TISSUE LIPID VARIATIONS UNDER LONG TERM DIETHYLSTILBESTROL ADMINISTRATION

by

Tai-Lee Ke

B.Sc., National Tsing-Hua University, Taiwan, 1970
M.S., (Chemistry), Kansas State University, 1973

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Graduate Biochemistry Group

Kansas State University
Manhattan, Kansas

1977

Approved by:

Major Professor: R. E. Clegg
Department of Biochemistry

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Much work has been aimed at the role of diethylstilbestrol (DES) as a carcinogen, as a growth factor in livestock and in the control of prostate carcinoma. Recent work has suggested that the variations in the serum lipid patterns brought about by the administration of large doses of DES for a limited time could be modified by high concentrations of ascorbic acid and vitamin E in the diet. This led to the question of what changes would occur, particularly in the tissues, of cockerels fed smaller doses of DES for an extended period. In the following study the effect of prolonged administration of lower levels of DES on the lipid composition of the tissues of rapidly growing and adult cockerels was investigated. Because of the large number of analyses the investigation was undertaken as a survey of the lipid variations and of the fatty acid distribution in each tissue selected. The results of this survey are to be used to plan future work, particularly work involving ascorbic acid and vitamin E.

LITERATURE REVIEW

Many investigators have studied the relationship of diet to the lipid changes in the blood serum and tissues of a variety of subjects. Only a few of these reports will be discussed. In all cases when enough lipid was added to the diet this change was reflected in the blood serum lipids, and in some cases in the tissues. Camejo et al. (4) reported early changes in the plasma lipoproteins in rabbits fed a high cholesterol diet. The cholesteryl ester of density less than 1.09 g/ml was the most prominent change. The lipoproteins of the cholesterol fed rabbits contained lipoproteins with a higher lipid-to-protein ratio. Corey et al. (7) concluded that the response of certain primate species to dietary changes in fat differed. For example, both cebus and squirrel monkeys were hypercholesterolemic on diets high in coconut oil, but only the squirrel monkeys were sensitive to extra cholesterol. Diets containing 10% safflower oil were not hypercholesterolemic. The source of plasma-free fatty acids was investigated by Heimberg (13). Human volunteers were fed either safflower oil or coconut oil, and the serum lipids compared. After safflower oil feeding the 18:2 in the free fatty acids increased. Similar observations were made in the triglycerides of the VLD lipoproteins. After coconut oil feeding the percentage of 12:0 and 14:0 increased in both the free fatty acids and the triglycerides. Renaud and Gautheron (21) fed cholesterol from a variety of fat sources and reported that the fats could be classified according to their atherogenicity as follows, in decreasing order: butter, olive oil, coconut oil, cacao butter and corn oil. The severity was correlated with the plasma cholesterol.

Guenter et al. (12) reported that in laying hens higher levels of dietary linoleic acid would increase egg yolk, liver and adipose tissue

linoleic acid, and the oleic and saturated fatty acids in yolk and tissue showed an inverse relationship to linoleic acid. Kruski et al. (17) reported that the greatest change in liver lipids of male chickens fed a hypercholesterolemic diet was found in the cholesterol ester, and that the concentration of cholesterol in the liver may be the principal factor controlling cholesterol metabolism. Widdowson et al. (37) suggested that the difference in the body fat of British and Dutch infants was due to the fatty acid content of the milk. The British were feeding cows' milk while the Dutch were removing the cow milk fat and replacing it with maize oil. The result was a much higher percentage of unsaturated fatty acids in the body fat of the Dutch infants. The British infants had a high concentration of cholesterol. Recently several authors have reported blood serum lipid variations when high levels of ascorbic acid were fed (8,11,15,25,29). Ascorbic acid decreased the serum cholesterol in healthy young adults (29). In patients with atherosclerosis ascorbic acid enhanced the activity of lipoprotein lipase and lowered the triglycerides (25).

The above discussion shows that dietary lipids do affect the lipids of both the blood and tissues. The changes resulting from DES administration are different in that the DES apparently affects the metabolism of the subject and causes lipid variations even in the case of low fat diets. Most of the studies on the effects of DES are concerned with the use of the compound in the control of prostate carcinoma (10,20), as a carcinogen (14,16,22,23,24), its use in livestock feeding (3,28), or its concentration in the tissues.

Kudzma et al. (18) have emphasized that the chick is a good laboratory model for the study of estrogen-induced hyperlipidemia. A recent article by Clegg, Klopfenstein and Klopfenstein (5) emphasizes that high dosages of DES do, as has been reported many times, cause a serum lipid rise, and

ascorbic acid and vitamin E decrease the levels of triglycerides and phospholipids of the serum. For birds receiving both vitamins, DES seems to produce a smaller percentage increase of oleic acid in the total esterified fatty acid. The administration of high levels of DES for several days has been standard procedure to produce a high concentration of lipids in the serum of cockerels (9,18,19). No reports have shown that short term administration of DES has an effect on the tissue lipids since most workers were interested only in the production of blood lipids and not the tissues.

An interesting investigation would be a determination of what effect low levels of DES administration for an extended period of time would have on the lipid spectrum of the blood serum and tissues, and what effect ascorbic acid and vitamin E would have on this change. Before attempting the above mentioned investigation, a determination of the effect of low level DES administration on serum and tissue lipids was undertaken. The purpose of this study was to determine what lipids in the tissue are affected by the lower DES administration so that in subsequent investigations the lipids that demonstrate changes may be followed when the high vitamin supplementation is employed.

ILLEGIBLE DOCUMENT

THE FOLLOWING DOCUMENT(S) IS OF POOR LEGIBILITY IN THE ORIGINAL

THIS IS THE BEST COPY AVAILABLE

MATERIALS AND METHODS

I. Treatment of Animals:

In the first series, 15 adult cockerels (6 month old, white Leghorn, Babcock strain) were divided into three groups of 5 cockerels each. The controls (group 1) were fed a commercial grower ration; the cockerels of group 2 were injected with 0.2 mg of diethylstilbestrol (DES) per day, and the cockerels of group 3 were injected with 0.4 mg of DES per day. Groups 2 and 3 received injections four times per week for a total of six weeks. In the second series 15 young cockerels eight weeks of age were compared. The controls (group 4) were fed a commercial grower ration; the cockerels of group 5 were injected with 0.1 mg of DES per day, and the cockerels of group 6 were injected with 0.2 mg of DES per day. Groups 5 and 6 were injected four times per week for a total of five weeks. In all cases the DES was dissolved in propylene glycol and injected into the leg of the chicken. At the end of each series blood samples were obtained, and then samples of liver, heart, adipose tissue, thigh tissue and breast tissue were removed, washed, wrapped in Saran wrap and stored in a freezer. The samples were then analyzed as subsequently described.

II. Blood Serum:

Approximately 10 ml of blood was taken from the wing vein of each bird. The blood was allowed to clot at 39°C for three hours and centrifuged at approximately 5000 rpm at 5°C. The serum (approximately 5 ml) was decanted and stored at 5 to 7°C.

The lipids were extracted from 2 ml of blood serum by mixing the

serum with 5.0 ml of methanol and 2.5 ml of chloroform and thoroughly shaking. Then 2.5 ml of chloroform and 2.5 ml of water were added, mixed well, and allowed to stand overnight at 5°C. The chloroform layer was removed and dried over anhydrous sodium sulfate. Aliquots were used for thin layer and gas liquid chromatography.

III. Extraction of Lipids from Tissues:

The tissues were weighed and cut into small pieces, blended with 15 ml of distilled water for five minutes and poured into a glass homogenizer. Another 5 ml of distilled water was employed to wash the blender and this portion was added to the homogenizer. The suspension was homogenized for 5 minutes and poured into a test tube. Five ml of the homogenate was returned to the homogenizer, mixed with 10 ml of methanol and 5 ml of chloroform and homogenized for 5 minutes. Another 5 ml of chloroform was added and this mixture homogenized for 5 additional minutes. The final homogenate was poured into a centrifuge tube and centrifuged at 5000 rpm for about 30 minutes. The chloroform layer was removed, measured and dried over anhydrous sodium sulfate. Aliquots were used for thin layer chromatographic (TLC) and gas liquid chromatographic (GLC) analyses.

In an investigation of this type where large numbers of determinations are to be run it is necessary to employ methods which are not time consuming. It should be emphasized at this stage that the interest was in surveying a broad spectrum of lipids and that it will be necessary to follow up results obtained by methods considered more reliable at a later date. The choices made here were TLC for the lipid spectrum and GLC for the fatty acid percentages. The TLC method is well adapted for large

numbers of samples but should be replaced by methods considered more reliable at a later date. The GLC technique is probably the best available for the fatty acids.

IV. Thin Layer Chromatography:

The apparatus used in this thin layer analysis was developed by Stahl (26). The TLC plates were Brinkmann Siliplate-22. Standard mixtures obtained from the Applied Science Laboratories (1) were run along with the unknown mixtures of lipids extracted from heart, liver, breast tissue, thigh tissue, adipose tissue and blood serum samples. These standards were: (a) cholesteryl ester, linoleic acid and trilinolein which were developed by hexane/ether/glacial acetic acid (90/10/1 by volume), and (b) cardiolipin, cholesterol, phosphatidyl ethanolamine, lecithin and lysolecithin which were developed by chloroform/methanol/water (60/25/4 by volume).

Standard mixtures were spotted along with the various chloroform extracted samples described previously. The plates were then developed in the solvent system appropriate for the standard. After development in the two different solvent systems the lipid spots were made visible by spraying with 18% phosphoric acid + 3% copper acetate in water (2), and heating in an oven at 112°C for 15 minutes. In order to estimate the amounts of various lipids in the sample, the thin layer plates were examined by means of a Photovolt Corp. photovolt recording densitometer.

The use of TLC to measure the amount of triglycerides may be questioned. The reagents employed to measure the amount of triglycerides are based on the effect of the reagents on the unsaturated fatty acids. Therefore it is possible that the total triglycerides are not measured,

and that only the changes in the degree of unsaturation of the fatty acids contained in the triglycerides were determined. This should be kept in mind when the triglycerides data are considered. The same may also be true of the lecithin and cephalin.

V. Gas Liquid Chromatography:

The esterification of lipid samples was accomplished by placing an aliquot of the chloroform solution in teflon lined cap culture tubes, adding 0.1 ml of 2 mg/ml solution of heneicosanic acid (internal standard), evaporating to dryness under nitrogen gas, and then adding 1.0 ml of a methanol solution of BCl_3. The tubes were tightly closed and heated at 57°C for one hour. The fatty acid esters were extracted with hexane and the resulting solution was employed in a Barber-Colman 500 GLC with an 8.0 foot column coated with 7.5% diethylene glycol succinate. The column temperature was 190°C and the nitrogen carrier gas flow was 70 ml per minute.

VI. Statistical Analysis:

The data were analyzed statistically by the Mann-Whitney test* for analysis of variance (6).

