TOTAL, NEUTRAL, AND PHOSPHOLIPID CONTENT IN RELATION TO DIELDRIN RESIDUE LEVELS IN SELECTED PORK MUSCLES

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ABSTRACT

TOTAL, NEUTRAL, AND PHOSPHOLIPID CONTENT IN RELATION TO DIELDRIN RESIDUE LEVELS IN SELECTED PORK MUSCLES

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To investigate the relationship between lipid composition and dieldrin residue levels in animal tissue, muscles were dissected from fresh hams of three York-Hampshire hogs. Two animals were administered oral doses of the pesticide prior to slaughter, while the third animal was fed no supplemental dieldrin. Muscles used were the adductor, quadriceps femoris, semimembranosus, biceps femoris, and semitendinosus.

Following roasting of muscles from the left hams to an internal temperature of 77°C, at an oven temperature of 177°C, both raw and cooked muscles were analyzed for lipid content. Total lipid was extracted using chloroform-methanol, and separated into neutral and phospholipid fractions on activated silicic acid. Phospholipid content was also estimated from an analysis of lipid phosphorus content. Both raw and cooked muscle tissue as well as cooking drip were analyzed for dieldrin residues using electron capture GLC.

Analyses of variance revealed significant differences among the muscles in total, neutral, and phospholipid content.

An inverse relationship existed between percentage of total lipid and phospholipid. Those muscles with a higher total lipid content had a smaller proportion of phospholipid than muscles with a lower percentage of total lipid. Lipid composition also appeared to be related to the color of the muscle.

Roasting resulted in a statistically significant increase in total lipid content, accompanied by a slight increase in percentage of phospholipid.

Dieldrin residue levels based on fat content did not differ significantly among the five muscles, but appeared to be slightly higher for the darker muscles with lower percentages of total lipid. Roasting resulted in an average reduction of 35% of residue levels in the muscle tissues; however only minute quantities of the dieldrin lost were found in the drip.

An association between percentage of phospholipid in the muscles and dieldrin levels was demonstrated. Positive correlations, statistically significant for two of the swine, were obtained between these two parameters for all three animals.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Pesticides have been used extensively in the past twenty-five years with unquestionable benefit to mankind, directly through the control of insect-vector-borne diseases, and indirectly in terms of greatly increased and improved agricultural production. In the last decade, however, public as well as professional attention has focused on the possible deleterious effects of extensive pesticide use.

The chlorinated hydrocarbon pesticides have been of particular interest, since their half-lives in the environment greatly exceed that of other types of pesticides. These organochlorines are highly soluble in fat, and thus are often linked with the lipid material in living tissues (Hayes, 1965). Little information is available, however, elucidating the mode of deposition in lipid fractions.

Lipids may be separated, according to structure and function, into neutral lipids and phospholipids (Chapman, 1969). The lipids of animal tissue contain both neutral and phospholipids, in varying proportions (O'Keefe et al., 1968; Hornstein et al., 1967). Variations in the percentages of each of these lipid fractions found in muscle tissue has been linked with the total lipid content of the

muscle (Turkki and Campbell, 1967), as well as with muscle color (Beecher et al., 1968). According to a recent study, light pork muscles were found to contain 20% more total lipid and 40% less phospholipid than dark muscles (Luddy et al., 1970).

Little information has been reported concerning the relationship of chlorinated hydrocarbon pesticides to lipid components in meats. Hugunin et al. (1969) suggested a relationship between organochlorine pesticides and phospholipid content in milk fat. The data of Ang (1970), however, did not indicate such a relationship. Zabik (1970) found dieldrin was more closely associated with neutral fats than with total lipids in eggs, whereas lindane was apparently better correlated with the phospholipid content.

To provide information which may elucidate the relationship, if any, between type of lipid material and level of chlorinated hydrocarbon pesticide residue in muscle tissue, dieldrin and total, neutral, and phospholipid analyses were conducted on selected muscles of pork rounds taken from hogs fed known quantities of dieldrin. Muscles were the adductor, quadriceps femoris, semimembranosus, biceps femoris, and semitendinosus. These muscles differ considerably in color and lipid composition (Luddy et al., 1970) and thus were considered ideal to use to illustrate any association between dieldrin and neutral or phospholipid fractions.

Several researchers have reported losses of residues from muscle tissues of various animals during cooking (Liska et al., 1967; Maul, 1969; Funk et al., 1971). This residue removal during the cooking process has been shown to accompany leaching of fat from the tissue (Ritchey et al., 1969; Yadrick et al., 1971).

An alteration in neutral and phospholipid content may also occur during cooking and other processing treatments (Younathon and Watts, 1960; Terrell et al., 1968). For example, the proportion of phospholipid in the total lipid has been found to increase in ground beef and pork during cooking (Campbell and Turkki, 1967). Therefore, the possible effects of cooking on total, neutral, and phospholipid and dieldrin levels in the present study were also examined to determine if any relationships existed between type of lipid present and ease of removal of pesticide.

REVIEW OF LITERATURE

Lipids of living tissue may be divided into neutral and phospholipids. These differ both in structure and function, and are distributed in varying amounts in different animal tissues.

The chlorinated hydrocarbon pesticides are generally found associated with the lipid fractions of animal tissue. Residue levels in tissues may be affected by a variety of treatments, some of which can also alter the lipid content of the tissue.

Lipid composition of muscle tissue was reviewed, emphasizing studies showing variations among total, neutral and phospholipid content of different animal tissues. Evidence of association between lipid composition and organochlorine residues, particularly dieldrin, was presented, following a discussion of the occurrence of residues in animal tissues and means to reduce residue levels.

Lipids

The lipids most commonly present in living tissue may be separated into the categories of neutral lipids and phospholipids. Neutral lipids, which include triglycerides,

waxes, cholesterol and vitamin A esters, are "variable" elements which serve as energy reserves for the animal, whether they are found in tissue or depot fat (Chapman, 1969). Phospholipids, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidic acid (PA), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), and sphingomyelin (Sph), are essential components of cell structure and are generally found complexed as lipoproteins. Phospholipids appear to be synthesized and catabolized in each tissue. During fasting, neutral fats are depleted, but phospholipids are not generally used as reserve supplies of energy.

Lipid content of animal tissue

Various studies have been done to determine total lipid content as well as the distribution of neutral and phospholipids in animal tissue. O'Keefe et al. (1968) reported a total lipid content of 3.48 and 6.21% in bovine semitendinosus and longissimus dorsi muscles, respectively. Phospholipid comprised 13% of the semitendinosus muscle lipid and 10% of that of the longissimus dorsi muscle, with the remainder of the lipid in each muscle present as neutral lipid. Hornstein et al. (1967) reported somewhat different muscle lipid distributions. For bovine semitendinosus and longissimus dorsi muscles, respectively, phospholipid was found to compose 15.9 and 25.6% of the total lipid. Percentages of total

lipid averaged 4.2 for the semitendinosus muscle and 5.6 for the longissimus dorsi muscle in this study.

There is evidence that percentage of phospholipid decreases with an increase in total lipid content in skeletal muscle. Turkki and Campbell (1967) reported that phospholipid comprised 17.2% of the lipid of the bovine psoas major muscle, with a total lipid content of 4.17% of the wet weight of the muscle and 41.5% of the lipid of the extensor carpi radialis muscle, whose total lipid content was 1.5%. This relationship between phospholipid content and percentage of total lipid seems to be supported by the findings of O'Keefe et al. (1968), but the values reported by Hornstein et al. (1967) do not conform to this hypothesis.

Phospholipids may be separated into several fractions, according to chemical composition. A number of researchers have determined the percentages of individual phospholipids comprising the total phospholipid content in various beef and pork muscles. These values are presented in Table I. Kuchmak and Dugan (1963) reported that an increase in lipid content was accompanied by a decrease in percentage of PE and an increase in percentage of Sph in samples taken from the center belly, a ham cross section, the center cut loin, and the 4-6 rib section of York-Hampshire hogs.

