AN ABSTRACT OF THE THESIS OF

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(Name) (Degree)

in ZOOLOGY presented on July 7, 1967
(Major) (Date)

Title: THE EFFECT OF ESTRADIOL ON THE FATTY ACID METABOLISM OF THE RAT

Abstract approved Redacted for Privacy
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Female Sprague-Dawley rats (220-240 g) were ovariectomized and seven days later were either treated as controls or received a single injection of either 0.1 μg, 100 μg, or 2 mg of estradiol-17β dissolved in 0.1 cc propylene glycol. These rats were autopsied either 1, 3, 6, 24, 48, or 72 hours after injection. Some of the ovariectomized animals were also hypophysectomized on either the fifth or sixth day after castration. Of these, some were injected with a single dose of either 2 mg estradiol, or 10 or 20 IU of ACTH.

Plasma samples of all of the treatments included in this study were analyzed by gas-liquid chromatography for the percent composition of fatty acids present. The percent composition of fatty acids present in adipose tissue for some control and 100 μg and 2 mg ovariectomized estradiol treated animals are included. Identification of fatty acids was based on relative retention times and separation by thin layer chromatography. Free fatty acid levels were determined

for ovariectomized animals which received 2 mg estradiol. Both whole body and dorso-lumbar fat pad weights were recorded.

It was determined that estradiol at all levels tested brings about a change in the composition of the plasma fatty acids 24 hours after injection. As dosage increased the change becomes more pronounced. At the lowest dose (0. 1 μg) only slight changes appear at 24 hours after injection. With 100 μg the fatty acid composition starts to change by 12 hours after injection, is complete by 24 hours and then returns to the control levels by 72 hours. With 2 mg the fatty acid composition remains altered for the duration of time (72 hours) studied. The changes in fatty acid levels at 24 hours after injection of 2 mg estradiol are as follows: palmitic acid changes from 16. 61 ± 0. 56% in the non-injected controls to 23. 69 ± 0. 74% in the treated animals, stearic acid changes from 16. 44 ± 0. 43% to 12. 81 ± 0. 54%, oleic acid changes from 13. 66 ± 0. 39% to 20. 58 ± 0. 76%, linoleic acid changes from 19. 48 ± 0. 51% to 26. 01 ± 0. 56% and arachidonic acid changes from 33. 30 ± 0. 92% to 16. 91 ± 1. 08%.

No change was noted in the fatty acid composition of adipose tissue, nor were the dorso-lumbar fat pad weights affected by estradiol treatment. Plasma free fatty acids showed no significant changes. A slight decrease was observed in the body weights of those animals treated with 2 mg estradiol. Plasma fatty acid composition in hypophysectomized plus estradiol or ACTH treated animals

was similar to that of ovariectomized estradiol treated animals.

Mechanisms are presented for the altered plasma fatty acid compositions brought about by estradiol and hypophysectomy treatment. Their significance is discussed.

The Effect of Estradiol on the Fatty Acid Metabolism of the Rat

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

June 1968

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ACKNOWLEDGMENT

I would like to express my sincere thanks to the many persons who have contributed to this investigation. The willingness in which Mr. James Saddler gave of his interest and advice has been particularly appreciated. The gas-liquid chromatograph which I used belonged to Dr. David C. Church. It is questionable whether this study would have been possible without his generosity. Finally, I would like to thank Dr. F. L. Hisaw, Jr. for his personal encouragement.

TABLE OF CONTENTS

	Page
INTRODUCTION	1
METHODS AND MATERIALS	9
Estradiol Experiment	11
Plasma fatty acid composition	11
Plasma free fatty acid concentration	16
Hypophysectomy Experiment	18
RESULTS	19
Gas-Liquid Chromatography	19
Identification of peaks	19
Calculation of correction factors	27
Estradiol Experiment	27
Composition of fatty acids in blood plasma	27
Composition of fatty acids in adipose tissue	45
Concentration of plasma free fatty acids	50
Body weight and dorso-lumbar fat pad weight	57
Hypophysectomy Experiment	68
DISCUSSION	72
SUMMARY	81
BIBLIOGRAPHY	86

LIST OF FIGURES

Figure		Page
1	Gas-liquid chromatography of rat plasma fatty acids.	22
2	Logarithm of retention time vs. carbon chain length for fatty acid standards and rat plasma samples.	24
3	Effect of two milligrams estradiol on plasma fatty acid composition.	44
4	Effect of 100 μg estradiol on plasma fatty acid composition.	46
5	Effect of 0.1 μg estradiol on plasma fatty acid composition.	47
6	Effect of two milligrams estradiol on plasma free fatty acid concentration using the method of Itaya and Ui.	52
7	Effect of two milligrams estradiol on plasma free fatty acid concentration using the method of Dole and Meinertz.	56
8	Body and dorso-lumbar fat pad weights following estradiol treatment.	66