---

*The test was suggested by Dr. Kemp of the Department of Statistics, Kansas State University.

RESULTS AND DISCUSSION

The lipid distribution analyses are summarized in Table 1 and the fatty acid distribution in Table 2. The data were compared by the method of Mann-Whitney (see Materials and Methods). First, the 0.0 level DES vs low level DES and the 0.0 level vs high level DES were compared in both the adult and the young cockerels. The results of the statistical analyses are shown in Table 3. Second, the young and the adult birds were compared within each DES level. The results are in Table 4. In order to emphasize certain relationships in Table 1 and 2 the data have been plotted and these comparisons are found in Figures 1 through 6.

The following discussion is concerned with significant differences at the 0.1 level or better, and attention will be focused on Tables 3 and 4. The data from which these tables of significance were calculated are in Table 1 and 2.

Blood Serum: (Table 3) The adult birds showed no significant differences in either the fatty acid distribution or the lipid distribution when group 1 was compared against group 2. The only exception was a lower 16:0 fatty acid for group 2, but the difference was at the 0.1 level. In contrast, when the levels of group 1 were compared against group 3 (high DES level) the 18:1 fatty acid rose and the 18:2 fatty acid fell (both at the 0.05 level). In addition, the cholesterol and cholesteryl ester levels rose (0.05 and 0.1 levels, respectively).

In the case of the young birds, group 5 (low level DES) exhibited an increase in 16:0 fatty acid and a decrease in cholesteryl esters (both at the 0.05 level). Group 6 (high level DES) exhibited an increase in

Table 1: Lipids in the Tissues of Adult and Young Cockerels[1]

Blood Serum:

Lipids	Group[2] 1	2	3	4	5	6
	milligrams per milliliter					
Triglycerides	4.20±1.95	3.70±0.30	4.10±0.30	0.90±0.30	0.50±0.20	1.05±0.75
Cholesteryl Esters	0.20±0.05	0.15±0.05	0.35±0.10	1.80±0.25	1.30±0.10	1.45±0.15
Cholesterol	0.25±0.15	0.50±0.25	0.75±0.25	1.65±0.20	1.25±0.10	1.40±0.40
Cephalin	0.25±0.10	0.25±0.10	0.25±0.20	0	0	0
Lecithin	10.2±2.70	10.2±2.50	11.1±2.80	5.85±1.80	6.20±1.10	8.75±1.25

Heart Tissue:

Lipids	Group[2] 1	2	3	4	5	6
	milligrams per gram					
Triglycerides	3.01±2.39	4.97±3.39	2.19±1.11	2.38±1.13	3.30±2.25	4.00±0.21
Free Fatty Acids	1.13±0.36	2.88±0.97	3.81±0.76	6.13±1.12	7.96±1.17	7.05±0.25
Cholesterol	6.69±1.88	6.71±0.87	7.97±1.44	11.2±2.89	12.0±2.28	11.8±1.28
Cephalin	8.51±3.48	6.65±1.75	5.77±1.19	10.5±1.26	9.48±2.49	9.14±2.00
Cardiolipin	1.05±0.41	0.47±0.19	0.53±0.27	1.57±1.51	0	0
Lecithin	76.9±18.8	57.9±11.2	62.5±9.35	59.7±13.1	39.6±22.3	27.8±11.3

Liver Tissue:

Lipids	Group[2] 1	2	3	4	5	6
	milligrams per gram					
Triglycerides	0	0	5.93±3.59	0	0	2.33±1.87
Free Fatty Acids	0	0	0.67±0.58	3.82±0.09	4.45±1.68	5.70±3.40
Cholesterol	6.77±1.56	6.92±2.82	8.72±3.46	14.1±3.43	12.5±3.21	15.4±1.86
Cephalin	5.84±2.77	8.14±1.73	12.1±1.01	10.9±1.51	10.4±2.20	10.8±2.00
Cardiolipin	0	1.46±0.90	0.93±0.46	1.33±0.44	0.99±0.51	0.90±0.18
Cholesteryl Esters	0.47±0.05	0.24±0.09	0.69±0.49	0	0	0
Lecithin	51.4±15.5	54.5±14.1	83.7±12.5	101.20±5.68	84.2±20.4	87.9±15.9

- Continued -

- Continued -

Thigh Tissue:

Lipids	Group(2) 1	2	3	4	5	6
	milligrams per gram					
Triglycerides	18.2±6.83	12.0±8.36	14.6±12.6	2.97±0.87	0	0
Cholesterol	2.82±1.91	1.25±0.73	1.96±0.42	3.06±0.72	3.14±1.36	3.58±1.12
Cephalin	2.74±0.76	2.81±0.93	2.32±1.33	1.74±1.08	1.53±0.67	2.06±0.86
Cardiolipin	0.93±0.11	0.73±0.65	0	0	0	0
Lecithin	28.4±5.24	31.0±11.8	17.3±6.20	8.11±7.56	0	0

Breast Tissue:

Lipids	Group(2) 1	2	3	4	5	6
	milligrams per gram					
Triglycerides	6.11±3.39	7.64±4.34	4.15±4.06	2.59±1.49	2.57±1.69	1.19±0.77
Cholesterol	0.57±0.40	1.26±0.76	0.32±0.27	3.43±1.12	4.26±0.83	4.42±0.84
Cephalin	1.14±0.39	3.20±0.93	0.91±0.90	2.90±0.63	3.37±0.59	2.75±0.58
Lecithin	27.6±9.28	42.2±12.1	19.3±6.35	38.2±9.81	45.2±7.61	34.1±4.94

Adipose Tissue:

Lipids	Group(2) 1	2	3	4	5	6
Triglycerides	0.55±0.10	0.72±0.10	0.74±0.17	0.88±0.27	0.91±0.23	1.00±0.43

(1) Each figure represents 3 - 5 samples
(2) Group 1 = Adult Controls; Group 2 = Adults on 0.2 mg DES; Group 3 = Adults on 0.4 mg DES; Group 4 = Young Controls; Group 5 = Young on 0.1 mg DES; Group 6 = Young on 0.2 mg DES.

Table 2: Percentage of Fatty Acids in the Tissues of Adult and Young Cockerels

Blood Serum:	Group(2)					
Fatty Acids	1	2	3	4	5	6
	percentage					
16:0	23.7±1.0	20.7±1.9	22.0±2.3	24.1±1.2	25.8±1.5	25.0±1.6
16:1	0.8±0.1	0.6±0.1	0.7±0.3	1.8±0.7	2.7±1.3	2.8±0.4
18:0	18.8±1.0	18.7±0.6	18.0±1.1	15.8±0.5	16.8±1.7	15.7±1.0
18:1	21.8±1.9	22.0±4.5	29.2±3.5	20.8±1.4	20.7±2.4	25.7±3.8
18:2	26.4±2.5	26.2±4.0	21.7±3.5	28.0±1.7	27.2±1.8	23.0±2.8
18:3	0	0	0	1.7±0.6	0.8±0.5	1.1±0.9
20:4	7.4±2.3	10.8±4.8	8.1±2.1	7.8±1.4	7.6±3.7	6.7±0.8

Heart Tissue:	Group(2)					
Fatty Acids	1	2	3	4	5	6
	percentage					
12:0	0.5±0.1	0.5±0.1	0.6±0.3	0	0	0
14:0	1.6±0.8	1.6±0.6	1.4±0.2	2.2±0.4	2.0±0.6	2.0±0.6
16:0	21.2±1.5	20.3±2.6	21.1±1.4	18.8±1.0	19.3±0.5	19.9±1.8
16:1	1.3±0.7	1.1±0.4	1.1±0.2	0	0	0
18:0	15.4±1.9	16.5±1.0	16.0±0.9	18.9±0.9	18.1±1.1	16.3±0.3
18:1	20.4±4.1	17.5±1.2	19.2±1.6	13.4±1.6	14.6±2.4	15.9±2.7
18:2	23.4±1.7	24.7±2.4	23.1±1.1	21.4±1.0	22.6±1.7	23.1±1.1
20:4	16.3±5.1	17.6±3.1	17.5±1.8	25.3±1.1	23.4±2.9	22.8±3.2

Liver Tissue:	Group(2)					
Fatty Acids	1	2	3	4	5	6
	percentage					
16:0	22.2±2.7	23.6±2.8	21.6±2.2	21.9±1.7	22.7±1.4	25.1±2.1
16:1	0.8±0.3	0.8±0.1	1.2±0.5	0	0	0
18:0	25.4±4.8	25.0±1.7	25.0±1.9	27.2±1.0	25.9±0.6	24.9±2.0
18:1	17.0±5.6	17.8±0.7	19.8±1.8	12.9±0.7	14.9±2.6	17.2±6.4
18:2	20.8±1.6	21.5±2.6	19.1±1.2	21.7±1.5	22.0±2.2	19.5±2.8
20:4	12.1±2.4	12.9±2.5	12.9±1.1	16.2±1.4	14.4±1.5	13.2±3.5

- Continued -

- Continued -

Thigh Tissue:	Group(2)					
Fatty Acids	1	2	3	4	5	6
12:0	0.6±0.2	0.7±0.2	0.7±0.2	0.8±0.1	0.9±0.1	1.1±0.1
14:0	0.5±0.3	0.8±0.4	1.0±0.8	2.7±0.4	2.8±0.3	2.8±0.5
16:0	23.6±1.4	22.9±0.8	23.3±2.4	21.8±1.3	20.4±1.7	20.4±1.2
16:1	2.5±1.3	1.7±0.7	1.7±1.5	1.3±0.4	1.4±0.3	1.8±0.7
18:0	12.5±3.2	13.2±1.2	12.7±1.6	15.8±1.7	17.7±0.4	16.4±1.1
18:1	29.8±4.0	29.5±1.7	31.4±2.7	21.7±3.0	18.6±1.4	19.5±2.8
18:2	23.6±1.3	24.7±3.1	24.1±0.6	24.3±1.8	23.8±0.5	23.0±0.8
18:3	2.1±0.5	1.0±0.7	1.8±0.3	0	1.0±0.5	1.4±0.3
20:4	4.8±2.1	5.6±2.6	3.3±1.7	11.8±1.7	13.3±1.0	13.9±2.6

Breast Tissue:	Group(2)					
Fatty Acids	1	2	3	4	5	6
	percentage					
12:0	0.9±0.3	0.4±0.1	0.9±0.3	0.5±0.1	0.8±0.3	0.9±0.3
14:0	2.0±0.5	1.8±0.7	2.0±0.3	3.9±0.8	4.1±0.4	4.3±0.9
16:0	25.2±1.6	22.9±1.9	24.7±1.5	24.3±2.3	26.2±1.4	25.8±1.6
16:1	1.9±0.7	1.7±0.9	1.7±0.7	1.4±0.2	0.8±0.4	0.6±0.5
18:0	10.5±0.8	10.9±1.3	9.1±1.8	10.4±1.0	11.3±1.5	11.0±1.0
18:1	31.7±3.8	33.5±3.6	36.6±3.2	26.9±4.5	26.3±1.6	26.0±2.4
18:2	18.8±2.2	20.3±1.6	18.4±1.4	18.5±1.4	19.0±1.3	18.7±1.6
18:3	1.3±0.8	1.3±0.6	0.6±0.2	0	0	0
20:4	8.6±2.2	7.9±3.5	6.7±3.3	14.0±4.0	11.6±2.4	12.6±1.9