Kuchmak and Dugan (1965) studied differences in fatty acid composition among various phospholipid fractions of pork tissue. Unsaturated fatty acids composed 34.3, 52.5, and

TABLE 1

TABLE I

Lipid Composition of Various Beef and Pork Muscles

Study	Animal	Muscle	Total lipid %	Neutral lipid %	Phospho lipid %	Phospholipid Components PE %	PS %	PC %	Sph %	Protein %
Turkki & Campbell (1967)	Beef	Psoas major	4.1		17.2[a]	41.4[c]		61.6	7.0	
		Extensor carpi radialis	1.5		41.5[a]	29.0[c]		62.3	8.7	
Hornstein et al. (1961)	Beef		4.6	3.6[b]	1.0[b]	43.1		49.7[d]		7.1
	Pork		6.6	5.9[b]	0.7[b]	42.3		50.0[d]		7.6
Kuchmak & Dugan (1963)	Pork	Belly	7.74	7.16[b]	0.58[b]	32.8	4.7	58.6	3.9	
		Ham	2.67	2.20[b]	0.47[b]	34.2	7.8	54.7	3.3	
		Loin	2.88	2.43[b]	0.45[b]	33.3	4.7	60.8	1.2	
		Rib	6.18	5.61[b]	0.57[b]	28.4	2.5	63.0	6.1	
Lawrie et al. (1963)	Pork	Psoas major	1.66							
		Extensor carpi radialis	1.39							
		Longissimus dorsi (lumbar)	3.36							
		Longissimus dorsi (thoracic)	3.26							

[a]Based on percentage total lipid; [b]Based on wet weight of muscle; [c]PE and PS combined; [d]PC and Sph combined.

40.3 mole percent of fatty acids in PC, PE, and PS, respectively. Of the unsaturated fatty acids, oleic predominated in PC and linoleic in PE, and these two existed in equal amounts in PS. Variations of palmitic and stearic acid content existed among the fractions as well. The fatty acid distribution for Sph generally resembled that of PC. Hornstein et al. (1961) found a considerable difference in unsaturated fatty acid content of neutral and phospholipids of both lean beef and pork muscles. Fatty acids with two or more double bonds were shown to comprise 50% of the phospholipid fatty acids, but only 10% of the neutral lipid fatty acids. This difference was due primarily to the considerable difference in arachidonic acid content of the two lipid fractions.

The effect of processing on lipid content

An alteration in neutral and phospholipid content may occur following various processing treatments. Campbell and Turkki (1967) reported that phospholipid concentration was higher in cooked than in raw ground beef and pork, composing 2.2-2.6% of the total lipid in raw pork, and 4.6-4.9% of that in the cooked meat. No appreciable difference in fatty acid distribution existed between raw and cooked beef. However linoleate concentration of the phospholipid fatty acids of pork appeared to increase with cooking, accompanied by a correspondingly lower linoleic acid content in the drip

phospholipids. Younathon and Watts (1960) found phospholipid comprised 10% of the 27.2% total lipid content of cooked fresh hams, but these researchers did not cite values for the raw meat.

Broiling affected phospholipid fatty acid content more than the neutral lipid fraction in bovine longissimus dorsi muscles (Terrell et al., 1968). The percentages of linolenic (neutral fraction) and myristic and pentadecanoic acids (phospholipid fraction) were significantly smaller and caprylic acid (phospholipid fraction) significantly larger in the cooked steaks than in the raw.

Cooking of freeze-dried meats did not seem to alter their fatty acid composition (Giam and Dugan, 1965). In this study lipids were divided into "free" lipids, those extractable with such solvents as petroleum ether and diethyl ether, and "bound" lipids, those obtained only by using a methanol-chloroform extraction. The bound lipids, found to contain a higher percentage of polyunsaturates than the free lipids, were high in linoleic, behenic, and arachidonic acids, while the free lipids were determined to contain predominantly myristic, palmitoleic, and oleic acids.

The percentage of unsaturated fatty acids in lipid fractions of freeze-dried raw beef seemed to have an effect on storage quality of the meat (El-Gharbawi and Dugan, 1965). The phospholipid fraction, containing 33.81% unsaturates, was oxidized first, followed by more gradual oxidation of the

neutral lipid fraction, 6.22% of which was unsaturated fatty acids.

Lipid composition and muscle color

Lipid content, in particular percentage of total, neutral, and phospholipid present in muscle tissue, may be linked with muscle color. "White" muscles, which operate in short bursts of activity, have myoglobin-poor, glycogen-loaded, broad fibers. They are also characterized by a high phosphorylase content, a high ATP-splitting capacity, and little capacity for respiratory activity (Lawrie, 1966). Anaerobic glycolytic metabolism occurs within these muscles (Mommaerts, 1966). Activity in "red" muscles is the reverse of that in white. They are more or less constantly in action, and require a continuous supply of oxygen. Red muscles are composed of narrow fibers, surrounded by capillaries, and have numerous mitochondria so that oxidative phosphorylation can provide the conditions for a steady state of activity.

Total lipid content has been found to be higher for light (white) than for dark (red) muscles (Beecher et al., 1968; Beecher, et al., 1965). Luddy et al. (1970), in analyzing the light and dark portions of the semitendinosus muscle, the semimembranosus (light), and the quadriceps femoris (dark) muscles of pork, found that the light muscles contained 20% more lipid, 20% more glycerides, and 40% less phospholipids than the dark muscles. Fatty acid composition

of neutral lipids was identical for these muscles, but it differed significantly for the phospholipids. The phospholipid fatty acids of the light muscles were predominantly monoenes while the dark muscle phospholipids were higher in polyunsaturates.

Pesticides

In recent years, with the increased interest of the public in ecology, attention has become focused on the interaction of pesticides with the environment. This has transpired with the realization that minute quantities of pesticide residues exist in virtually every form of life (Paul, 1965; Durham, 1963).

The chlorinated hydrocarbon pesticides, due to their relatively long half-life, present a greater residue problem than some of the other more toxic pesticide groups. These organochlorines may be separated into a number of groups on the basis of their chemical composition: DDT and its derivatives, including DDE, DDD, Kelthane, and methoxychlor; cyclodiene compounds, such as aldrin, dieldrin, endrin, and heptachlor; and miscellaneous compounds such as lindane and benzenehexachloride (BHC). Dieldrin is not less than 85% of 1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-exo-5,8-dimethanonaphthalene (HEOD).

Residues in soil

Hetrick (1957) found that dieldrin applied to soil to control the Eastern subterranean termite maintained toxicity for seven years after application. After a period of four years, 65% of chlordane, 50% of dieldrin, and 24% of aldrin applied to African soils for control of the Argentine ant remained (Durr et al., 1958). Other investigators have reported similar results evidencing persistence of the chlorinated hydrocarbons (Banham, 1961; Lichtenstein et al., 1960).

Residues in animals and their products

Perhaps of more immediate interest is the fact that residues are present in minute quantities in most of our food supply. Cummings (1966) reported that 42% of meat, fish, and poultry samples examined contained dieldrin residues at levels of less than 0.001 to 0.006 ppm.

Contamination of animals and their products by pesticides results either from ingestion of contaminated feed or from direct application of insecticides on animals and their dwellings to control insect pests (Marth, 1965). The feeds become contaminated as a result of deliberate application of insecticides to feed crops, from translocation of insecticide into crops grown in contaminated soil, or by the unintentional contamination of crops by drift from pesticides applied to adjacent fields (Saha, 1969).

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Contamination of animals by the chlorinated hydrocarbons results in accumulation of the pesticide primarily in body fat, since these pesticides are lipid-soluble (Durham, 1963).

Accumulation of pesticides in animal tissues has been investigated by various researchers, both with regard to naturally-occurring residue levels, and through feeding of abnormally high levels of pesticide. Hogs fed 0.1-2.25 ppm dieldrin daily during a twelve week period showed generally increased residue levels in six tissues throughout the carcass with increased dosage (Gannon et al., 1959). Storage of the pesticide was in direct proportion to the fat content of the animals. After withdrawal of dieldrin-contaminated food, residue levels dropped by approximately 40% during a six-week period following the initial feeding period.

Davison (1970) compared accumulation of dieldrin in various tissues of sheep, following administration of 0.0-4.0 mg dieldrin/kg body wt/day for thirty-two weeks. Dieldrin accumulated equally in bone, carcass, adipose tissue, and heart, based on tissue fat content. Accumulation of dieldrin in the fat of muscle tissue and kidneys was similar, and slightly less than that for the above tissues. As in the earlier study, the level of dieldrin accumulated was directly related to the level of pesticide fed. Variations in the energy level of the diet fed the sheep did not significantly affect dieldrin accumulation. Following feeding of DDT, the

pesticide and its derivatives appeared to be evenly distributed throughout the extractable fat of beef cattle, based on analyses of thirteen different tissues (Rumsey et al., 1967). Residue levels in this study were also related to level of dosage of the pesticide and time elapsed after feeding.

