0.05$) and Rancid Feed ($P < 0.08$) Experiments. All other experiments showed no significant differences for this trait.

The mean bone breaking strength (Table XXIX) for the Rancid Fat Experiment showed that both the bones of birds fed rancid fat alone and those fed rancid fat and vitamin E had a significant higher mean bone breaking strength when compared to the birds fed the standard ration and the other lipotropic factors. The highest mean bone breaking strength (20.13 Kg) was recorded by bones of hens fed rancid feed and vitamin C.

TABLE XXVII
EFFECTS OF LIPOGENIC AND LIPOPOTROPIC FACTORS ON
EGG SPECIFIC GRAVITY

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride
	Mean Egg Specific Gravity Score					
Standard Ration	4.22	4.08	4.76	1.57	4.12	3.46
Without Lipotropic Factors	5.07	4.82	4.80	3.20	4.33	5.42
Vitamin E	4.60	4.19	4.66	3.61	3.83	5.41
Choline Chloride	4.38	4.60	3.63	2.70	3.76	4.25
Santoquin	5.13	4.75	4.43	3.19	4.29	4.21
Biotin	4.32	4.04	5.00	2.54	3.96	5.46
Linoleic Acid	4.10	4.22	3.68	3.70	4.54	5.42
Vitamin C	4.44	5.19				
Selenium	4.28	4.03				

TABLE XXVIII

ANALYSES OF VARIANCE FOR BONE STRENGTH FOR HENS
RECEIVING LIPOGENIC AND LIPOTROPIC FACTORS

Lipogenic Factors	Mean Square				
	Source of Variance				
	Total	Lipotropic Factors	Residual	F Value	Prob > F
High Fat	(35)*1873.91	(8) 1780.47	(27)1901.60	0.94	0.50
Rancid Feed	(35) 1842.29	(8) 3039.69	(27)1487.51	2.04	0.08
Rancid Fat	(27) 2369.07	(6) 4916.48	(21)1641.24	2.10	0.03
Orotic Acid	(27) 916.17	(6) 831.99	(21) 940.23	0.89	0.52
Aflatoxin	(27) 1350.22	(6) 1478.48	(21)1313.58	1.13	0.38
Carbon Tetrachloride	(27) 1904.24	(6) 1883.49	(21)1910.17	0.99	0.54
D1-ethionine	(27) 1103.40	(6) 1297.87	(21)1297.87	1.24	0.33

*Degrees of Freedom are given with the parentheses.

TABLE XXIX

EFFECTS OF LIPOGENIC AND LIPOPOTROPIC FACTORS ON
BONE STRENGTH

Lipotropic Factors	Lipogenic Factors					
	High Fat Ration	Rancid Feed	Rancid Fat	Orotic Acid	Aflatoxin	Carbon Tetrachloride
	Mean Bone Strength (Kilograms)					
Standard Ration	12.50	13.63 ^{b*}	13.63 ^{ab*}	12.38	12.30	14.15
Without Lipotropic Factor	18.35	11.58 ^b	18.58 ^a	11.85	12.98	15.00
Vitamin E	15.35	15.10 ^{ab}	19.68 ^a	10.23	11.40	13.23
Choline Chloride	16.15	13.63 ^b	12.05 ^b	14.58	10.45	19.00
Santoquin	14.93	13.70 ^b	11.30 ^b	13.03	13.63	15.60
Biotin	19.10	11.80 ^b	10.90 ^b	11.80	8.90	13.55
Linoleic Acid	15.50	15.65 ^{ab}	13.58 ^{ab}	10.78	14.48	17.73
Vitamin C	13.33	20.13 ^a				
Selenium	16.13	17.68 ^{ab}				

*Mean values in the same column which are followed by different superscripts are significantly different.

CHAPTER VI

SUMMARY

Experiments were conducted to compare the lipogenic and lipotrophic properties of selected treatments in laying hens.

"Lipogenic" factors studied were: high fat, rancid feed, rancid fat, orotic acid, aflatoxin, carbon tetrachloride and ethionine. "Lipotrophic" factors were: vitamins E and C, choline, santoquin, biotin, linoleic acid and selenium. With a few exceptions, each "lipotrophic" factor was tested in combination with each "lipogenic" factor.