- Continued -

- Continued -

Adipose Tissue:	Group[2]					
Fatty Acids	1	2	3	4	5	6
	percentage					
12:0	0.5±0.2	0.7±0.1	0.5±0.1	0.7±0.2	0.7±0.2	0.7±0.1
14:0	0	0	0	0.4±0.3	0.2±0.1	0.2±0.1
16:0	25.5±1.4	24.4±1.1	23.9±1.1	20.7±0.1	22.0±1.0	22.7±0.8
16:1	4.4±0.4	4.4±0.9	5.0±0.7	3.1±1.2	5.3±0.9	5.9±2.1
18:0	7.1±1.5	7.0±1.5	6.4±0.8	7.6±1.4	6.1±0.1	6.1±1.7
18:1	37.5±1.9	37.3±3.4	39.1±0.5	36.7±1.9	33.8±2.5	35.3±2.3
18:2	22.2±1.0	23.8±3.0	22.4±0.7	28.1±0.4	29.3±2.4	26.7±2.5
18:3	2.6±1.7	2.2±0.5	2.5±0.6	2.7±0.6	2.5±0.6	2.3±0.4

(1) Each figure represents 3-5 samples
(2) Same as (2) in Table 1

Table 3: Results of the Mann-Whitney Statistical Analyses of the Control vs the DES Treated Birds

	Fatty Acids									Lipids						
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	TG	CE	CH	PE	PC	CL	FA
Blood Serum:																
Adult 1 vs 2	–	–	+↓	0	0	0	0	–	0	0	0	0	0	0	–	–
Adult 1 vs 3	–	–	0	0	0	++↑	++↓	–	0	0	+↑	++↑	0	0	–	–
Young 4 vs 5	–	–	++↑	0	0	0	0	0	0	0	++↓	0	0	0	–	–
Young 4 vs 6	–	–	0	+↑	0	++↑	++↓	0	0	0	+↓	0	0	+↑	–	–
Heart Tissue:																
Adult 1 vs 2	0	0	0	0	0	0	0	–	0	0	–	0	0	0	++↓	0
Adult 1 vs 3	0	0	0	0	0	0	0	–	0	0	–	0	+↓	0	++↓	++↑
Young 4 vs 5	–	0	0	–	0	0	0	–	0	0	–	0	0	0	++↓	+
Young 4 vs 6	–	0	0	–	++↓	0	+↑	–	0	0	–	0	0	++↓	++↓	0
Liver Tissue:																
Adult 1 vs 2	–	–	0	0	0	0	0	–	0	0	0	0	0	0	++↑	0
Adult 1 vs 3	–	–	0	0	0	0	0	–	0	++↑	0	0	++↑	++↑	++↑	++↑
Young 4 vs 5	–	–	0	–	++↓	0	0	–	0	0	–	0	0	0	0	0
Young 4 vs 6	–	–	++↑	–	+↓	0	0	–	0	++↑	–	0	0	0	+↓	0

- Continued -

- Continued -

	Fatty Acids									Lipids						
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	TG	CE	CH	PE	PC	CL	FA
Thigh Tissue:																
Adult 1 vs 2	0	0	0	0	0	0	0	+↓	0	0	-	0	0	0	0	-
Adult 1 vs 3	0	0	0	0	0	0	0	0	0	0	-	0	0	+↓	++↓	-
Young 4 vs 5	0	0	0	0	+↑	+↓	0	++↑	0	++↓	-	0	0	++↓	-	-
Young 4 vs 6	++↑	0	0	0	0	0	0	++↑	++↑	++↓	-	0	0	++↓	-	-
Breast Tissue:																
Adult 1 vs 2	++↓	0	+↓	0	0	0	0	0	0	0	-	0	++↑	0	-	-
Adult 1 vs 3	0	0	0	0	0	+↑	0	0	0	0	-	0	0	0	-	-
Young 4 vs 5	++↑	0	0	+↓	0	0	0	-	0	0	-	0	0	0	-	-
Young 4 vs 6	++↑	0	0	++↓	0	0	0	-	0	0	-	+↑	0	0	-	-
Adipose Tissue:																
Adult 1 vs 2	0	-	0	0	0	0	0	0	-	++↑	-	-	-	-	-	-
Adult 1 vs 3	0	-	0	0	0	0	0	0	-	++↑	-	-	-	-	-	-
Young 4 vs 5	0	0	++↑	+↑	0	0	0	0	-	0	-	-	-	-	-	-
Young 4 vs 6	0	0	++↑	+↑	0	0	0	0	-	0	-	-	-	-	-	-

Adult 1 = Control; 2 = Adults on 0.2 mg DES; 3 = Adults on 0.4 mg DES;
Young 4 = Control; 5 = Young on 0.1 mg DES; 6 = Young on 0.2 mg DES.

0 = Not significantly different; - = Not measurable;
+ = Significant at the 0.1 level;
++ = Significant at the 0.05 level;
↑ or ↓ indicates the direction of the difference of the second group mentioned.

TG = Triglycerides; CE = Cholesteryl Esters; CH = Cholesterol; PE = Cephalin (phosphatidyl ethanolamine)
PC = Lecithin (phosphatidyl choline); CL = Cardiolipin; FA = Free Fatty Acids.

Table 4: Results of the Mann-Whitney Statistical Analyses of the Adult vs the Young Cockerels

	Fatty Acids									Lipids						
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	TG	CE	CH	PE	PC	CL	FA
Blood Serum:																
Group 1 vs 4	-	-	0	++↓	++↑	0	0	++↓	0	+↑	++↓	++↓	++↑	+↑	-	-
Group 2 vs 5	-	-	++↓	+↓	++↑	0	0	++↓	0	++↑	++↓	++↓	++↑	++↑	-	-
Group 3 vs 6	-	-	+↓	++↓	++↑	0	0	++↓	0	++↑	++↓	++↓	++↑	0	-	-
Heart Tissue:																
Group 1 vs 4	++↑	0	++↑	++↑	++↓	++↑	++↑	-	++↓	0	-	++↓	0	0	0	++↓
Group 2 vs 5	++↑	0	0	++↑	+↓	+↑	0	-	++↓	0	-	++↓	+↓	0	0	++↓
Group 3 vs 6	++↑	0	0	++↑	0	++↑	0	-	++↓	0	-	++↓	++↓	++↑	0	0
Liver Tissue:																
Group 1 vs 4	-	-	0	++↑	0	0	0	-	+↓	0	++↑	++↓	++↓	++↓	++↓	++↓
Group 2 vs 5	-	-	0	++↑	0	0	0	-	0	0	++↑	++↓	+↓	++↓	0	++↓
Group 3 vs 6	-	-	++↓	++↑	0	0	0	-	0	0	++↑	++↓	0	0	0	++↓

- Continued -

- Continued -

	Fatty Acids									Lipids						
	12:0	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:4	TG	CE	CH	PE	PC	CL	FA
Thigh Tissue:																
Group 1 vs 4	+↓	++↓	+↑	0	+↓	++↑	0	++↑	++↓	++↑	-	0	0	++↑	++↑	-
Group 2 vs 5	+↓	++↓	++↑	0	++↓	++↑	0	0	++↓	++↑	-	+↓	++↑	++↑	++↑	-
Group 3 vs 6	++↓	++↓	++↑	0	++↓	++↑	0	++↑	++↓	+↑	-	++↓	0	++↑	0	-
Breast Tissue:																
Group 1 vs 4	++↑	++↓	0	0	0	0	0	++↑	++↓	0	-	++↓	++↓	0	-	-
Group 2 vs 5	++↑	++↓	++↓	0	0	++↑	0	++↑	+↓	0	-	++↓	0	0	-	-
Group 3 vs 6	0	++↓	0	0	0	++↑	0	++↑	++↓	0	-	++↓	++↓	++↓	-	-
Adipose Tissue:																
Group 1 vs 4	0	++↓	++↑	0	0	0	++↓	0	-	0	-	-	-	-	-	-
Group 2 vs 5	0	++↓	++↑	0	0	0	++↓	0	-	0	-	-	-	-	-	-
Group 3 vs 6	++↓	++↓	0	0	0	++↑	++↓	0	-	0	-	-	-	-	-	-

Group 1 = Adult Control; Group 2 = Adults on 0.2 mg DES; Group 3 = Adults on 0.4 mg DES;
Group 4 = Young Control; Group 5 = Young on 0.1 mg DES; Group 6 = Young on 0.2 mg DES.

0 = Not significantly different; - =Not measurable;
+ = Significant at the 0.1 level;
++ = Significant at the 0.05 level;
↑ or ↓ indicates the direction of the difference of the first group mentioned.

TG = Triglycerides; CE = Cholesteryl Esters; CH = Cholesterol; PE = Cephalin (phosphatidyl ethanolamine)
PC = Lecithin (phosphatidyl choline); CL = Cardiolipin; FA = Free Fatty Acids.

16:1 and 18:1 fatty acids and lecithin (0.10, 0.05 and 0.10 levels respectively), and a decrease in 18:2 fatty acid (0.05 level), and cholesteryl esters (0.1 level).

Heart Tissue: (Table 3) In the adult the low DES level (group 2) showed no difference in all comparisons over the control except cardiolipin which fell significantly (0.05 level). The heart tissue of the birds administered high levels of DES (group 3) still showed no significant difference in the fatty acids distribution, but the cardiolipin and phosphatidyl ethanolamine decreased (0.05 and 0.1 level respectively) and the free fatty acids increased (0.05 level).

The fatty acid percentage and the lipid component of the young heart tissue showed no difference between the control and the low DES treatment except that the free fatty acid value of group 5 was higher than the control (0.1 level) and cardiolipin fell significantly (0.05 level). When the high level DES (group 6) was compared with the control a decrease in 18:0 fatty acid, cardiolipin and lecithin (all at the 0.05 level) and an increase in 18:2 fatty acid (0.1 level) occurred. In fact, the cardiolipin was not measurable in either DES level.

Liver Tissue: (Table 3) No significant differences in fatty acid percentage distribution occurred either for the low DES level or the high DES level over the control adult birds. For the low DES (group 2) there was an increase in cardiolipin, while for high DES (group 3) there was an increase in triglyceride, free fatty acids, phosphatidyl ethanolamine, cardiolipin and lecithin (all at the 0.05 level).