LIST OF TABLES

Table		Page
1	Distance traveled, relative retention time and logarithm of retention time of fatty acid standards and blood plasma samples.	20
2	Thin-layer chromatography of fatty acid standards and lipid extract of rat blood plasma.	25
3	Retention times for a standard solution of fatty acids and rat plasma after separation by thin-layer chromatography.	26
4	Determination of correction factors:	
	A. Palmitic, oleic and arachidonic fatty acid methyl esters--Mixture No. 1.	28
	B. Applied sciences standard K-108.	29
	C. Summary of correction factors for methyl esters of fatty acids computed from Mixture No. 1 and applied sciences standard K-108.	30
5	The effect of a single dose of 0.1 μg estradiol at varying time intervals after injection on the percent composition of fatty acids in the plasma of seven day castrate female rats.	31
6	The effect of a single dose of 100 μg estradiol at varying time intervals after injection on the percent composition of fatty acids in the plasma of seven day castrate female rats.	34
7	The effect of a single dose of two milligrams estradiol at varying time intervals after injection on the percent composition of fatty acids in the plasma of seven day castrate female rats.	36
8	Summary of the effects of estradiol on the percent composition of fatty acids in the plasma of seven day castrate female rats.	38

Table		Page
9	Percent composition of fatty acids in the plasma of seven day castrate, noninjected and propylene glycol control female rats.	40
10	Percent composition of fatty acids in dorso-lumbar fat pads from seven day ovariectomized noninjected control and estradiol injected rats.	48
11	Theoretical percent composition of plasma fatty acids after dilution with various amounts of adipose tissue fatty acids.	49
12	Plasma free fatty acid concentrations (μeq/liter) determined by the method of Itaya and Ui at various time intervals after injection of a single dose of two milligrams estradiol.	51
13	Body weight (g) and free fatty acid concentrations (μeq/liter) determined after the method of Dole and Meinertz in noninjected controls, propylene glycol and estradiol treated seven day ovariectomized rats.	54
14	Body and dorso-lumbar fat pad weights of propylene glycol and noninjected castrate female rats.	58
15	The effect of a single dose of 0. 1 μg estradiol at varying time intervals after injection on body and dorso-lumbar fat pad weights of seven day castrate female rats.	60
16	The effect of a single dose of 100 μg estradiol at varying time intervals after injection on body and dorso-lumbar fat pad weights of seven day castrate female rats.	62
17	The effect of a single dose of two milligrams estradiol at varying time intervals after injection on body and dorso-lumbar fat pad weights of seven day castrate female rats.	64

Table		Page
18	Percent composition of plasma fatty acids in ovariectomized, hypophysectomized rats noninjected and injected with either estradiol or adrenocorticotropin hormone (ACTH).	70

THE EFFECT OF ESTRADIOL ON THE FATTY ACID METABOLISM OF THE RAT

INTRODUCTION

As recently as thirty years ago adipose tissue was considered to be a connective tissue filled, by chance, with fat droplets. Fat stores were believed to be purely passive in nature and in no way involved in the energy metabolism except under starvation conditions. Some of the early workers who were instrumental in dispelling this underestimation of the importance of adipose tissue included Wells (1940), Rosenfeld (1925), Schoenheimer (1942) and particularly Wertheimer and Shapiro (1948). These latter two authors were the first to review the physiology of adipose tissue. This review proved to be the impetus for much interest in the subject and there has been no end to the rapid development of events in this field of physiology. The most complete review to date is the volume on adipose tissue (Section 5) in the Handbook of Physiology series published under the auspices of the American Physiological Society and the editorship of Renold and Cahill (1965).

One of the important early developments which revolutionized opinions about adipose tissue was the discovery of the intimacy of carbohydrate metabolism with that of adipose tissue. Approximately 30% of the dietary carbohydrate in animals maintaining constant body

weight is converted to fat before it is further metabolized. In 1954, Favarger and Gerlach confirmed that carbohydrate is converted into fat in adipose tissue and not in the liver as was earlier believed.