Metabolism of pesticides

Limited information is available regarding the metabolism of chlorinated hydrocarbons in animals. Accumulation of dieldrin in sheep tissues tended to reach a plateau with time (Davison, 1970). This has been found to occur generally in mammals, suggesting that dieldrin may be metabolized and/or excreted. Matthews and Matsumara (1969) demonstrated the presence of a urinary and a fecal metabolite of dieldrin when oral doses of the pesticide were administered to rats during a one month period. Use of ^{14}C-labeled dieldrin in sheep suggested the presence of two of its metabolites in urine, one a glucuronic acid conjugate of transdiol and the other containing glucuronic acid and glycine (Hedde et al., 1970). Feel et al. (1970) identified two dieldrin metabolites in sheep urine: trans-6,7-dihydroxy-dihydroaldrin and 9-(syn-epoxy)hydroxy-1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4-endo-5,8-exo-dimethanonaphthalene. It has been suggested that the excretion of dieldrin occurs slowly, based on evidence that the pesticide is

recycled from blood to the gastrointestinal tract in ruminants (Cook, 1970).

Dieldrin may bind with solid proteins of circulating blood. Labeled dieldrin and Telodrin added to blood of rats and rabbits was found mainly to associate with erythrocytes and plasma, rather than leucocytes, platelets, or stroma (Moss and Hathaway, 1964). Intraperitoneal injection of ^{14}C dieldrin and Telodrin in rats resulted in a very rapid initial rate of removal from the blood, followed by a roughly logarithmic removal. Proportionation of these two organochlorine pesticides between blood plasma and cells in vivo at various times and concentrations suggested free permeability of the erythrocyte surface to dieldrin and Telodrin.

Placental transfer of dieldrin occurred in dairy heifers contaminated during gestation (Braund et al., 1968). The approximately 0.9% level of dieldrin recovered from the calves did not appear to vary either with the stage of gestation at which the dams were contaminated or the concentration of the pesticide in the body fat of the dams.

Excretion of heptachlor epoxide, DDT, and dieldrin occurred in the milk of dairy cows to which these pesticides were fed (Bruce et al., 1965). Portions of the residues were also present in the omental fat of the animals.

Reduction of pesticide residues

At the present time, the significance of any residues remaining in the food in relation to human health is largely unknown. Our food supply serves as a continual source of minute quantities of chemical residues which accumulate in the body (Durham, 1963). The U.S.D.A. and F.D.A. indicate that the average United States diet contains 0.02, 0.003, 0.05, and 0.003 ppm of chlorinated hydrocarbon, organophosphate, carbamate, and chlorophenoxy insecticides, respectively (Liska and Stadelman, 1968). In 1966-67, 35% of the chlorinated hydrocarbon residues in the diet were supplied by meat, fish, and poultry.

Reduction of these residues through any possible means would seem to be advantageous. Decontamination can be accomplished by actual removal of the pesticide from the animal itself, or by removal from the foodstuff through some type of processing.

Liska and Stadelman (1969) suggested three methods of approach to decontamination of animals and birds. These are (1) changes in the diet to accelerate fat removal, and with it, removal of residues; (2) use of chemicals to increase detoxification of ingested residues; and (3) prevention of residue absorption from the intestinal tract.

DDT removal from contaminated hens was slightly enhanced by use of a high protein ration following a 48 hr starvation period (Wesley et al., 1966). In a second series of

experiments, hens on a low fat diet with added vitamins retained 15% less DDT residues than hens on a low fat diet without vitamins, and 40% less residues than hens on a high fat ration (Wesley, 1968).

DDT was found to induce enzyme systems in rat liver to detoxify dieldrin more rapidly when fed simultaneously, prior to, and after ingestion of dieldrin (Liska and Stadelman, 1969). Activated carbon, which adsorbs dieldrin, seemed to prevent animal absorption of the pesticide in ruminants (Wilson and Cook, 1970). Animals fed activated carbon excreted three to ten times the amount of dieldrin eliminated by control animals. A variety of mammals treated with phenobarbital demonstrated decreased storage time of dieldrin, probably through the mechanism of induction of the liver microsomal enzymes which metabolize the insecticide (Cook and Wilson, 1970). The long-term effects of the treatment of animals with these chemicals is unknown, however. Implications of their effect on nutritional and general health status of the animal would have to be considered prior to extensive use.

Removal of residues from foodstuffs may offer a more practicable means of decontamination. The chlorinated hydrocarbon pesticide residues may be removed, in normal processing, when the fat tissues in which they concentrate are rendered out, trimmed off, or concentrated in one fraction of the food (Duggan, 1968). Destruction of residues

may occur in heat processing, irradiation, or bacterial fermentation.

Nearly quantitative removal of residues of a number of chlorinated hydrocarbon pesticides has been achieved in samples of cottonseed, soybean, and rapeseed oil through commercial processing techniques (Gooding, 1966; Saha et al., 1970; Smith et al., 1968). Elimination of the residues occurred primarily during the deodorization step, which involves a high heat treatment. Some removal of endrin was reported during hydrogenation, presumably by adsorption by activated carbon in the catalyst or by destruction by dehalogenation. Removal during deodorization may have been achieved by volatilization, which is supported by the volatilization characteristics of the pesticides (Smith et al., 1968).

Dieldrin was decomposed by ultraviolet light at 2537 A. (Henderson and Crosby, 1967). However, one of the photo decomposition products, photodieldrin, was found to be five times more toxic to mice than dieldrin itself. Such decomposition in sunlight is negligible, however, since the atmosphere absorbs uv light at 2863 A.

The effect of various treatments on chlorinated hydrocarbon residues in milk has been studied by a number of researchers. Irradiation of milk with ultraviolet light resulted in some changes in structure and destruction of methoxychlor and DDT, but rendered the milk unacceptable in

flavor (Li and Bradley, 1967). Li et al. (1970) later reported decreases in residue levels during spray-drying.

Langlois et al. (1964) studied the effect of processing of a variety of dairy products on DDT, lindane, endrin, dieldrin, and heptachlor residues in milk. Butter, ice cream, Swiss cheese, and condensed spray and roller-dried milk were prepared. Some decrease in residue levels for all the pesticides occurred during drying, with a significant loss of DDT and lindane during spray-drying, and of lindane only during roller-drying. A loss of heptachlor epoxide and dieldrin occurred during condensing. Heptachlor and heptachlor epoxide were the only residues remaining in the processed skim milk, and endrin and dieldrin the only residues found in cream.

Molecular distillation was found to remove 95-99+% of organochlorine residues from milk fat, with nearly quantitative recovery of the distilled pesticides (Bills and Sloan, 1967). Kroger (1968) also demonstrated removal of chlorinated hydrocarbon insecticides from butteroil using a 180-195^{o}C heat treatment.

Freeze-drying has been suggested as a means of removing organochlorine residues from eggs (Zabik and Dugan, 1971). A reduction of 79, 37, and 57% of lindane, dieldrin, and p,p'-DDT respectively occurred in whole eggs after freeze-drying, whereas the level of DDE increased. Removal of

residues seemed to be related to vapor pressure of the pesticides.

Various cooking treatments have been shown to be effective in reducing residue levels of chlorinated hydrocarbons in meat tissues. This is thought to occur through removal of the lipid-soluble pesticide as the fat is leached out. Reduction in levels of DDT, dieldrin, chlordane, telodrin, ovex, and lindane have been demonstrated to occur in chicken tissues cooked by various methods (Ritchey et al., 1967; Liska et al., 1967; McCaskey et al., 1968). Frying and steaming were shown to be more effective than baking in reducing residue levels in these tissues (Ritchey et al., 1969). Dieldrin levels in pork loin chops and roasts, sausage, and bacon were reduced following cooking (Maul, 1969; Funk et al., 1971; Yadrick et al., 1971). Residues were detected in the cooking drip from these tissues, indicating a loss of pesticide with the lipid lost from the meat during the cooking process.

Association of pesticide with lipid

Evidence of the association of the chlorinated hydrocarbon pesticides with lipid material of animal tissues has been presented. The nature of this association has yet to be elucidated however.

A relationship between organochlorine pesticides and phospholipid content in milk fat has been suggested (Hugunin et al., 1969). Data obtained by Ang (1970) do not

demonstrate this association, however. Zabik (1970) found dieldrin was more closely associated with neutral fats than with total lipids in eggs, whereas lindane was apparently better correlated with the phospholipid content.