In the case of the young birds the low level of DES compared against the control showed no difference in the lipid distribution and a decrease

in 18:0 fatty acid (0.05 level). For the high level DES there was an increase in 16:0 fatty acid and triglycerides (both at the 0.05 level) and a decrease in 18:0 fatty acid and cardiolipin (both at the 0.1 level). Triglycerides in both young and old were not measurable by the densitometer in the control or low DES groups.

Thigh Tissue: (Table 3) In the adult the lipid and fatty acid distribution between the control and the low level DES (group 2) showed no differences except the 18:3 fatty acid of low DES treatment was lower than the control (0.1 level). The fact that the high DES level does not go this low would make this value suspect, but it should be emphasized that the 18:3 fatty acid of high DES is lower, although not significantly, at the 0.1 level. The high DES level (group 3) showed no difference in fatty acid distribution but a decrease in cardiolipin and lecithin over the control (at the 0.05 and 0.1 levels respectively).

For the young tissue when the levels in the low DES (group 5) were compared against the control the 18:3 fatty acid and 18:0 fatty acid rose (0.05 and 0.1 levels respectively) and the triglyceride, lecithin, and 18:1 fatty acid decreased (0.05, 0.05 and 0.1 levels respectively). The levels in the high DES (group 6) showed an increase in 12:0 fatty acid, 18:3 fatty acid and 20:4 fatty acid, and a decrease in triglycerides and lecithin; all differed significantly at the 0.05 level when compared to the control.

Breast Tissue: (Table 3) The adult birds receiving low DES (group 2) exhibited phosphatidyl ethanolamine significantly higher (0.05 level) and 12:0 and 16:0 fatty acids lower (0.05 and 0.1 levels respectively) but the fact that the higher DES level did not echo this, would make these values suspect. At present we have no explanation for this. For the high DES

treatment (group 3) the only difference was an increase in 18:1 fatty acid at the 0.1 level.

The young birds administered low level DES (group 5) showed no difference in lipid distribution but did exhibit an increase in 12:0 fatty acid (0.05 level) and a decrease in 16:0 fatty acid (0.1 level). For the birds administered high DES level (group 6) the 12:0 fatty acid and cholesterol increased (0.05 and 0.1 levels respectively), and the 16:1 fatty acid decreased (0.05 level).

Adipose Tissue: (Table 3) For the adult birds there were no differences in fatty acid percentage distribution either for low DES level or for high DES level against the control. In both the low and high DES levels there was an increase in triglyceride (0.05 level).

The adipose tissue of the young birds showed no difference in major lipid constituents for both the low DES and high DES levels against the control, but an increase in 16:0 fatty acid (0.05 level) and 16:1 fatty acid (0.1 level) at both DES levels.

In an investigation of this type in which trends are sought it is necessary to search the data for differences that do not appear to be significant as well as those that meet the level of significance originally set. In the above summary only those differences which were at the 0.1 level or better were discussed. In the following paragraphs trends which do not meet this requirement are outlined. For this purpose consult Figs. 1-6.

In the serum results (Fig. 1) of the young cockerels the rise in the 16:1 fatty acid at the low DES level was almost as great as in the high level young birds. Therefore the trend was up in this case. In the

Figure 1: Lipid and Fatty Acid Distribution in Blood Serum of Normal and DES Treated Cockerels*

(a) Lipids: (upper bar graphs)

A - Triglycerides

B - Cholesterol

C - Cholesteryl Esters

D - Lecithin (phosphatidyl choline)

E - Cephalin (phosphatidyl ethanolamine)

(b) Fatty Acids: (lower bar graphs) The number before the colon is the number of carbons and the number after the colon is the number of double bonds

1 - Control Adult Cockerels

2 - Adults Administered 0.2 mg DES

3 - Adults Administered 0.4 mg DES

4 - Control Young Cockerels

5 - Young Cockerels Administered 0.1 mg DES

6 - Young Cockerels Administered 0.2 mg DES

*The open bars are the adult cockerels; the black bars are for the young cockerels. The vertical line within the top part equals standard deviation.

FIGURE 1

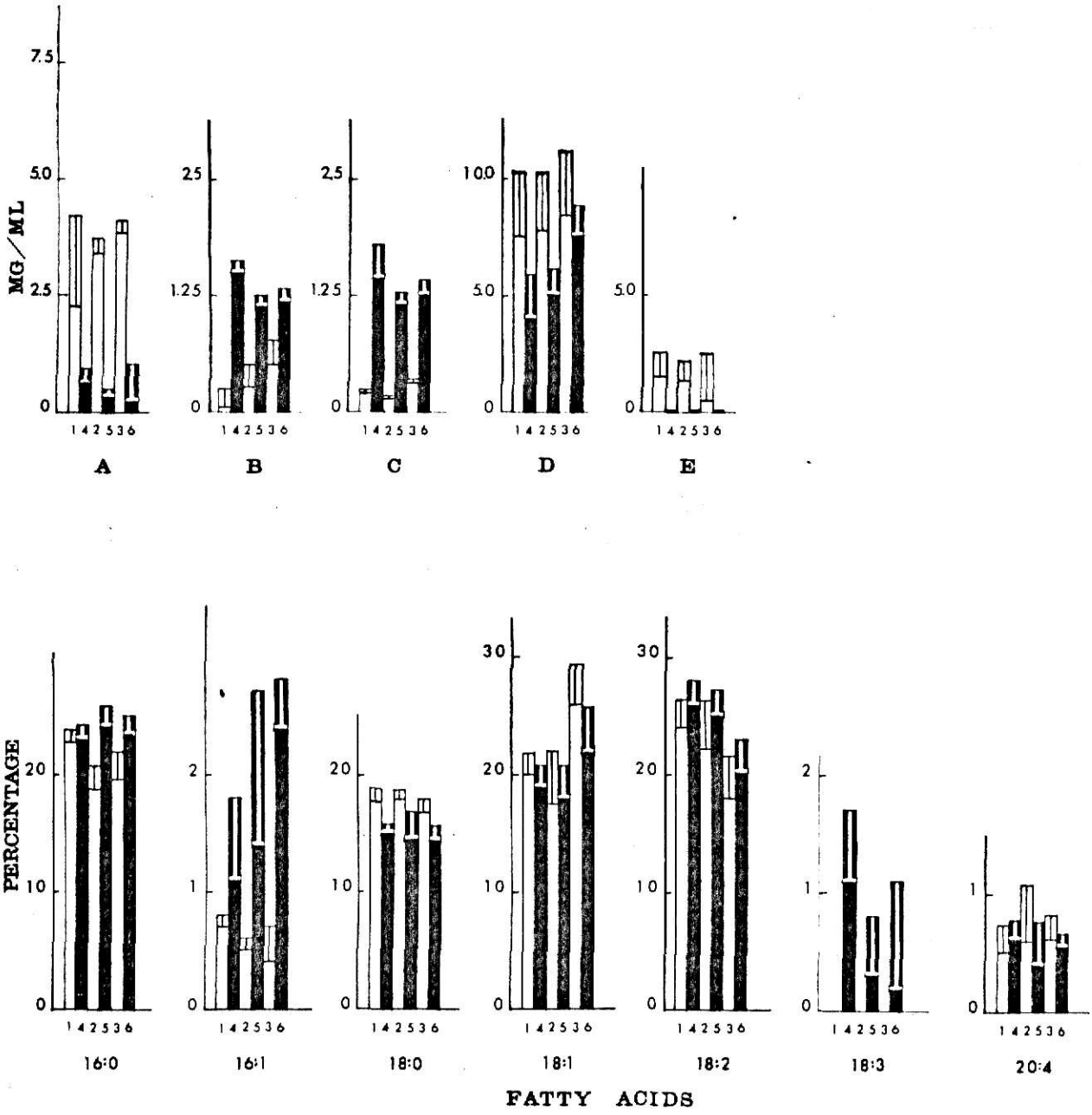

MG/ML
7.5
5.0
2.5
0
2.5
1.25
0
2.5
1.25
0
10.0
5.0
0
5.0
0
1 4 2 5 3 6
A
B
C
D
E
PERCENTAGE
20
10
0
2
1
0
20
10
0
30
20
10
0
30
20
10
0
2
1
0
1
0
16:0
16:1
18:0
18:1
18:2
18:3
20:4
FATTY ACIDS

Figure 2: Lipid and Fatty Acid Distribution in Heart Tissue of Normal and DES Treated Cockerels*

(a) Lipids: (upper bar graphs)

A - Triglycerides

B - Free Fatty Acids

C - Cholesterol

D - Cephalin (phosphatidyl ethanolamine)

E - Cardiolipin

F - Lecithin (phosphatidyl choline)

(b) Fatty Acids: (lower bar graphs) The number before the colon is the number of carbons and the number after the colon is the number of double bonds

1 - Control Adult Cockerels

2 - Adults Administered 0.2 mg DES

3 - Adults Administered 0.4 mg DES

4 - Control Young Cockerels

5 - Young Cockerels Administered 0.1 mg DES

6 - Young Cockerels Administered 0.2 mg DES

*The open bars are the adult cockerels; the black bars are for the young cockerels. The vertical line within the top part equals standard deviation.