More recently Shapiro, Chowers and Rose (1957), and Leboeuf, Flinn and Cahill, Jr. (1959) have discovered that fat tissue has a conspicuous absence of the enzyme glycerokinase which catalyzes the conversion of glycerol to α-glycerophosphate. Since there is no indigenous source of α-glycerophosphate, it is these authors' conclusion that the extent of re-esterification of fatty acids depends on the amount of α-glycerophosphate formed from the catabolism of glucose. It is, thus, felt that glucose metabolism regulates the blend (relative amount of fatty acid to glucose) of the caloric demand, but that it does not affect the total caloric output.

One of the early unanswered questions concerns the substance which is mobilized from adipose tissue and how it is transported to and oxidized by the various organs. Studies of this problem primarily by Dole (1956), and Gordon and Cherkes (1956) have revealed that adipose tissue releases a ceaseless stream of free fatty acids (FFA) into the bloodstream. These FFA are sometimes referred to as unesterified fatty acids (UEFA). They are transported mostly on albumin molecules (Goodman, 1958) and comprise only about 5% of the total fatty acid fraction of blood plasma. Although this is a rather small percent of the fatty acid fraction of blood plasma, it

appears to have a rapid turn-over rate as calculated from the rate of removal of palmitate-C^{14}. On the basis of this work the half life of FFA in plasma has been estimated to be 0. 6 minutes in the fasting rat (Laurell, 1959).

One important fate of FFA is that they may be immediately taken up into the peripheral tissue and either metabolized directly to carbon dioxide or deposited as esters. The fatty acid esters may then be subsequently oxidized. A large fraction (as much as 30%) of the circulating FFA is assimilated by the liver. As in the peripheral tissues the fatty acids may be converted to carbon dioxide. This amounts to only a small fraction of the total FFA taken up by the liver cell. The largest fraction is deposited within the liver cell as triglycerides, some as phospholipids and a very small amount as cholesterol esters. Some are converted to ketone bodies which go back into the plasma and can then be utilized by the peripheral tissues. Finally, some of the FFA taken up by the liver are transformed into triglycerides and are then built into lipoproteins. These lipoprotein triglycerides can probably be utilized by many peripheral tissues, but this has been very inadequately studied and deserves more attention. Thus, FFA in the metabolism of lipids plays a role similar to that of glucose in the metabolism of carbohydrates.

One of the interesting problems at present concerns the composition of the plasma fatty acids. The plasma fatty acid composition is

effected primarily by that of its major source, adipose tissue. However, the extent to which such factors as intravascular lipolysis, non-uniform release or uptake by different tissues can alter the composition of fatty acids in plasma is not known. Miller, Gold and Spitzer (1962) reported differences in the rates of release of individual fatty acids by subcutaneous epigastric adipose tissue in the dog and non-uniform uptake of fatty acids by different anatomical sites. The preferential extraction of oleic acid from arterial blood by the human and dog heart has been reported by Rothlin and Bing (1961). Just what the physiological significance of these findings is and the mechanism responsible for the consequent differences in fatty acid composition remain unanswered questions.

The concept that certain hormones have the capacity to cause release of the triglycerides stored in adipose tissue was first introduced by the investigations of Barrett, Best and Ridout (1938), Best, Haist and Ridout (1939), and Stetten and Salcedo (1944). Two terms which are used to denote this property are adipokinetic and lipolytic. Some of the hormones which have exhibited adipokinetic activity include the catecholamines (Gordon and Cherkes, 1958), various pituitary polypeptides (Raben, _et al._, 1961; White and Engel, 1958; Frienkel, 1961; Rudman, _et al._, 1962; Friesen, Barrett and Astwood, 1962) and the pancreatic polypeptide glucagon (Vaughn, 1960; Hagen, 1961).

Many of the experiments in which adipokinetic properties have been attributed to these hormones have been carried out on adipose tissue *in vitro.* The effects which these hormones exhibit on the fat cell are complex and in addition to triglyceride hydrolysis with concomitant release of fatty acids, alteration in the rates of the following processes have been observed: triglyceride synthesis, glucose uptake, glucose oxidation and, finally, increase in the activity of the phosphorylase enzymes and one or more of the intracellular lipases (Vaughn, 1961; Steinberg and Vaughn, 1961; Engel, 1962; Jeanrenaud, 1961). In addition, there are differences between *in vitro* and *in vivo* conditions which make it difficult to assess the physiological significance of these effects. These differences include the presence or absence of blood flow, extra-adipose effects which over-ride FFA release, and the possible degradation of the hormones *in vivo.* Although there is a general agreement today as to the importance of glyceride breakdown as a major point of metabolic control of fat mobilization, the fact that there is such a multiplicity and seeming unrelatedness of agents concerned contributed to the difficulty of evaluating the significance of adipokinetic hormones. It is possible that this difficulty will be resolved by finding a common effector mechanism, perhaps involving adenyl cyclase and cyclic adenylic acid or one of the sympathetic amines.