Little if any information is available concerning the association of the neutral lipids and phospholipids of pork muscle tissue with pesticide residues. This study was conducted to establish whether or not any type of preferential relationship actually existed.

EXPERIMENTAL PROCEDURE

To investigate the relationship between lipid composition and dieldrin residue levels in selected roasted and raw pork muscles, uncured ham samples were obtained from three crossbred York-Hampshire hogs. The near-term pregnant hogs were ten months of age at slaughter and ranged in weight from 340 to 390 lbs. The hogs had been on a standard ration; however, during the 13-day period immediately preceding slaughter, two of the hogs, designated as animals 1 and 2, were fed orally by capsule on nine randomly selected days a total of 14.04 g of dieldrin, divided into 1.56 g portions. Animal 3, randomly selected as the control, was fed no dieldrin.

The hams were removed immediately after slaughter, skinned, wrapped first in Saran and then in waxed freezer wrap, frozen and stored at -29°C for approximately 12 mos. Following thawing at 3°C for 72 hrs, the adductor, quadriceps femoris group (including the rectus femoris, vastus lateralis, vastus medialis, and vastus intermedius muscles) semimembranosus, biceps femoris, and semitendinosus were dissected from both the right and left hams. Muscles from the left side were roasted, and both the cooked muscles and the raw muscles of the right side stored at 2°C for 3-48 hrs.

Both raw and cooked muscles were separately ground using a Toledo grinder, model 5010, fitted with a plate having holes 1/8 inch in diameter to obtain homogeneous samples for analysis.

Roasting

Muscles from the left hams were individually roasted, prior to analyses, in a Hot Point deck oven, model HJ225 with the grids set on medium, the damper half open, and the temperature maintained at 177°C ± 1° by a Versatronik controller. For roasting, each muscle was placed on a wire rack in an aluminum pan, and an iron constantan thermocouple lead was inserted into the center of the muscle. Time-temperature relationships were recorded continuously during cooking using a Brown Electronic Potentiometer High Speed Multiple Recorder. Muscles were removed from the oven when the internal temperature reached 77°C, and allowed to stand undisturbed until the maximum internal temperature had been recorded. Total, volatile, and drip losses during roasting were determined according to the method outlined by Funk et al. (1966). Cooking drip was collected for analysis in 2-oz jars with aluminum foil-lined lids by scraping the rack and pan with a chemical spatula and rinsing with a hexane-acetone mixture (2:1). The hexane-acetone solvent mixture was removed by evaporation, and the samples were stored at -29°C.

Chemical Analyses

Duplicate determinations were made for each analysis, except for the dieldrin analysis of cooking drip, in which the entire drip sample was used for a single determination. All chemicals used were ACS reagent grade.

Moisture analysis

Moisture in the muscle tissue was determined by drying samples, weighed to the nearest 0.001 g for 6 hrs at 90°C and a vacuum of 27 ins of Hg and reweighing following cooling. Percentage moisture in the samples weighing approximately 2 g was calculated according to the following formula.

$$\% \text{ moisture} = \frac{\text{wt of moisture lost (g)}}{\text{wt of original sample (g)}} \times 100$$

Lipid analyses

Total lipid was extracted from muscle tissue samples, and separated into neutral lipid and phospholipid fractions. Phospholipid in the samples was estimated from an analysis of lipid phosphorus content.

Total lipid extraction. The total lipid extraction was carried out using modifications of the procedure described by Bligh and Dyer (1959). This involved a chloroform-methanol extraction based on an 80% water content in the food. Distilled water in 34 and 50 ml portions was added to

66 g samples of raw and 50 g samples of cooked pork, respectively, in order to approximate an 80% water content. Samples were weighed to the nearest 0.001 g, using a Mettler balance, model 1-9111, and blended two times with 100 ml chloroform and 200 ml methanol in a Waring blendor at low speed. An additional 100 ml chloroform and 100 ml water were added separately, and the sample blended 30 sec after each of these additions.

The extract was placed in a 500 ml separatory funnel, following filtration over a Coors No. 3 Buchner funnel with Whatman No. 1 filter paper and slight suction. The lipid-containing chloroform layer was decanted into a 250-ml volumetric flask, following washing of the blender, residue, and filter paper with 10-15 ml aliquots each of chloroform and methanol and addition of the washings to the separatory funnel. Additional chloroform was used to bring the extract to volume. Chloroform was evaporated from 10 ml aliquots of the fat extract, and percentage lipid in the muscle calculated based on the weight of the lipid content of the aliquot, according to the following formula.

$$\% \text{ lipid} = \frac{\text{wt of lipid (g)}/10 \text{ ml} \times 25}{\text{wt of sample (g)}} \times 100$$

Separation of neutral and phospholipids. The total lipid was separated into neutral and phospholipid fractions using modifications of the procedure described by Zook (1968). This involved a separation on silicic acid in which

neutral lipids were preferentially removed by washing with chloroform, followed by removal of pnospholipids with methanol.

A slurry prepared by addition of 5-10 ml chloroform to 5 g activated silicic acid and a 10 ml aliquot of lipid extract in a 250 ml beaker was allowed to stand 30 min at room temperature, then washed with 300-400 ml chloroform on a 150 ml -60 M Buchner funnel with a fritted disk using slight suction. The remaining phospholipid-containing silicic acid was washed with 200-300 ml methanol. Percentages of neutral and phospholipids in the total lipid sample were calculated according to the following formulas after evaporation of the solvents over a steam bath.

$$\% \text{ neutral lipid} = \frac{\text{wt of neutral lipid (g)/10 ml aliquot}}{\text{wt of total lipid (g)/10 ml aliquot}} \times 100$$

$$\% \text{ phospholipid} = \frac{\text{wt of phospholipid (g)/10 ml aliquot}}{\text{wt of total lipid (g)/10 ml aliquot}} \times 100$$

Separation of phospholipids. Phospholipid fractions were separated with thin layer chromatography using modifications of a procedure described by Parker and Peterson (1965). Thin layer plates were coated with Silica Gel G to a thickness of 0.25 mm using a Desaga TLC spreader and activated for 1 hr at 100°C. Approximately 50 µg portions of the lipid extract were used to spot the plates, which were developed in chloroform-methanol-distilled water (65:25:4 ml). Following drying at room temperature, the plates were sprayed

with ninhydrin, heated to develop color, then sprayed with ammonium molybdate. Sprays were prepared according to the procedure of Dittmer and Lester (1964). Five phospholipid fractions were identified using reference standards. These included LPE and PE, identified with ninhydrin, and LPC, PC, Sph, and PA, identified using ammonium molybdate. Values for LPC and LPE were combined since the TLC spots were too close together to be separated. Spots were marked and thin layer plates stored in a dessicator until subsequent phosphorus analysis.

Analysis for phosphorus. To estimate phospholipid content and percentage of each phospholipid fraction, aliquots of total lipid extract and the phospholipid spots from thin layer plates were analyzed for phosphorus content, using modifications of the procedure of Parker and Peterson (1965).

Two-tenths ml of total lipid extract or the entire phospholipid fraction spot was used for the phosphorus analysis. Samples were digested in 100 ml micro-Kjeldahl flasks with 0.9 ml of 70% perchloric acid for 30 min on a micro-Kjeldahl digestion apparatus at medium heat, then cooled. The sides of the flasks were rinsed with 5 ml distilled water; one ml portions each of 2.5% ammonium molybdate and 10% ascorbic acid were added; and the samples shaken gently after each addition. An additional 2 ml of distilled water were added prior to heating in a boiling water bath for 5 min to develop the color produced by the reaction of molybdic acid with

ascorbic acid. Samples scraped from thin layer plates were centrifuged at 2400 rpm for 5 min following cooling, and the absorbance of all samples read on a Beckman DB-G grating spectrophotometer at 820 nm. Phosphorus content was determined using a standard curve, prepared using standards with 1-100 μg of phosphorus, and phospholipid content estimated by multiplying percentage phosphorus values obtained from lipid extracts by a factor of 25, as outlined by Wittcoff (1951). The percentage of total phospholipid represented by each phospholipid fraction was calculated according to the following formula, as described by Rao (1970).

$$\% \text{ phospholipid fraction} = \frac{\text{absorbance of phospholipid fraction}}{\text{total absorbance of all fractions}} \times 100$$

Pesticide analyses

Pesticide extraction and analysis for dieldrin were carried out according to the procedure described by Yadrick et al. (1971). Muscle samples weighing from 6-11 g and the entire available drip sample were used, after weighing to the nearest 0.001 g on a Mettler balance, model H15.