FIGURE 2

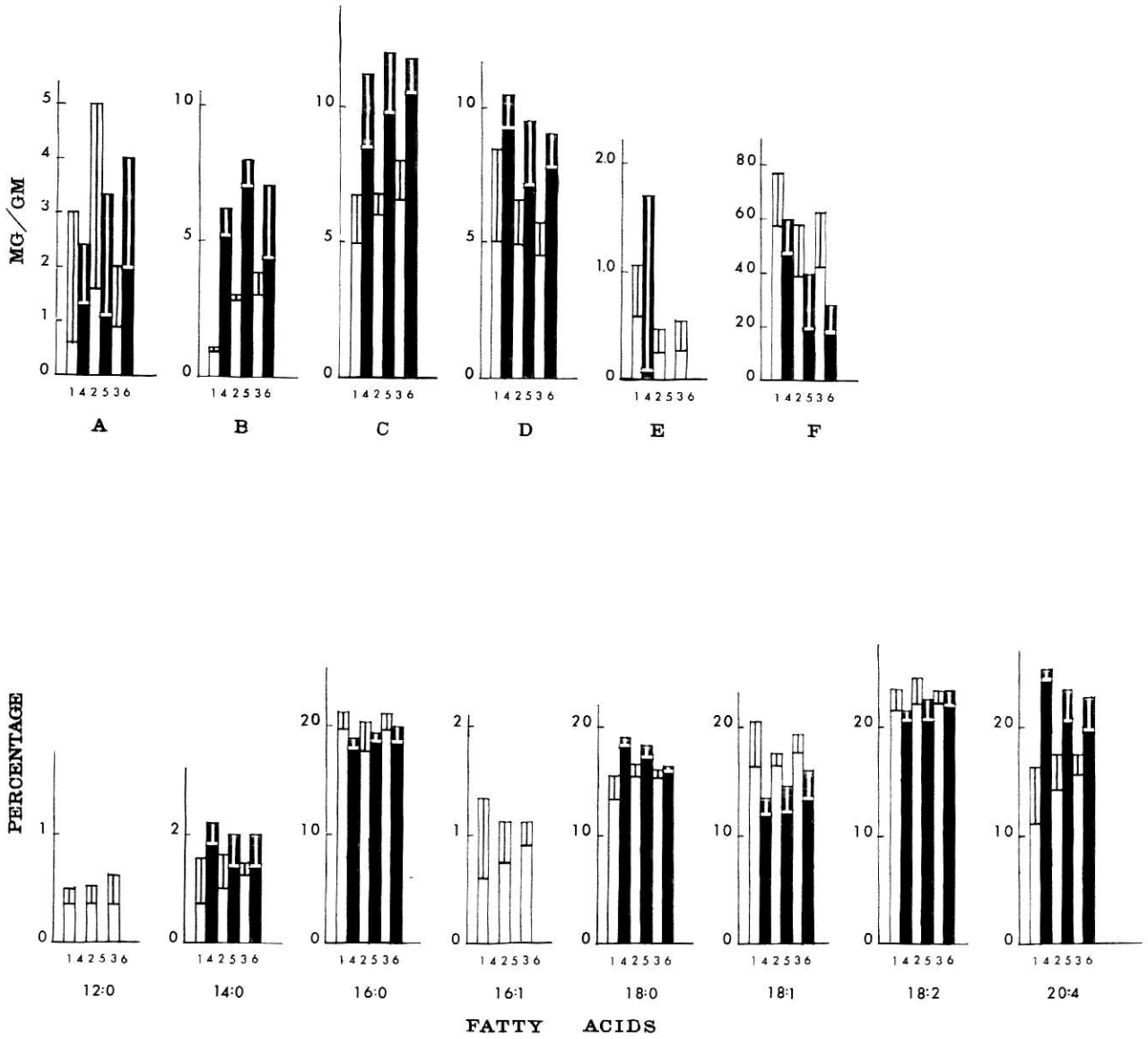

MG/GM
PERCENTAGE
1 4 2 5 3 6
A
B
C
D
E
F
12:0
14:0
16:0
16:1
18:0
18:1
18:2
20:4
FATTY ACIDS

Figure 3: Lipid and Fatty Acid Distribution in Liver Tissue of Normal and DES Treated Cockerels*

(a) Lipids: (upper bar graphs)

A - Triglycerides

B - Free Fatty Acids

C - Cholesterol

D - Cephalin (phosphatidyl ethanolamine)

E - Cardiolipin

F - Lecithin (phosphatidyl choline)

G - Cholesteryl Esters

(b) Fatty Acids: (lower bar graphs) The number before the colon is the number of carbons and the number after the colon is the number of double bonds

1 - Control Adult Cockerels

2 - Adults Administered 0.2 mg DES

3 - Adults Administered 0.4 mg DES

4 - Control Young Cockerels

5 - Young Cockerels Administered 0.1 mg DES

6 - Young Cockerels Administered 0.2 mg DES

*The open bars are the adult cockerels; the black bars are for the young cockerels. The vertical line within the top part equals standard deviation.

FIGURE 3

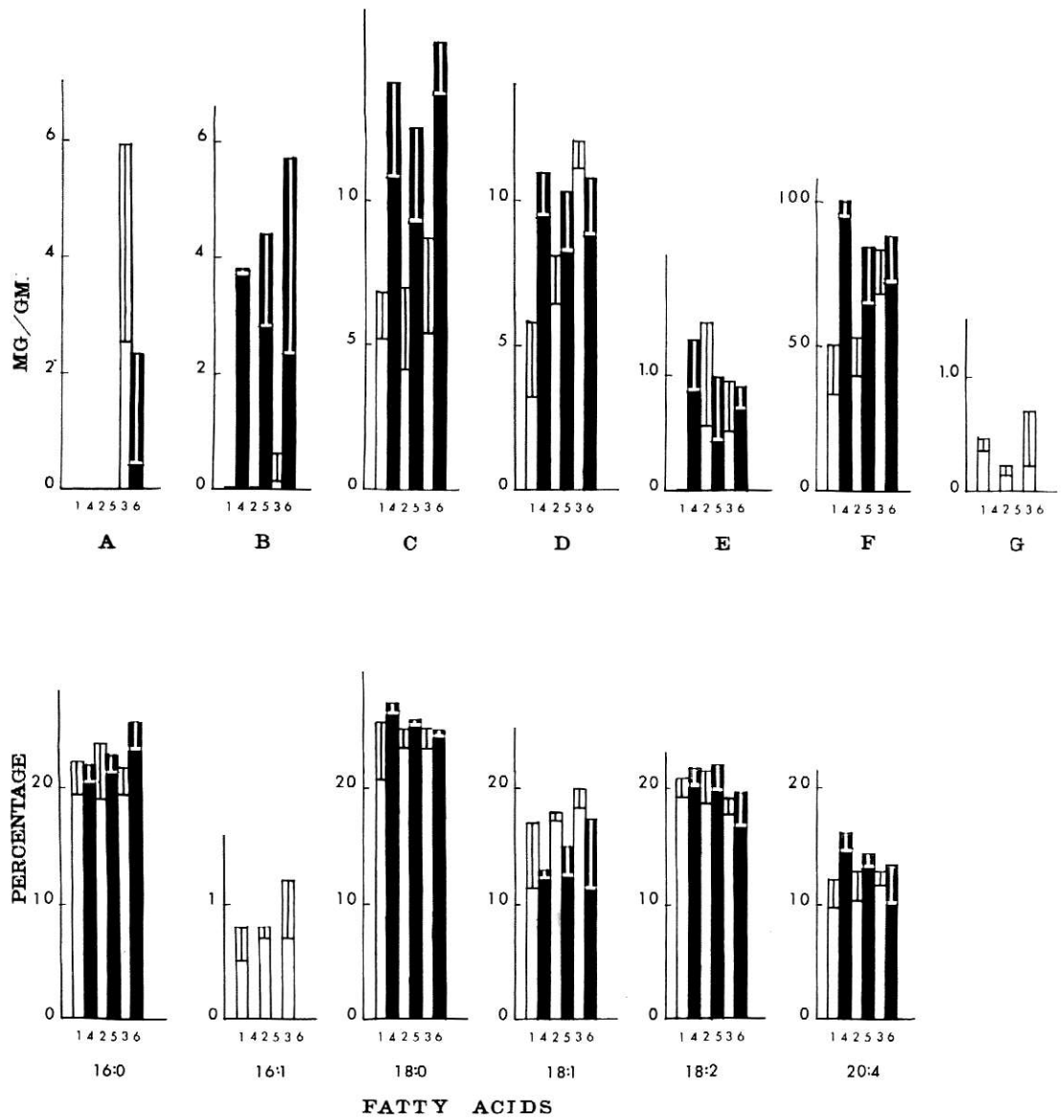

MG/GM.
PERCENTAGE
1 4 2 5 3 6
A
B
C
D
E
F
G
16:0
16:1
18:0
18:1
18:2
20:4
FATTY ACIDS

Figure 4: Lipid and Fatty Acid Distribution in Thigh Tissue of Normal and DES Treated Cockerels*

(a) Lipids: (upper bar graphs)

A - Triglycerides

B - Cholesterol

C - Lecithin (phosphatidyl choline)

D - Cephalin (phosphatidyl ethanolmaine)

E - Cardiolipin

(b) Fatty Acids: (lower bar graphs) The number before the colon is the number of carbons and the number after the colon is the number of double bonds

1 - Control Adult Cockerels

2 - Adults Administered 0.2 mg DES

3 - Adults Administered 0.4 mg DES

4 - Control Young Cockerels

5 - Young Cockerels Administered 0.1 mg DES

6 - Young Cockerels Administered 0.2 mg DES

*The open bars are the adult cockerels; the black bars are for the young cockerels. The vertical line within the top part equals standard deviation.

FIGURE 4

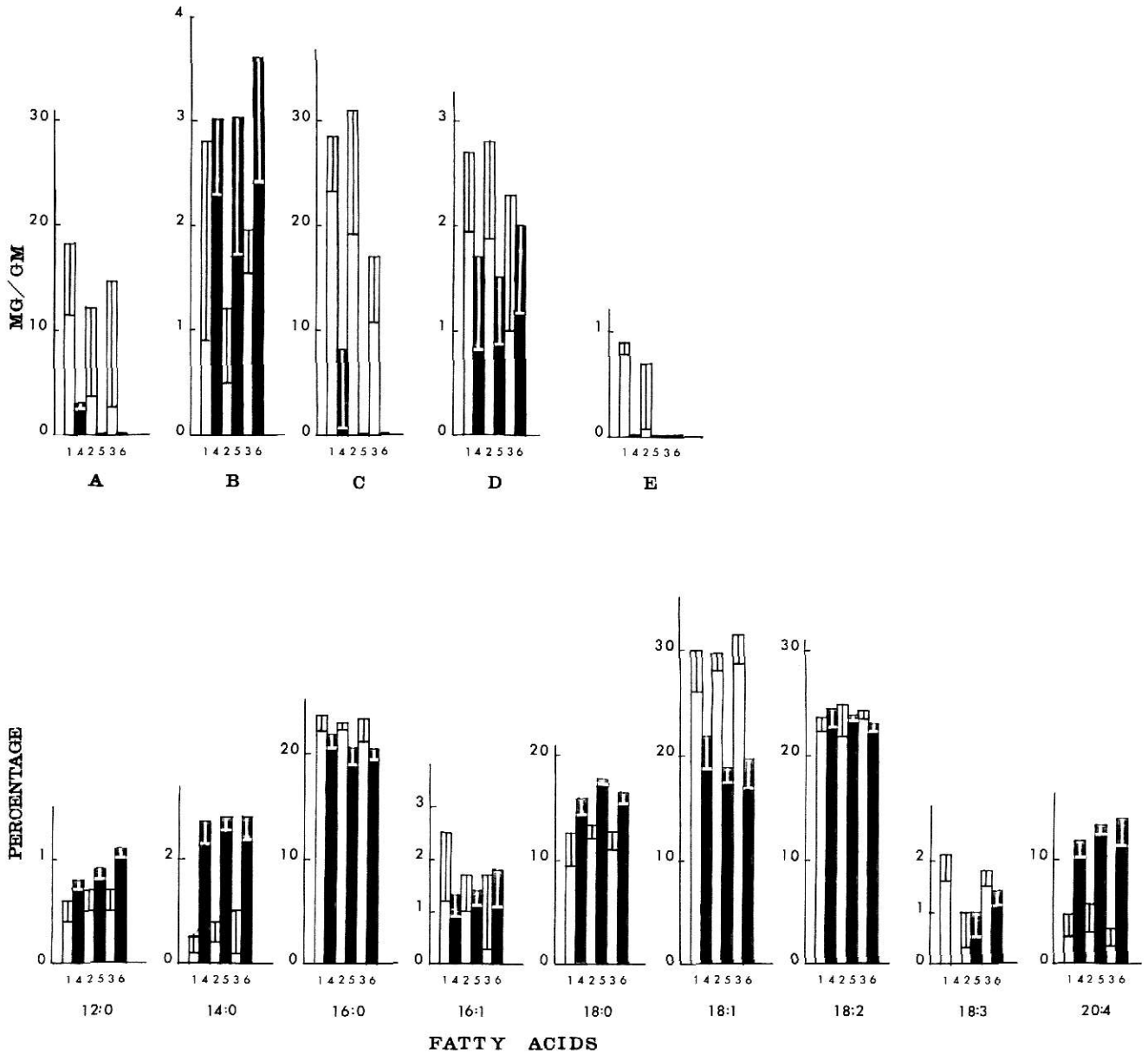

MG/GM
PERCENTAGE
1 4 2 5 3 6
A
B
C
D
E
12:0
14:0
16:0
16:1
18:0
18:1
18:2
18:3
20:4
FATTY ACIDS

Figure 5: Lipid and Fatty Acid Distribution in Breast Tissue of Normal and DES Treated Cockerels*