Finally, in any review of the hormonal control of lipid metabolism mention should be made of the species differences in response to adipokinetic hormones. Rudman (1963) has compared the minimal effective dose[1] of eight adipokinetic hormones upon the adipose tissue of six species of vertebrates. To cite one example of a species difference to an adipokinetic hormone, whereas adipose tissue from the rabbit, guinea pig, hamster and rat all exhibited increases in FFA levels in response to adrenocorticotropin (ACTH) the dog and pig adipose tissue did not release FFA into the media even at the highest concentrations of ACTH tested (Rudman, 1963). There is no explanation for these species differences at present. A more complete knowledge of the mobilization of lipids within the individual species will be required before we can understand why they are different.

The rather extensive list of agents which share the adipokinetic property might be related to the variety of physiological uses of FFA. The mobilization of stored fat appears to play a role in the adaptation of mammals to a number of different situations, such as exposure to cold, fright, starvation, migration, reproduction and arousal from hibernation.

Studies on the relationship of reproduction to lipid metabolism have been difficult to assess. This is not surprising in view of the

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[1] Minimal effective dose is considered the concentration of hormone that produces a statistically significant ($p < 0.05$) increase in FFA production.

complex interrelationships among pituitary, gonadal and adrenal secretions. The purpose of this study was to examine one aspect of the relationship of reproduction to lipid metabolism.

It has been shown that estrogen treatment increases the levels of plasma FFA simultaneously with those of the total lipids and lipophosphoprotein in the immature fowl. Administration of gonadotropin increased total lipids, lipophosphoproteins and plasma FFA in the mature laying bird. Similar changes were found in moulting birds, but no such changes were obtained in immature pullets (Heald and Rookledge, 1964). These results suggest that the increase in the FFA is a direct result of estrogens due to follicular stimulation and only indirectly of the pituitary hormones.

A lipid mobilizing effect of estrogens has been shown in mammals (Laron and Kowadlo-Silbergeld, 1965); however, this was under pharmacological conditions (10 mg per dose) and was brought about by using estradiol benzoate. Further, no attempt was made to evaluate the fatty acid composition of the plasma.

Lyman, Shannon and Ostwald (1962) and Monsen, Okey and Lyman (1962) have demonstrated that the sex of the animal affects the fatty acid composition of the plasma lipids. They reported that in plasma phospholipids females had a higher level of stearic acid than did males, whereas in males the predominate fatty acid of this fraction was palmitic acid. Lyman, Shannon and Ostwald (1962)

demonstrated that in intact animals, estradiol benzoate facilitates the removal of unsaturated fatty acids from hepatic cholesterol esters and also increases the level of plasma cholesterol arachidonate.

Aftergood and Alfin-Slater (1965) studying gonadal hormone effects on lipid metabolism in the rat concluded that:

> "the effect of estrogenic deficiency on lipid metabolism includes: (a) decreased hepatic cholesterol biosynthesis; (b) increased hepatic sterol ester and decreased phospholipid concentration; (c) increased depletion of unsaturated fatty acid in plasma and liver during essential fatty acid deficiency; and (d) increase in severity of essential fatty acid deficiency symptoms, using as criteria the ratio of trienoic to tetraenoic fatty acids in plasma and liver lipids."

None of these studies have been devoted to the effect of a short term treatment with estrogens on the composition of plasma fatty acids. The present investigation was designed to find out whether treatment with various dosages of estradiol of durations comparable to that of the estrus cycle would effect either the level or the composition of plasma fatty acids. It was felt that with this design it would be possible to speculate on the possible adaptive value of estrogens in the mobilization of energy supplies during reproduction. In addition, some work has been carried out on castrate and hypophysectomized animals in an effort to understand how the lipid mobilizing effects of estrogens is brought about.

METHODS AND MATERIALS

Many different factors, although not always well understood, are important in determining the triglyceride fatty acid pattern. Among these, phylogenetic and dietary factors appear to be the most important, whereas ambient temperature, site of adipose tissue within a given organism, age, photoperiod, etc., are less important.

With the exception of two groups of animals, all animals used in this study were purchased either from Packard Research, Inc., Beaverton, Oregon, or Berkeley Pacific Laboratories, Berkeley, California. The two groups which were not supplied from these dealers were reared in our own laboratory and were offspring of the Harvard University rat colony. All animals used were from the Sprague-Dawley strain.