Dieldrin-containing lipid was extracted from the samples using hexane-acetone (2:1), washed with 10% sodium chloride solution to remove the acetone, and the pesticide partitioned into acetonitrile. The hexane-fat layer was decanted, and the acetonitrile removed by washing with 10% sodium chloride

solution following partitioning of the dieldrin into additional hexane. Samples were stored at -15°C following a Florisil-Celite column clean-up, until subsequent analysis by electron capture gas chromatography.

Gas chromatographic analyses were carried out using a Varian Aerograph Series 1200 instrument equipped with a tritium foil electron capture detector. It was fitted with a 6 ft (1.83 m) x 1/16 inch (2.0 mm) I.D. stainless steel column packed with 11% QF-1 and OV-17 on 80/100 mesh Gas Chrom Q and was operated at column, injector, and detector temperatures of 200, 240, and 210°C, respectively. Nitrogen flow rate was 100 ml/min for the discharge side of the detector. Quantitations were based on peak heights of standards prepared analytical grade dieldrin (recrystallized, 99+%, Shell Chemical Company, New York).

Parts per million of dieldrin based on fat content and total micrograms of pesticide were calculated for each muscle and drip sample.

Analyses of Data

Analyses of variance among muscles, animals, and/or roasting were calculated for the following factors: total, volatile, and drip cooking losses; total lipid based on the chloroform-methanol extraction, and neutral and phospholipid content based on the silicic acid separation; and dieldrin

concentration based on fat content. Duncan's multiple range test (1957) was used to sort out significant differences revealed by analyses of variance. Simple correlation coefficients were calculated between the pesticide concentration (based on the percentage fat in the sample) and the percentage of neutral and phospholipid in the sample.

RESULTS AND DISCUSSION

Five muscles were dissected from the right and left fresh hams of three York-Hampshire hogs. Two of the hogs were fed oral doses of dieldrin prior to slaughter, but no dieldrin was administered to the third animal, designated as Animal 3 in the tables. Muscles from the left fresh hams were roasted, and both raw and cooked muscles analyzed for lipid composition. Muscle tissue samples and cooking drip were also analyzed for dieldrin content.

Roasting of Muscles

The left ham muscles were roasted in a 177°C oven to a final internal temperature of 77°C. Pork has traditionally been cooked to an internal temperature of 85°C, to insure destruction of the Trichinella spiralis organism. However, recent research has shown that roasting to an internal temperature of 77°C, in addition to destroying the organism, produced organoleptically acceptable pork roasts while minimizing cooking losses (Carlin et al., 1968; Bramblett et al., 1970).

Roasting time and rates of temperature rise appeared to be dependent on both the size and shape of the muscle as illustrated in Figure 1. Average muscle weights are

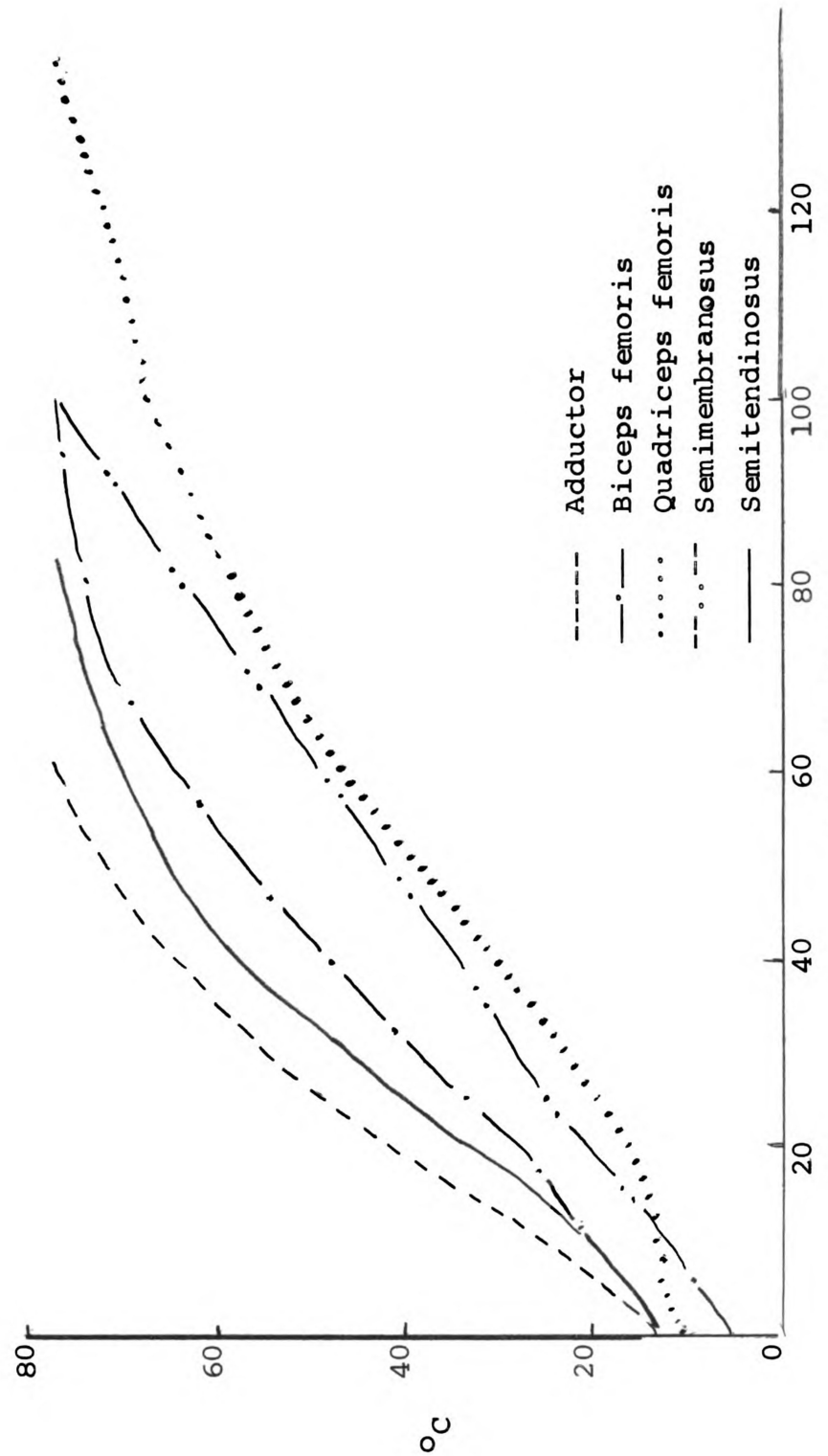

Figure 1. Rates of temperature rise during roasting of pork round muscles.

presented in Table II. The shortest roasting time was recorded for the adductor, the smallest muscle of the round. Roasting time increased by a factor of 1.5 for the biceps femoris, a large flat muscle about four times the weight of the adductor, and by a factor of 2 for the quadriceps femoris, a group of four small muscles which as a unit were approximately equal to the weight of the biceps femoris, but are considerably thicker.

TABLE II

Average Weights of Raw and Cooked Pork Round Muscles[a]

Muscle	Right fresh ham	Left fresh ham	
	Raw g	Raw g	Cooked g
Adductor	280	264	172
Quadriceps femoris	1161	1155	752
Semimembranosus	925	916	638
Biceps femoris	1195	1079	714
Semitendinosus	450	359	252

[a]Average of 3 replications.

Cooking loss data are presented in Table III. Analysis of variance established no significant differences in cooking losses among the muscles. Total cooking losses for the five muscles averaged 32.48%, with the major portion (30.47%)

occurring as volatile losses. Drip losses accounted for only 2.21% of the total losses; muscles were trimmed of all external fat, however, which would ordinarily contribute to the drip. Drip losses may also have been lowered due to spattering as the drip came into contact with the hot roasting pan. Some loss of drip weight may also be attributed to charring of organic material which was evident in the baking pan.

TABLE III

Total, Volatile, and Drip Cooking Losses[a]

Muscle	Total %	Volatile %	Drip %
Adductor	34.52	31.57	2.95
Quadriceps frmoris	34.81	33.90	1.91
Semimembranosus	30.01	28.31	1.70
Biceps femoris	33.39	30.69	2.70
Semitendinosus	29.67	27.86	1.81

[a]Average of 3 replications.