(a) Lipids: (upper bar graphs)

A - Triglycerides

B - Cholesterol

C - Lecithin (phosphatidyl choline)

D - Cephalin (phosphatidyl ethanolamine)

(b) Fatty Acids: (lower bar graphs) The number before the colon is the number of carbons and the number after the colon is the number of double bonds

1 - Control Adult Cockerels

2 - Adults Administered 0.2 mg DES

3 - Adults Administered 0.4 mg DES

4 - Control Young Cockerels

5 - Young Cockerels Administered 0.1 mg DES

6 - Young Cockerels Administered 0.2 mg DES

*The open bars are the adult cockerels; the black bars are for the young cockerels. The vertical line within the top part equals standard deviation.

FIGURE 5

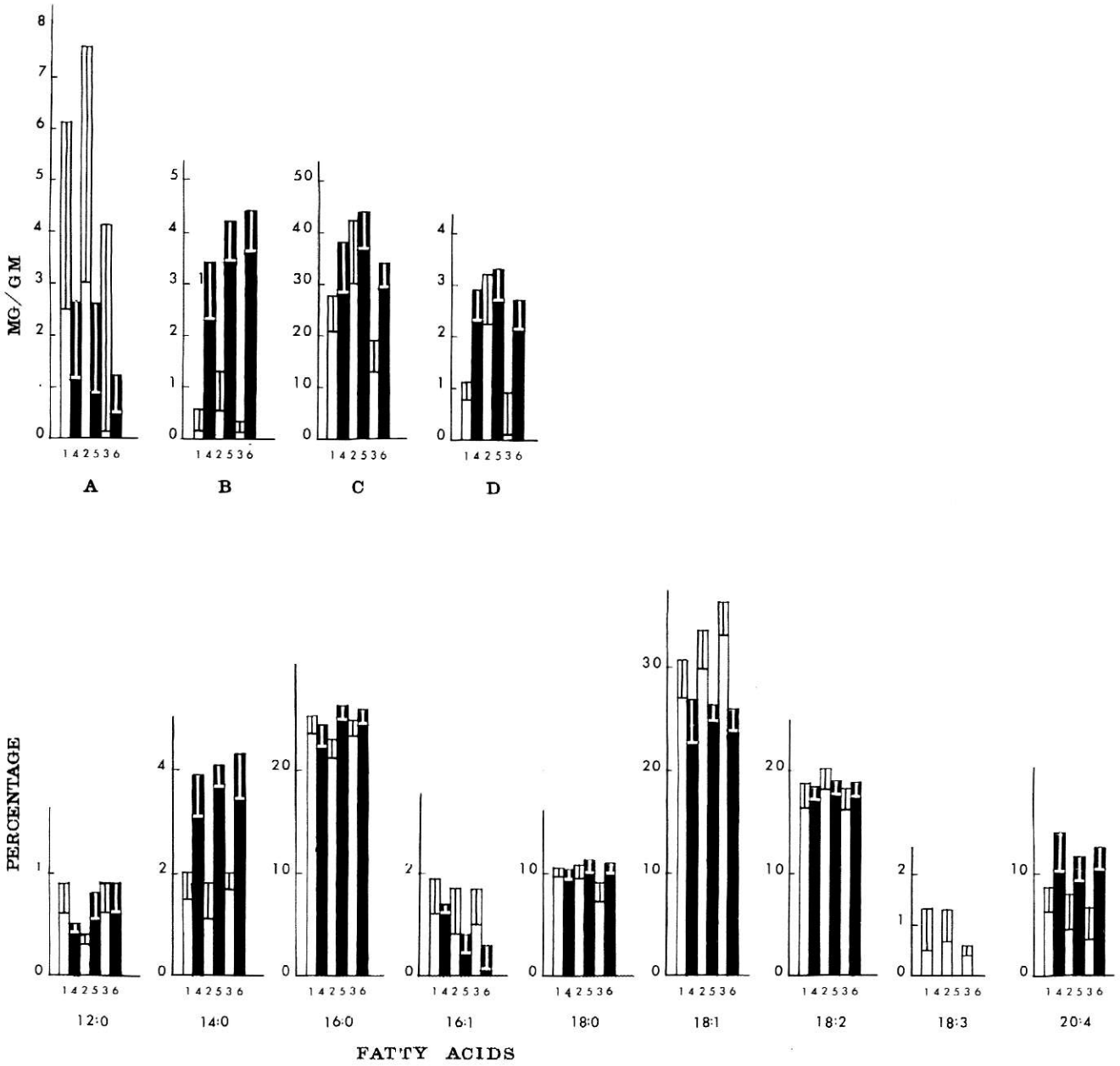

MG/GM
1 4 2 5 3 6
A
B
C
D
PERCENTAGE
12:0
14:0
16:0
16:1
18:0
18:1
18:2
18:3
20:4
FATTY ACIDS

Figure 6: Lipid and Fatty Acid Distribution in Adipose Tissue of Normal and DES Treated Cockerels*

(a) Lipids: (upper bar graphs)

A - Triglycerides

(b) Fatty Acids: (lower bar graphs) The number before the colon is the number of carbons and the number after the colon is the number of double bonds

1 - Control Adult Cockerels

2 - Adults Administered 0.2 mg DES

3 - Adults Administered 0.4 mg DES

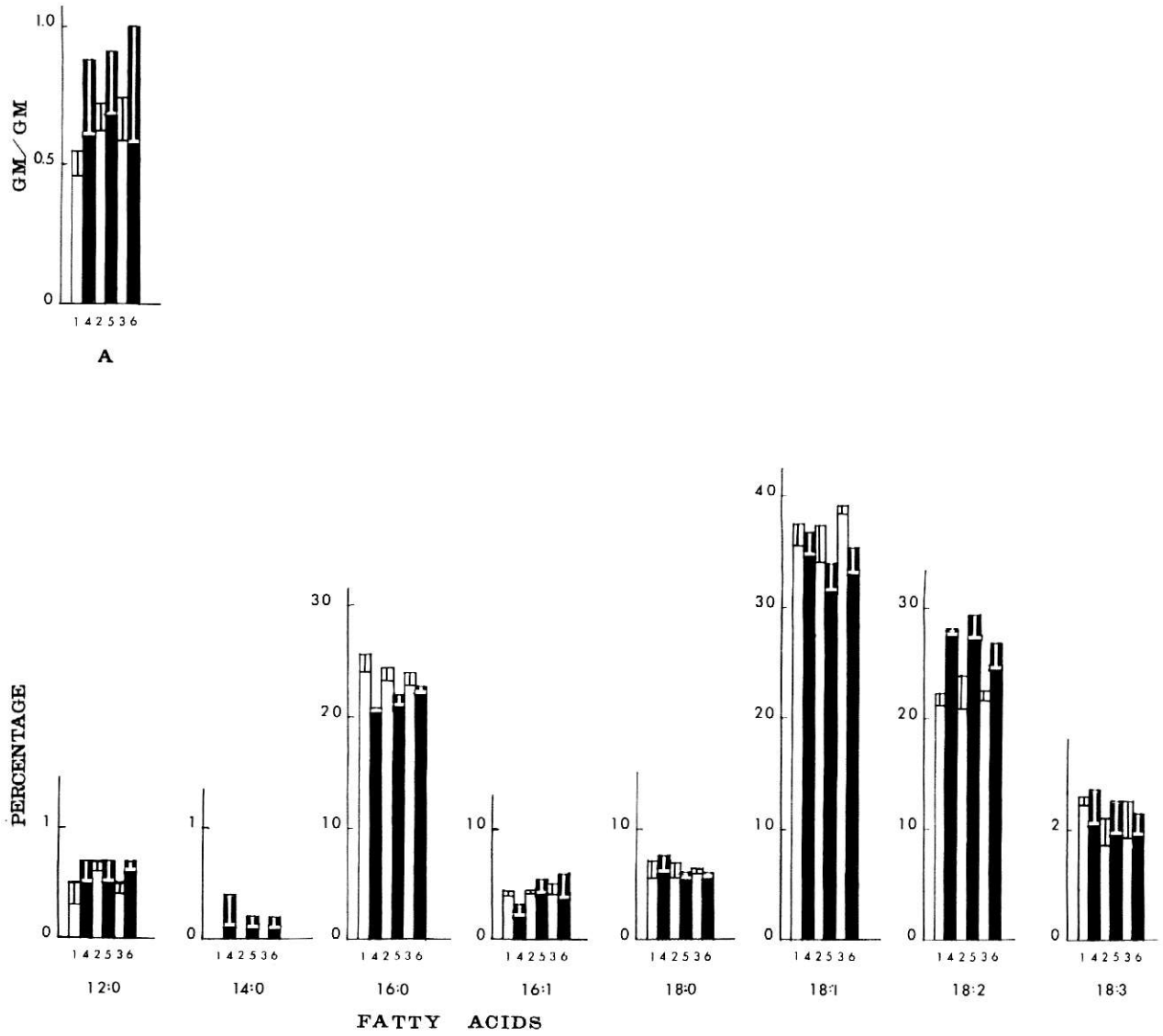
4 - Control Young Cockerels

5 - Young Cockerels Administered 0.1 mg DES

6 - Young Cockerels Administered 0.2 mg DES

*The open bars are the adult cockerels; the black bars are for the young cockerels. The vertical line within the top part equals standard deviation.

FIGURE 6


GM/GM
1.0
0.5
0
1 4 2 5 3 6
A
PERCENTAGE
12:0
14:0
16:0
16:1
18:0
18:1
18:2
18:3
FATTY ACIDS

young cockerels the 18:3 fatty acid decreased at both DES levels but the difference did not meet the 0.1 level. In both of these cases the total amount present was small and the standard deviation relatively large.