Two-hundred and nineteen Leghorn type pullets, 30 weeks of age, were used in seven experiments. The hens were housed in individual laying cages and received standard management procedures.

Physiological parameters measured included the following: per cent liver fat, liver weight, liver color, liver texture, liver haemorrhages, abdominal fat, comb color, blood prothrombin time, BSP clearance, egg production, egg weight, egg specific gravity and bone strength.

When compared with the standard laying ration, the rancid fat ration without additions significantly increased the per cent liver fat, liver weight and the incidence of liver haemorrhages. The rancid feed ration also resulted in an increase in liver fat. BSP clearance rate was significantly reduced by the ethionine treatment. Egg weight

was reduced by the high fat ration and egg specific gravity was reduced by the carbon tetrachloride treatment. Other differences were non-significant.

The effects of the "lipotropic" factors on most of the traits were highly variable and were influenced by the type of "lipogenic" treatment with which they were combined.

Choline exerted a lipogenic effect when fed in combination with a high fat ration - the per cent of liver fat was increased. Choline reduced liver fat in the rancid feed and rancid fat treatments. The percent liver fat was decreased by vitamins C and E, Santoquin and biotin in the rancid feed treatment and by choline in the rancid fat treatment.

Liver color was significantly influenced by "lipotropic" factors in the ethionine treatment only. Choline, santoquin and biotin resulted in livers with a greater degree of yellow color.

Choline in the ethionine treatment resulted in livers that were less fragile while linoluc acid combination with ethionine increased the fragility of the liver.

Choline reduced the incidence of haemorrhages in the rancid fat treatment. Liver haemorrhages were increased by biotin and linoleic acid in the high fat treatment and santoquin in the rancid feed treatment.

The degree of abdominal fatness was reduced only by the feeding of biotin in the rancid feed treatment.

Prothrombin time was significantly increased by a wide variety

of the "lipotropic" factors. Choline, santoquin, biotin, linoleic acid, vitamin C and vitamin E in the rancid feed treatment increased prothrombin time, as well as linoleic acid in the high fat treatment. Biotin reduced the prothrombin time in the ethionine treatment.

BSP clearance rate was lowered by choline but was raised by linoleic acid in the ethionine treatment.

Bone strength was reduced by choline, santoquin and biotin in rancid fat treatment but was increased by vitamin C in the rancid feed treatment.

These results suggest that rancidity in laying rations is a most likely cause of abnormally high fat deposition in the livers of hens and of other traits commonly associated with the "fatty liver syndrome". Of the substances tested in this experiment, the most effective were those known to possess anti-oxidative properties or those subject to destruction by developing feed rancidity.

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VITA

Emmanuel D. Tebong was born in Batibo, United Republic of Cameroon. He attended Presbyterian Schools in Bali, Diche, Mbengwi and graduated from the Cameroon Protestant College (high school), Bali, in December, 1958. He taught at Presbyterian School, Bali, in 1959, and in 1960 he was admitted to the Cameroon Institute of Agriculture from which he graduated in 1961 to work for the then West Cameroon Department of Agriculture in both extension and research.

In 1964 he studied at Modesto Junior College, Modesto, California, returning home in 1965 to work in agriculture extension, concentrating on poultry extension programs.

In July 1970 he earned the National Diploma in Poultry from the National Diploma Board in England and the College Diploma in Poultry from Harper Adams Agricultural College after studying there from 1968.

He was admitted to Berea College, Berea, Kentucky in the Fall of 1970 and in 1971 he was awarded the B. S. degree in Agriculture.

He commenced graduate studies at the University of Tennessee in 1971 and in August 1972, he was awarded the M. S. degree with a major in Poultry Science and minors in Agriculture Extension Education, and Agriculture Economics. In August, 1974 he completed the requirements for the Doctor of Philosophy degree in Animal Science, specializing in physiology with collateral areas in zoology and agriculture economics.

He was awarded the Layman Memorial Prize by Berea College in 1971. He is a member of Alpha Gamma Sigma, Delta Tau Alpha, and Gamma Sigma Delta.

He was married in July, 1959, and he has two sons and three daughters.