No cooking loss values for individual pork round muscles were obtained from the literature. Lukianchuk (1960) reported total, volatile, and drip losses of 33.14, 31.10, and 2.04%, respectively, for bovine semimembranosus muscles roasted to 80°C. These values are slightly higher than for

the same muscle in this study perhaps due to differences in size and/or composition. The small percentage of drip losses reported by Lukianchuk probably reflect the fact that these beef muscles were also trimmed of external fat.

In the roasting of pork loins to 75°C, Pengilly and Harrison (1966) observed total, volatile, and drip losses of 20.3, 14.4, and 7.3% respectively. That these losses are somewhat lower than those reported in this study may be attributed in part to the slightly lower internal temperature used and to the relatively larger size of the loin roasts compared to individual round muscles. The presence of bone in the roasts and a protective layer of surface fat may have also contributed to smaller losses. Funk et al. (1968) found that the presence of surface fat reduced total cooking losses. The higher drip losses reported by Pengilly and Harrison may be explained by the presence of surface fat on the roasts.

Lipid Composition

Two methods were utilized for extraction of total lipids. The first of these, a chloroform-methanol extraction based on an 80% adjusted moisture content in muscle tissue, has been demonstrated to be an efficient method for nearly quantitative lipid removal (Bligh and Dyer, 1959). Average moisture content before adjustment was 72.20% for raw and 61.25% for cooked muscles. A hexane-acetone lipid extraction,

following the recommendations of Shell Development Company (1965) with slight modifications, was used to extract dieldrin from the samples. Parts per million of dieldrin in the fat were calculated on the basis of total lipid values obtained from the hexane-acetone extraction. The remainder of the calculations and any comparisons made among lipid values are based on the chloroform-methanol extraction data, since slightly lower total lipid values consistently obtained with the hexane-acetone extraction show this method to be less efficient for total fat.

Percentages of neutral lipid and phospholipid were determined using a separation on activated silicic acid, while percentage of phospholipid was also estimated from phosphorus content of total lipid samples. Values for phospholipid from the silicic acid separation were arbitrarily chosen for use in comparisons since neutral lipid values from the same separation were also used, although phospholipid values obtained by both methods are in most cases within 1-3% of each other.

Values for total, neutral, and phospholipids in the raw and cooked muscles are presented in Table IV, and analyses of variance to determine significant differences among these lipid values in Table V. Significant differences among muscles and animals were pinpointed using Duncan's multiple range test (1957); the results for muscles are included in Table IV.

TABLE IV

Total, Neutral, and Phospholipid in Selected Pork Muscles[a,b]

Muscle	Total[c]		Percentage of Total Lipid		
			Neutral	Phospholipid	
	Chloroform-methanol %	Hexane-acetone %	Silicic acid %	Silicic acid %	Phosphorus %
Raw:					
Adductor	2.12[A]	1.67	69.23	30.78	30.50
Quadriceps femoris	3.19[B]	2.75	79.05[C]	20.53[E]	17.90
Semimembranosus	2.96[AB]	2.67	80.01[C]	19.87[E]	16.80
Biceps femoris	4.44[B]	4.19	89.51[D]	10.50[F]	11.84
Semitendinosus	5.86	5.31	88.01[D]	11.99[F]	9.77
Cooked:					
Adductor	3.01[A]	1.91	68.70	31.63	33.95
Quadriceps femoris	6.47[B]	4.51	82.36[C]	17.63[E]	19.30
Semimembranosus	4.26[AB]	3.25	77.58[C]	20.76[E]	17.76
Biceps femoris	6.53[B]	5.49	84.95[D]	15.05[F]	15.89
Semitendinosus	10.63	8.65	89.50[D]	10.50[F]	13.54

[a]Average of 3 replications.

[b]Values with the same superscript are not significantly different ($P < 0.01$). Analyses were conducted only for chloroform-methanol and silicic acid determinations.

[c]Based on wet weight of muscle.

TABLE V

Analyses of Variance Among Muscles, Roasting, and Animals of Total, Neutral, and Phospholipid Content

Source	dif.	Mean Square		
		Total Lipid	Neutral Lipid	Phospho-lipid
Total	29			
Muscle	4	27.97**	379.02**	374.31**
Roasting	1	45.49**	2.80	1.08
Animal	2	7.25*	122.54**	103.68**
Muscle-Roasting	4	3.72	14.51	12.06
Muscle-Animal	8	0.99	13.34	12.00
Roasting-Animal	2	2.59	7.26	10.37
Error	8	1.20	6.07	1.27

*Significant at the 0.05 level of probability.
**Significant at the 0.01 level of probability.

Lipid composition for animals 1, 2, and 3, respectively, was found to include the following: 5.00, 5.77, and 4.07% total lipid; of which 81.82, 83.78, and 76.98% was neutral lipid; and 18.06, 16.23, and 22.49% was phospholipid. The percentage of total lipid for animal 3 was demonstrated by Duncan's multiple range test to be significantly lower ($P < 0.05$) than that of animal 2. In addition, animal 3 had values significantly lower ($P < 0.01$) than the other two

animals for neutral lipid and significantly higher ($P < 0.05$) for phospholipid. It is suggested that these differences are due to differences in the metabolism of the animals, rather than to the fact that animal 3 was the control animal.

Roasting resulted in a significant increase in total lipid content ($P < 0.01$) relative to the wet muscle weight. The five cooked muscles contained an average total lipid content of 6.18%, of which 19.11% was phospholipid. The increase in total lipid may be attributed primarily to the moisture loss occurring during roasting. Campbell and Turkki (1967) reported an increase of approximately 2% in percentage of phospholipid in total lipid when ground beef and pork were cooked. Muscles used in this study showed an average increase in the proportion of phospholipid in the total lipid of less than 1% following cooking. Since phospholipids form part of the cell structure, and neutral lipids are more likely to be associated with expendable fat stores, one might expect neutral fats to be more easily rendered during cooking. The raw ground meat used in the study cited above contained a higher percentage of the expendable neutral fats than the trimmed muscles of this study.

The five muscles of the round were found to have an average total lipid content in the raw state of 3.71%, of which 18.73% was phospholipid. Kuchmak and Dugan (1963), using swine of the same breed as those used in this study, extracted the lipid from a cross-section of the uncured hams,

and found a total lipid content of 2.7%, 17.6% of which was phospholipid.

Duncan's multiple range test revealed significant differences in total, neutral, and phospholipid among the five muscles (Table IV). The semitendinosus contained a significantly higher ($P < 0.01$) level of total lipid than all other muscles, and the biceps femoris and quadriceps femoris had significantly higher ($P < 0.01$) values for total lipid than the adductor. The adductor had a significantly higher ($P < 0.001$) level of phospholipid and lower ($P < 0.001$) level of neutral lipid than all other muscles. Phospholipid values for the quadriceps and semimembranosus were significantly higher ($P < 0.01$) than those for the biceps femoris and semitendinosus muscles.

Several researchers have associated differences in total, neutral, and phospholipid content with differences in muscle color. Pork muscles may be classified as either dark (red) muscles, which have been characterized as being high in respiratory activity and constantly in action, or light (white) muscles, which generally operate in short bursts of activity with little capacity for respiratory activity (Lawrie, 1966).

This study included five muscles of the pork round, of which two (the adductor and the quadriceps femoris) were dark and two (the semimembranosus and the biceps femoris) were light. The fifth muscle, the semitendinosus, may contain both

a light and a dark portion. Beecher et al. (1968) found differences in the lipid composition of the light and dark portions, as demonstrated by the 9.3% total lipid content of the light portion, compared to 4.6% for the dark portion. Luddy et al. (1970) observed similar differences in total lipid between light and dark portions of porcine semitendinosus muscles. The semitendinosus muscles used in this study did not appear to easily lend themselves to separation into light and dark portions, and in fact resembled more closely the appearance of the two light muscles used in the study. Therefore, the semitendinosus muscles were analyzed in their entirety, rather than divided into two sections.

The raw adductor, a dark muscle, contained the highest percentage of phospholipid (30.78%) and the white biceps femoris, a light muscle, only 10.50%. Phospholipid levels in the quadriceps femoris (dark) and semimembranosus (light) muscles fell between those for the two muscles cited above, and were not significantly different from each other. The semitendinosus muscle, observed to be the color of a light muscle, had a phospholipid content of 11.99% thus falling close to that for the light biceps femoris muscle.