In the heart tissue (Fig. 2) there was a general upward trend in the free fatty acids from the control to the low DES to the high DES of the adult birds but only the high DES cockerels value was significantly higher than the control. In addition, the drop of the phosphatidyl ethanolamine in the young birds was not as great as in the older birds; and, although the trend was present, the difference was not significant. The rise in the triglyceride of the low DES adult was caused by a wide variation of results and probably should be questioned. Again, we observe a trend upward in the triglyceride of the young birds which is not significant.

In the liver data (FIg. 3) the trend for the 18:1 fatty acid is up in both the young and adult birds and in the young birds the trend for the 20:4 acid is down. Both of these look to be more significant than the decrease in the 18:0 acid, and the larger standard deviations do not enable them to meet the 0.1 level of significance.

General trends in the thigh muscle (Fig. 4) for the adult were up in the 14:0 acid and in the young cockerels for the 12:0 and 20:4 acids but again these values were relatively low and the standard deviation did not enable these differences to reach the 0.1 level of significance.

In the breast tissue (Fig. 5) there was a trend downward in the triglycerides of the young and in the 18:3 and 20:4 fatty acids of the adult, but their standard deviations did not enable these differences to be significant.

In the adipose tissue (Fig. 6) the trend for triglycerides was up

for the young cockerels but the standard deviation was too big to meet the 0.1 level of significance.

Table 4 summarizes the statistical analyses of the adult vs the young birds in each category. This comparison was made to observe the difference in the young and adult, and to observe the effect of the DES on rapidly growing birds compared with growth stabilized adults.

Blood Serum: (Table 4) In the blood serum 16:1 FA, 18:3 FA, cholesteryl esters and cholesterol of the adults were significantly lower than the young (0.05 level). The 18:0 acid and phosphatidyl ethanolamine were higher in the adult birds (0.05 level); the triglycerides were higher, but at the 0.1 level. These same trends were observed in the zero, low and high levels of DES. Therefore these differences may be attributed to the age of the birds.

The 16:0 acid was lower in the DES treated adult birds, and in the case of phosphatidyl choline there was no significant difference between the young and old birds when the high DES groups were compared. These differences may possibly be attributed to the administered DES.

Heart Tissue: (Table 4) The levels of the control in the adults were compared against the young and 12:0, 16:1 and 18:1 acids were higher and the 20:4 acid and cholesterol were lower in the adults (0.05 level). The same trends were observed for both the low and high levels of DES; all of these may be due to age difference.

The 16:0 and 18:2 acids were higher in the adult controls but no difference was observed in low and high DES. The phosphatidyl ethanolamine was lower for the adults on the DES treatment. The 18:0 acid and free fatty acids were lower for the adult controls and the low level of DES but

showed no significant difference for the birds on the high DES treatment. This may be due to the DES administration.

Liver Tissue: (Table 4) The liver tissues of the adult control birds were higher in 16:1 FA and cholesteryl esters and contained less free fatty acid and cholesterol than the young birds (0.05 level). The same results were obtained when the low and high levels of DES were compared; therefore, the differences may be due to the differences between the ages of the birds.

When the adult controls were compared against the young, the adult tissues contained lower cardiolipin and 20:4 FA. A lower 16:0 FA for high DES treatment of the adult and lower phosphatidyl choline and phosphatidyl ethanolamine for the adult controls and the low level DES were observed. These changes may be attributed to the administration of DES.

Thigh Muscle: (Table 4) The levels of the adult control were lower than the levels of the young birds in 12:0 FA, 14:0 FA, 18:0 FA and 20:4 FA and were higher in 16:0 FA, 18:1 FA, triglyceride and phosphatidyl choline (0.05 level). For both the low and high level DES treated birds the same significant differences were observed. This probably was the consequence of the age difference.

However, the adults showed an increase in phosphatidyl ethanolamine for low DES only and a decrease in 18:3 FA for high DES. Also when the adults were compared against the young there was an increase in cardiolipin for the controls and low level DES but not for the high DES treatment. A decrease in cholesterol for both the low and high DES treated birds was observed. The administration of DES possibly was responsible for the latter changes.

Breast Muscle: (Table 4) The comparison between the adult and the young birds showed that the adult was lower in 14:0 FA, 20:4 FA and cholesterol and higher in 18:3 FA (0.05 level) and the same trend was observed in the DES treated birds. This indicates that the differences probably may be attributed to age.

For the adult birds the 16:0 FA was lower for low DES and the phosphatidyl choline was lower for high DES. In addition, the adults showed an increase in 12:0 FA for controls and the low level DES group and a decrease in phosphatidyl ethanolamine for controls and high DES and an increase in 18:1 FA for DES treated birds. These results may possibly be attributed to the effect of DES administration.

Adipose Tissue: (Table 4) The control of the adult was lower in 18:2 FA and 14:0 FA (0.05 level), and the same results were observed for both the low and high levels of DES. These differences may be due to age.

In addition, the adult was higher in 12:0 FA and lower in 18:1 FA for the high level DES only. The 16:0 FA of the adult was higher for the control and the low level of DES. There was no difference in the case of the high DES. Therefore the above changes may be attributed to the effect of DES.

Generally the importance of the data has been discussed in the following decreasing order: data significant at the 0.05 level and at the 0.10 level, and, finally, trends that do not meet either level of significance. It is apparent that the young and adult cockerels did not react the same to the administration of the DES. As a conclusion to this discussion only the most important differences, those meeting the 0.05 level, will

be summarized. This will be followed by a short discussion of the basic differences between the adult and young cockerels.

In the blood serum when DES was administered both the adult and young exhibited an increase of the 18:1 acid and a decrease in the 18:2 acid at the high level. However, only the adults exhibited an increase in cholesterol at the high level, while in the young birds a decrease in cholesteryl esters was evident at both levels.

In the heart tissue of the adults the cardiolipin decreased at both levels, and the free fatty acid increased at the high level. In the young cockerels there were decreases in the 18:0 acid and lecithin at the high level, and a decrease in cardiolipin at both levels.

In the liver tissue of the adults the cardiolipin increased at the low level while the triglycerides, free fatty acid, phosphatidyl ethanolamine, cardiolipin and lecithin increased. The pattern in the young birds was different. In this case there was a decrease in 18:0 acid at the low level. At the high level the 16:0 acid and triglycerides increased.

In the thigh tissue of the adults the cardiolipin decreased at the high level. For the young birds the 18:3 acid rose and the triglycerides and lecithin decreased at the low level, and at the high level the 12:0, 18:3 and 20:4 acids increased and the triglycerides and lecithin decreased.

In the breast tissue of the adults on low level DES the phosphatidyl ethanolamine increased and the 12:0 acid decreased, but the fact that the higher level did not show these differences makes these results questionable. The low level of DES in the young birds did exhibit an increase in 12:0 fatty acid; at the high levels the 12:0 acid increased and the 16:1 acid decreased.

The adult adipose tissue exhibited only an increase in triglycerides at both levels. On the other hand, the young birds did show an increase in the 16:0 at both levels.

The above would tend to indicate that the responses of the adult and young cockerels were quite dissimilar. It must be remembered that for the most part the adults have reached a stable situation and changes would be attributed to turnover. The young birds are in an actively growing situation and are laying down new tissue in addition to any turnover of tissue already there.

It should also be obvious that there are basic differences between the tissues of the adult and young birds. In the following discussion only the controls will be emphasized since the situation is not complicated by DES. Only the most obvious differences will be emphasized, and for this discussion Figs. 1-6 should be consulted.

In the serum (Fig. 1) the triglycerides, phosphatidyl choline and phosphatidyl ethanolamine were much higher in the adults. The cholesterol and cholesteryl esters were higher in the young birds. In the fatty acid analysis the 16:1 and 18:3 acids of the young were higher than in the adults. The 18:0 acid was significantly higher in the adults.

In the heart tissue (Fig. 2) the free fatty acid and cholesterol were significantly higher in the young birds. In the fatty acid analysis the adult had more 12:0, 16:0, 16:1 and 18:1 acids; in fact, the young had no measurable 12:0 and 16:1 acids. The young birds had significantly more 18:0 and 20:4 acids.

The liver tissue (Fig. 3) of the young cockerels was higher in free fatty acid, cholesterol, phosphatidyl ethanolamine, phosphatidyl choline

and cardiolipin, whereas the adults were higher in cholesteryl esters. The older birds contained 16:1 acid, but the young birds exhibited no measurable amount. No other difference at the 0.05 level was observed.

In the thigh tissue (Fig. 4) the triglycerides, phosphatidyl choline and cardiolipin of the adults were significantly higher than in the young birds. In the fatty acids the tissue of young birds was higher in 14:0 and 20:4, and lower than the adults in 18:1 and 18:3 acids.

In the breast tissue (Fig. 5) the adults exhibited a higher percentage of 12:0 and 18:3 acids while the young birds contained a higher percentage of 14:0 and 20:4 acids. In the young birds the cholesterol and phosphatidyl ethanolamine were higher (0.05 level). From the graph it would appear that the triglycerides of the adults should be higher than the young but a large standard deviation here did not permit this difference to be significant (see Tables 1 and 4).

The adipose tissue (Fig. 6) of the young birds contained a greater amount of triglycerides but the difference was not significant and this was probably due to the large standard deviations. In fatty acids the young tissue was significantly higher in 14:0 and 18:2 acids, and the adult tissue was higher in 16:0. It is interesting that the only measurable amounts of 14:0 acid were found in the adult tissue.

The results indicate that the lipid and the fatty acid spectra of the adults and young cockerels were different in many respects, and that the responses were also different when DES was administered. Future work should include the preparative samples of the esterified substances (triglycerides, phosphatidyl choline and ethanolamine and cholesteryl esters), and the fatty acid distribution in each should be examined

closely. This would enable us to decide if the variations were due to the rise in each or to the change of the degree of unsaturation in each. It could be a combination of both, but it would be a good check on the TLC results, and would also give additional data about the fatty acid distribution in each category. Our present results apply only to the total fatty acids present. In any future work great care in the choice of age is in order since our results clearly indicate age differences both in the control and DES treated birds.

SUMMARY

For adult cockerels there was an increase in triglycerides of liver and adipose tissue at high and both low and high level DES respectively; in cholesterol of blood serum at high level DES; in cephalin of liver tissue at high level DES; in lecithin of liver tissue at high level DES; in cardiolipin of liver tissue at both low and high level DES and in free fatty acids of heart and liver tissue both at high level DES and a decrease only in cardiolipin of heart and thigh tissue at both low and high and high level DES respectively. For the young cockerels there was an increase only in triglycerides of liver tissue at high level DES and a decrease in triglycerides of thigh tissue at both low and high level DES; cholesteryl ester of blood serum at both low and high level DES; lecithin of heart and thigh tissue at high and both low and high level DES respectively, and cardiolipin of heart tissue at both high and low level DES.

The only difference in fatty acid distribution in the adult was an increase in 18:1 fatty acid and a decrease in 18:2 fatty acid of blood serum at high level DES. For the young cockerels there was an increase in 12:0 fatty acid of thigh tissue at high level and breast tissue of both low and high level DES; in 16:0 fatty acid of liver tissue at high level and adipose tissue at low and high level DES; in 18:1 fatty acid of blood serum at high level DES; in 18:3 fatty acid of thigh tissue at both low and high level DES and in 20:4 fatty acid of thigh tissue at high level DES and a decrease in 16:1 acid of breast tissue at high level DES; in 18:0 acid of heart tissue at high level and liver tissue at both low and high level DES and in 18:2 fatty acid of blood serum at high level DES.

The lipid and fatty acid distributions of adult and young cockerel tissues were quite different in many respects. They also responded differently to the DES administration. More of the adult lipids were changed by DES while more of the young cockerel fatty acids were changed by DES. In both the adult and young cockerels the high level DES stimulated more changes in lipid and fatty acid distribution than the low level DES.

ACKNOWLEDGEMENTS

The author wishes to express her appreciation and gratitude to her major professor, Dr. R. E. Clegg, for his guidance and assistance throughout this investigation. The author is also indebted to Carol Klopfenstein for direct and indirect assistance during the course of the investigation.

BIBLIOGRAPHY

1. Applied Science Laboratories, Inc. Catalog No. 19, 1976. P.O. Box 440, State College, Pennsylvania 16801.

2. Brinkmann Instruments, Inc. Instructions for Using Brinkmann Precoated TLC Layers, Cantiague Road, Westbury, N.Y. 11590.

3. Calder, F. W., J.W.G. Nicholson and J. E. Langiele. Comparison of Silages Made from Grass, Grass-Legume and Corn Fed to Steers with and without Diethylstilbestrol. Can. J. Anim. Sci. 56:57. 1976.

4. Camejo, G., V. Bosch, C. Arreaga and H. C. Mendez. Early Changes in Plasma Lipoprotein Structure and Biosynthesis in Cholesterol-fed Rabbits. J. Lip. Res. 14:61. 1973.

5. Clegg, R. E., C. F. Klopfenstein and W. E. Klopfenstein. Effect of Diethylstilbestrol, Ascorbic Acid and Vitamin E on Serum Lipid Patterns. Poult. Sci. 55:1104. 1976.

6. Conover, W. J. Practical Nonparametric Statistics. 1971. Wiley, New York.

7. Corey, J., K. C. Hayes, B. Dorr and D. M. Hegsted. Comparative Lipid Response of Four Primate Species to Dietary Changes in Fat and Carbohydrate. Atherosclerosis. 19:119. 1974.

8. Crawford, G.P.M., C. P. Warlow, B. Bennett, A. A. Daroson, A. S. Douglas, D. F. Kerridge and D. Ogston. The Effect of Vitamin C Supplements on Serum Cholesterol, Coagulation, Fibrinolysis and Platelet Adhesiveness. Atherosclerosis. 21:451. 1975.

9. Entenman, C. F., W. Lorenz and I. L. Chaikoff. Endocrine Control of Lipid Metabolism in the Bird (1). Effects of Pregnant Mare Serum on the Blood and Lipids of the Domestic Fowl. J. Biol. Chem. 134:495. 1940.

10. Giarelli, L. and A. Ferlito. Bilateral Breast Metastases from Oatcell Lung Carcinoma in a Man Treated with Diethylstilbestrol for Prostatic Adenocarcinoma. J. Amer. Geriat. Soc. 24:511. 1976.

11. Ginter, E., P. Bobek, J. Babala and E. Barbierikova. Effect of Ascorbic Acid on the Lipid Metabolism of Guinea Pigs Fed on Atherogenic Diet. Cor. Vasa 11:65. 1969.

12. Guenter, W., D. B. Bragg and P. A. Kondra. Effect of Dietary Linoleic Acid on Fatty Acid Composition of Egg Yolk, Liver, and Adipose Tissue. Poult. Sci. 50:845. 1971.

13. Heimberg, M., G. D. Dunn and H. G. Cox. Derivation of Plasma Free Fatty Acids from Dietary Neutral Fat in Man. J. Lab. Clin. Med. 83:393. 1974.

14. Huseby, R. A. Estrogen-Induced Leydig Cell Tumor in the Mouse: A Model System for the Study of Carcinogenesis and Hormone Dependency. J. Toxicol. Environ. Health Suppl. 1:177. 1976.

15. Hutagalung, R. I., G. L. Cromwell, V. W. Hays and C. H. Chaney. Effect of Dietary Fats, Protein, Cholesterol, and Ascorbic Acid on Performance, Serum and Tissue Cholesterol Levels and Serum Lipid Levels of Swine. J. Anim. Sci. 29:700. 1969.

16. Khudolei, V. V. and V. S. Ermoshchenkov. Carcinogenic Action of Diethylstilbestrol on Frog. Bull. Exp. Biol. Med. Engl. Trans. 81:898. 1977.

17. Kruski, A. W. and K. A. Narayan. Effect of Dietary Supplementation of Cholesterol and Its Subsequent Withdrawal on the Liver Lipids and Serum Lipoproteins of Chickens. Lipids. 7:742. 1972.

18. Kudzma, D. J., P. M. Hegstad and R. E. Stoll. Chick as a Laboratory Model for the Study of Estrogen-Induced Hyperlipidemia. Metab. Clin. Exp. 22:423. 1973.

19. Linsay, S. F., W. Lorenz, C. Entenman and I. L. Chaikoff. Production of Atheromatosis in Aorta of Chicken by Administration of Diethylstilbestrol. Proc. Soc. Expt. Biol. Med. 62:315. 1946.

20. Nolten, W. E., L. J. Sholiton, L. S. Srivastava, H. C. Knowles and E. E. Werk. Effects of Diethylstilbestrol and Medroxyprogesterone Acetate on Kinetics and Production of Testosterone and Dihydrotestosterone in Patients with Prostatic Carcinoma. J. Clin. Endocrinol. Metab. 43:1226. 1976.

21. Renaud, S. and P. Gautheron. Influence of Dietary Fats on Atherosclerosis, Coagulation and Platelet Phospholipids in Rabbits. Atherosclerosis. 21:115. 1975.

22. Robboy, S. J., R. E. Scully, W. R. Welch and A. L. Herbst. Intrauterine Diethylstilbestrol Exposure and Its Consequences, Pathologic Characteristics of Vaginal Adenosis, Clear Cell Adenocarcinoma, and Related Lesions. Arch. Path. Lab. Med. 101:1. 1977.

23. Shellabarger, C. J., J. P. Stone and S. Holtzman. Synergism between Neutron Radiation and Diethylstilbestrol in the Production of Mammary Adenocarcinomas in the Rat. Cancer Res. 36:1019. 1976.

24. Sirota, D. K. and S. C. Marinoff. Endometrial Carcinoma in Turner's Syndrome Following Prolonged Treatment with Diethylstilbestrol. Mount Sinai J. Med. 42:586. 1975.

25. Spittle, C. R. Atherosclerosis and Vitamin C. Lancet. 2:1280. 1971.

26. Stahl, E. Thin Layer Chromatography. A Laboratory Handbook, Academic Press. New York and London. p. 5. 1965.

27. Widdowson, E. M., M. J. Dauncey, D.M.T. Gairdner, J.H.P. Konxis and M. Pelikan-Filipkova. Body Fat of British and Dutch Infants. Br. Med. J. 1:653. 1975.

28. Williams, D. B., R. L. Vatter, W. Burroughs and D. G. Topel. Effects of Ration Protein Level and Diethylstilbestrol Implants on Early-Weaned Beef Bulls. J. Anim. Sci. 41:1525. 1975.

29. Zaitsev, V. F., L. A. Mayasnikov and L. Scheikman. Effect of Ascorbic Acid on Distribution of Cholesterol-4-C-14 in Tissues of Animals with Experimental Atherosclerosis. Kardiologiya. 4:30. 1964.

TISSUE LIPID VARIATIONS UNDER LONG TERM DIETHYLSTILBESTROL ADMINISTRATION

by

Tai-Lee Ke

B.Sc., National Tsing-Hua University, Taiwan, 1970
M.S., (Chemistry), Kansas State University, 1973

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Graduate Biochemistry Group

Kansas State University
Manhattan, Kansas

1977

The effect of long term administration of DES on tissue lipids of both rapidly growing and adult cockerels was investigated. The tissues (heart, liver, thigh, breast and adipose) and blood serum were obtained after administering DES (0, 0.2 mg and 0.4 mg respectively) for six weeks to the adults and administering DES (0, 0.1 mg and 0.2 mg respectively) for five weeks to the young. Lipid spectrum (triglycerides, cholesteryl ester, cholesterol, lecithin, cephalin, cardiolipin and free fatty acids) were determined by TLC, and fatty acid distribution (12:0, 14:0, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, and 20:4) was determined by GLC. The data were compared by the Mann-Whitney statistical method.

For adult cockerels there was an increase in triglycerides of liver and adipose tissue at high and both low and high level DES respectively; in cholesterol of blood serum at high level DES; in cephalin of liver tissue at high level DES; in lecithin of liver tissue at high level DES; in cardiolipid of liver tissue at both low and high level DES and in free fatty acids of heart and liver tissue both at high level DES and a decrease only in cardiolipin of heart and thigh tissue at both low and high and high level DES respectively. For the young cockerels there was an increase only in triglycerides of liver tissue at high level DES and a decrease in triglycerides of thigh tissue at both low and high level DES; cholesteryl ester of blood serum at both low and high level DES; lecithin of heart and thigh tissue at high and both low and high level DES respectively, and cardiolipin of heart tissue at both low and high level DES.

The only difference in fatty acid distribution in the adult was an increase of 18:1 fatty acid and a decrease in 18:2 fatty acid of blood

serum at high level DES. For the young cockerels there was an increase in 12:0 fatty acid of thigh tissue at high level and breast tissue of both low and high level DES; in 16:0 fatty acid of liver tissue at high level and adipose tissue at low and high level DES; in 18:1 fatty acid of blood serum at high level DES; in 18:3 fatty acid of thigh tissue at both low and high level DES and in 20:4 fatty acid of thigh tissue at high level DES and a decrease in 16:1 acid of breast tissue at high level DES; in 18:0 acid of heart tissue at high level and liver tissue at both low and high level DES and in 18:2 fatty acid of blood serum at high level DES.

The lipid and fatty acid distributions of adult and young cockerel tissues were quite different in many respects. They also responded differently to the DES administration. More of the adult lipids were changed by DES while more of the young cockerel fatty acids were changed by DES. In both the adult and young cockerels the high level DES stimulated more changes in lipid and fatty acid distribution than the low level DES.