Luddy et al. (1970) reported similar variations in total, neutral, and phospholipid content that were related to pork muscle color. The semimembranosus (light) was found to have a lipid content of 5.4%, of which 19.8% was phospholipid, while the quadriceps femoris (dark) contained 3.9%

total lipid, 34.3% of which was phospholipid. O'Keefe et al. (1968) reported bovine semitendinosus muscles had a 3.48% total lipid content, 13% of which was phospholipid. Hornstein et al. (1967) found values of 4.2% and 15.9% for total and phospholipid respectively in the same muscle.

The findings of this study did not reveal the considerable differences between the semimembranosus and quadriceps femoris muscles that were demonstrated by Luddy et al.; however, a similar trend toward differences in the proportion of neutral and phospholipids in light and dark muscles seems to support the conclusions of these researchers.

The percentages of each lipid fraction found in the muscles and for the animals of this study seemed to be even more closely related to total lipid content in the muscle or animal than to muscle color itself. Muscles with lower total lipid possessed the greatest proportion of phospholipid while muscles with higher total lipid had less phospholipid (Table IV). Turkki and Campbell (1967) reported that an increase in neutral lipid and a decrease in phospholipid accompanied an increase of total lipid in bovine psoas major and extensor carpi radialis muscles. The data of Luddy et al. (1970) also reflected this relationship.

The phospholipid portion of each muscle was further separated into lysophosphatidylcholine and lysophosphatidylethanolamine, sphingomyelin, phosphatidylcholine, phosphatidylethanolamine, and phosphatidic acid. Percentages of

each of these fractions are reported in Table VI.

TABLE VI

Phospholipid Fractions in Selected Pork Muscles

Muscle	State	LPC & LPE %	Sph %	PC %	PE %	PA %
Adductor	Raw	18.36	18.97	32.96	18.84	10.83
	Cooked	17.45	18.97	32.11	21.27	15.80
Quadriceps femoris	Raw	11.11	19.02	37.67	18.64	14.57
	Cooked	15.66	34.29	20.37	16.11	19.19
Biceps femoris	Raw	13.64	14.87	34.77	18.85	17.89
	Cooked	12.52	24.44	33.06	16.69	13.29
Semimembranosus	Raw	16.75	28.49	23.54	12.19	12.87
	Cooked	17.63	37.33	22.63	21.86	15.44
Semitendinosus	Raw	23.40	13.20	31.04	19.34	14.52
	Cooked	11.45	9.60	40.97	24.02	14.46

Averages in the raw muscle for LPC and LPE, Sph, PC, PE, and PA were 16.65, 18.91, 31.99, 17.57, and 14.15% respectively. These contrast with levels of 3.3, 54.7, 34.2, and 7.8% for Sph, PC, PE, and PS (phosphatidyl serine) respectively, reported by Kuchmak and Dugan (1963) for entire ham muscles. Average levels in the cooked muscles varied only slightly from those present in the raw muscles.

Pesticide Analyses

Parts per million of dieldrin were determined for muscles and drip, both on a wet tissue and total drip basis, and based on fat content. These values are reported in Table VII.

Residue levels based on fat content in animal 3 were significantly different from those in the other two animals ($P < 0.05$). This would be expected, since pig 3 was the control animal and was not administered supplemental dieldrin. Residues found in its tissues may be assumed to have accumulated from the animal's normal diet.

An analysis of variance revealed that the adductor had a significantly higher level of parts per million of dieldrin based on fat content. This significant difference appears to be due entirely to an abnormally high level of dieldrin present in the cooking drip for animal 2. In comparing the weight of the cooking drip for the adductor of this animal with that of other muscles and animals, there is evidence that an error in weighing occurred, which would account for the high dieldrin level. It is therefore suggested that figures for dieldrin in the drip of this muscle be disregarded.

An analysis of variance showed no significant reduction of parts per million of dieldrin in the fat of the muscle tissues occurred with roasting. Residue levels in pork have been reported to decrease with cooking, primarily by

TABLE VII

Dieldrin Levels in Raw and Cooked Pork Muscles and in Cooking Drip

Muscle	Animal	Raw		Cooked		Drip	
		ppm/wet	ppm/fat	ppm/wet	ppm/fat	ppm/wet	ppm/fat
Adductor	1	0.80	38.35	0.67	35.87	0.11	58.82
	2	0.91	59.16	0.78	34.53	4.08	251.62
	3	0.07	5.72	0.05	3.33	0.13	59.39
	Mean	0.59	34.41	0.50	24.58	0.12	56.61
Quadriceps femoris	1	1.25	42.19	1.56	37.71	0.31	34.94
	2	1.29	50.30	1.75	30.13	0.65	11.28
	3	0.07	2.56	0.08	2.17	0.17	12.76
	Mean	0.87	31.68	1.13	23.34	0.37	19.66
Semimem-branosus	1	0.97	30.11	1.03	29.05	0.09	18.39
	2	0.95	33.70	0.90	27.16	0.10	33.86
	3	0.06	3.01	0.09	3.01	0.02	5.39
	Mean	0.66	22.27	0.67	19.74	0.07	19.21
Biceps femoris	1	1.12	26.09	1.25	18.73	0.02	22.23
	2	1.39	28.49	1.40	24.34	0.37	11.61
	3	0.08	2.41	0.03	0.82	0.02	2.91
	Mean	0.87	18.99	0.90	14.63	0.14	12.25
Semiten-dinosus	1	1.90	30.65	1.93	27.21	0.24	16.88
	2	1.63	31.76	1.75	14.35	0.72	20.39
	3	0.10	2.05	0.06	0.95	0.04	1.36
	Mean	1.21	21.49	1.25	14.17	0.33	12.87

accompanying leaching of fat as drip (Ritchey et al., 1969; Yadrick et al., 1971). The lack of surface fat on the muscles appears to have limited reduction of residue levels through this mechanism.

A consistent reduction in residue levels based on fat, though not significant, did occur with roasting however. These data would seem to support the conclusions of the studies cited above, which indicate that cooking is an effective means of reducing levels of chlorinated hydrocarbon residues in meats.

Recovery of dieldrin in the cooked muscles and drip compared to residue levels in the raw meat is presented in Figure 2. The average recovery calculated based on total μg of dieldrin in the raw and cooked wet tissue and drip, was 69.58% ± 18.89, most of which was present in the cooked meat, since very little drip was obtained during roasting. Most of the loss of residues apparently accompanied volatile losses in the meat during cooking. Zabik and Dugan (1970) have indicated a possible relationship between vapor pressure of pesticides and their removal from eggs by freeze-drying. Levels of recovery in each animal are presented in Table VIII. Levels of recovery in each muscle for pigs 1 and 2 were more consistent than those for animal 3.

Recovery of dieldrin in the cooked muscles appeared to be related to the size and shape of the muscle. The highest levels of recovery were obtained in the quadriceps femoris

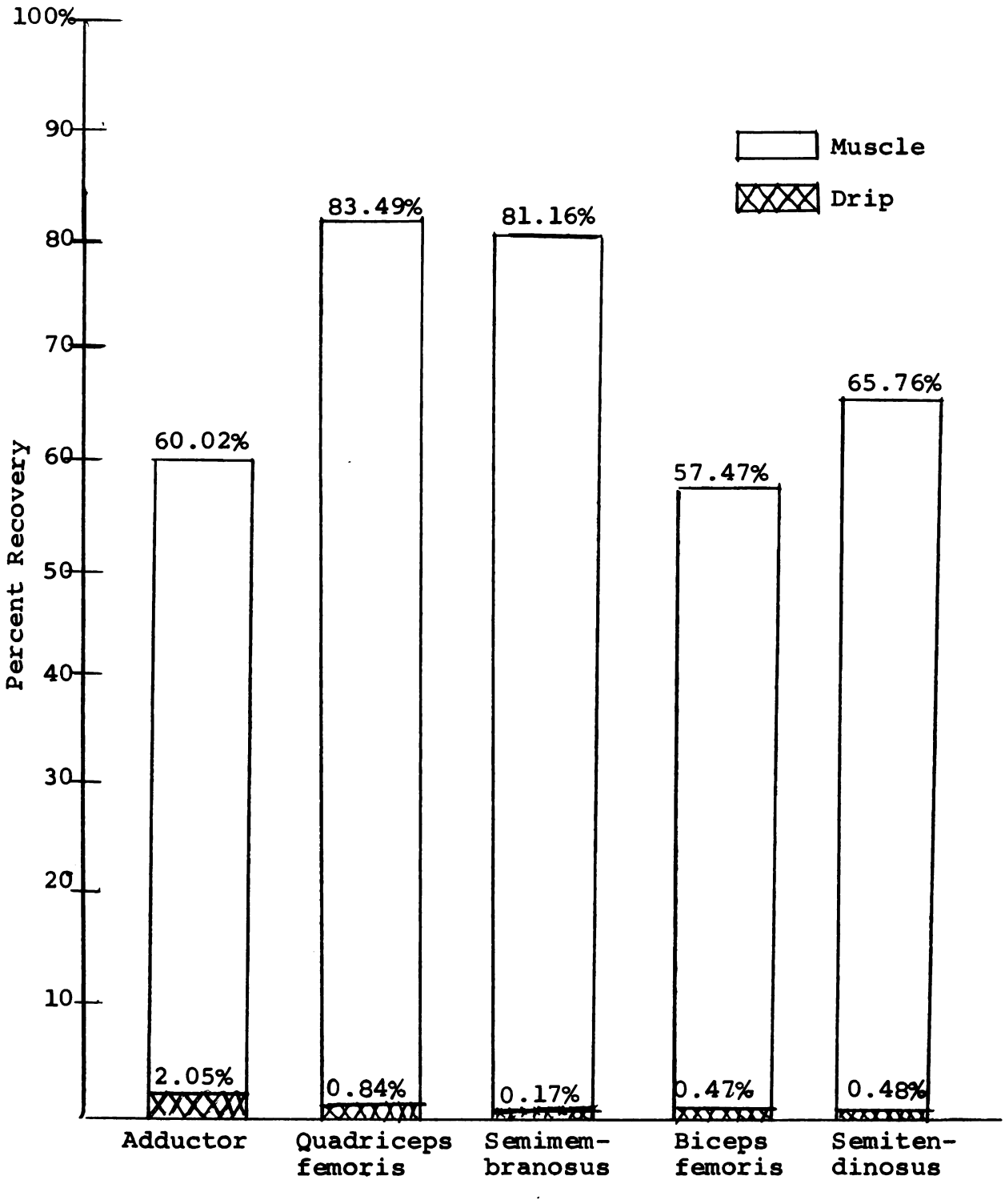

Figure 2. Dieldrin recovered in cooked pork muscles and drip.

TABLE VIII

Recovery of Dieldrin in Roasted Pork Muscles and Drip

Muscle	Animal	Recovery %
Adductor	1	67.95
	2	58.53
	3	53.59
Quadriceps femoris	1	82.58
	2	66.21
	3	23.63
Semimembranosus	1	84.01
	2	88.45
	3	78.02
Biceps femoris	1	72.49
	2	69.20
	3	101.79
Semitendinosus	1	75.50
	2	75.96
	3	45.83

and the semimembranosus, both of which are relatively large, thick muscles. Lower recovery levels occurred in the smaller semitendinosus and adductor muscles, and in the biceps femoris which, though comparable to the quadriceps femoris in weight, is a very thin, flat muscle.

Dieldrin Residue Levels in Relation to Lipid Composition

Correlation coefficients were calculated between parts per million based on fat content and neutral and phospholipid content of the muscles. These are reported in Table IX.

TABLE IX

Correlation Coefficients of PPM (based on lipid) of Dieldrin with Percentage of Neutral or Phospholipid

Animal	Percentage Neutral Lipid	Percentage Phospholipid
1	-0.76**	0.74**
2	-0.44	0.44
3	-0.79***	0.78***

**Significant at the 0.01 level of probability.
***Significant at the 0.001 level of probability.

Correlation coefficients indicate an association between phospholipid content and dieldrin residue levels in muscle tissues. The mechanism of metabolism of dieldrin by swine is unknown. However, the significant correlations between the phospholipid and dieldrin of animals 1 and 3 may indicate some preferential deposition of the dieldrin into the phospholipid component of the fat. Although the correlation coefficient for animal 2 is not significant, it supports the trend demonstrated in the other two animals. Hugunin et al. (1969) reported a similar association between phospholipid and levels of chlorinated hydrocarbon residues in milk fat.

Ingestion of dieldrin in quantities higher than those naturally occurring in pork tissues did not appear to affect the correlation of phospholipid with dieldrin, since coefficients were similar for animal 1, fed 14.02 g of dieldrin

prior to slaughter, and animal 3, the control animal fed no dieldrin. The correlations may be attributed in part to the fact that doses of dieldrin were fed immediately prior to slaughter. It is unknown whether correlations would exist for animals 1 and 2 had dosages of the pesticide ceased some time prior to slaughter.

SUMMARY AND CONCLUSIONS

This study investigated the relationship between the lipid composition of raw and cooked muscles of the pork round and dieldrin residue levels in the muscle tissue. Dieldrin residues accumulated in the animal tissues through the normal diet, and in the case of two of the swine, through additional oral doses of the pesticide in capsule form.

The adductor, quadriceps femoris, semimembranosus, biceps femoris, and semitendinosus muscles of the round were included in this study. Muscles from the left fresh hams were individually roasted at 177°C to an internal temperature of 77°C. These cooked samples as well as raw muscles from the right hams were analyzed for total, neutral, and phospholipid composition. All muscle tissue samples and cooking drip from the roasted muscles were analyzed for dieldrin content using electron capture gas chromatography.

Difference in total, neutral, and phospholipid content were found to exist among the five muscles. The total lipid content of the raw muscles ranged from 2.12% in the adductor to 5.80% in the semitendinosus. As total lipid increased in the five muscles, the proportion phospholipid in total lipid tended to decrease. Phospholipid content for the raw

adductor was 30.78% of the total lipid, and only 11.99% for the semitendinosus. This relationship was also reflected in the cooked muscles.

In general, those muscles designated by observation of color as dark (red) muscles tended to have a lower total lipid content and a higher percentage of phospholipid based on total lipid than those muscles observed to be light (white). This is reflected in the figures cited above for the dark adductor muscle and for the light semitendinosus.

A significant increase in total lipid content based on the wet weight of the muscles occurred with roasting, accompanied by a slight increase in the proportion phospholipid in total lipid. The cooked adductor contained 3.01% total lipid, 31.63% of which was phospholipid; values for the cooked semitendinosus were 10.63% total lipid of which 10.50% was phospholipid.

Average levels of dieldrin based on fat content were 34.41, 31.68, 22.27, 18.99, and 21.49 parts per million for the raw adductor, quadriceps femoris, semimembranosus, biceps femoris, and semitendinosus, respectively. An analysis of variance revealed no significant differences among muscles; however those muscles designated as dark seemed to have slightly higher residue levels than did the light muscles.

Although roasting resulted in a reduction of dieldrin levels in the muscle tissues, these differences were not

statistically significant. Average values for the cooked adductor, quadriceps femoris, semimembranosus, biceps femoris, and semitendinosus were 24.58, 23.34, 19.74, 14.63, and 14.17 ppm based on fat, respectively.

The average dieldrin recovery based on total micrograms of dieldrin in the raw and cooked muscle tissue and drip was 65.58%. Residue losses apparently accompanied volatile losses in the meat during cooking, and appeared to be related to the size and shape of the muscle. Recovery was greater for the quadriceps femoris and semimembranosus, large, thick muscles, than for the smaller adductor muscle.

An association was evident between the levels of dieldrin and phospholipid in the muscle tissue. The correlation coefficients for animals 1, 2, and 3, respectively were 0.74, 0.44, and 0.78, indicating that the association did not appear to be dependent on an abnormal pesticide intake.

The data obtained in this study seem to indicate that:

1. Total lipid content of pork muscles is related to muscle color.
2. An inverse relationship exists between total lipid and phospholipid content in pork round muscle tissue.
3. Dieldrin residues are more closely associated with the phospholipids than with the neutral lipids of pork round muscles.
4. Dieldrin residues in pork muscles may be reduced by roasting.

SUGGESTIONS FOR FUTURE RESEARCH

Any conclusions drawn from data obtained in this research were based on the analysis of only three animals. To establish the validity of these findings, the analyses conducted herein should be extended to a much larger number of animals. The trends noted in this study suggest the following additional research:

1. The association established between lipid composition and muscle color should be explored further in pork round muscles and in other muscles and species of animals that exhibit this color difference.

2. The association between total lipid content and percentage of phospholipid should be elaborated further, with respect to metabolism and deposition of each lipid fraction.

3. The mechanism of metabolism of dieldrin in swine and other animals should be explored, based on the findings that it is preferentially associated with phospholipids in pork round tissue.

4. The implications of cooking for reduction of pesticide residue levels warrants further investigation, both in terms of reduction of residues in foods during commercial processing and at a consumer-homemaker level.

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