All of the animals while in our laboratory were maintained on Purina Lab Chow. Those animals which were obtained from Packard Research, Inc. were also reared on the Purina diet. The animals obtained from the Berkeley Pacific Laboratories were reared on a ration which contained no fish meal.[2] This is in contrast to the Purina Lab Chow which does contain fish meal. This difference in diet is believed to be the reason for the differences which were

[2] The Berkeley Pacific Laboratories animals were reared on Feed Stuff formulated by Feedstuffs Processing Company, 1240 Minnesota Street, San Francisco 7, California.

observed in the plasma fatty acid composition in the animals obtained from the two different suppliers.

An attempt was made to minimize the effect of a variable photoperiod by introducing an artificial photoperiod of fourteen hours light and ten hours of darkness. The animal room was not, however, shut off from the natural light source so in order to minimize the variability in the photoperiod, the time was adjusted so that the artificial and natural photoperiods overlapped. It is felt that the changes in temperature which occurred with the facilities available were moderate and inconsistant enough so as not to alter the fatty acid composition of the animals.

In most cases, the body weight range of the animals varied from approximately 220 to 240 grams. It was necessary, however, to use some animals with body weights outside this weight range due to difficulties in obtaining rats at certain times during the progress of this study.

All of the rats were females and were ovariectomized prior to treatment. For convenience in assessing the action of the hormones, the timing of the treatment is considered to start with the injection of the hormone rather than with the ovariectomy of the animals.

Estradiol Experiment

In these experiments, a single injection of estradiol-17β was administered as a solution in 0.1 cc propylene glycol on the seventh day following castration. The dosage levels of the hormone included 0.1 μg, 100 μg, or 2 mg. The intervals of time from injection to autopsy included 1, 3, 6, 24, 48 and 72 hours. All of these animals had their food removed and were placed on clean bedding material as close to 12 hours prior to autopsy as possible except those groups of animals at the 1, 3 and 6 hour intervals following injection. These animals had their food removed prior to autopsy at 13, 15 and 18 hours respectively.

In addition to those animals which received estradiol, two types of control animals were included as a part of this experiment. With one type of control the animals were treated exactly like the estradiol treated animals except that they received no injection. The second type of control was made up of animals which were injected with propylene glycol 1, 48 and 72 hours prior to autopsy.

Plasma fatty acid composition.

At autopsy, whole body weights were taken and while under deep ether anesthesia, approximately 6 to 8 cc of blood was drawn from the vena cava vein in a heparinized syringe. Following centrifugation,

3 ml of plasma was pipetted into a mixture of 3. 75 ml of redistilled chloroform and 7. 5 ml of redistilled methanol. This mixture was then immediately placed in a deep freeze to await final extraction. Following the removal of blood a sample of fat associated with the uterus was removed and quickly placed in a deep freeze. Finally, the left fat pad which adheres to the dorsal wall of the peritoneal cavity and which extends from the groin region to about the first rib was removed and its weight recorded.

The procedure for the extraction and purification of the plasma lipids prior to gas liquid chromatographic (GLC) analysis for the fatty acids has been described by Bligh and Dyer (1959). With this method, chloroform and methanol in the appropriate proportions form a miscible system with the water in the sample. Following filtration with a medium porosity fritted ware filter, this will separate into a diphasic solution in which the chloroform layer contains all of the lipids and the methanol-water layer contains all the non-lipids. The only modification of this method which was introduced involved extracting in 100 ml volumetric flasks rather than in a Waring Blender.

The preparation of ester derivatives of fatty acids provides for a rapid and convenient method of their analysis by GLC. Because most of the literature dealing with the identification of the recorder

peaks produced by fatty acids is based on methyl ester derivatives, this method was chosen.

The initial step in the preparation of fatty acid methyl esters was the evaporation of the chloroform in which they were dissolved. This was done by placing them under a slow stream of nitrogen gas. At the same time they were also slightly warmed by subjecting them to the warm air flow of a hair dryer. At this time the fatty acids were redissolved in 0. 5 ml of super dry methanol (Weissberger, 1949) which was approximately 3. 8% HCl.[3] To this was added 0. 5 ml of redistilled hexane. The samples were then placed in an 80°C water bath for three hours. It was necessary to construct special plastic liners for the caps in order to prevent the loss of solution at the pressure generated under these conditions. This also prevented contamination of the samples by the commercially available cap liners. The samples were then cooled and extracted with approximately 1. 5 ml of redistilled hexane. They were then concentrated under a flow of nitrogen with slight warming to a volume of approximately 10 to 20 μl. These samples were then stored in a deep freeze for future GLC analysis. No samples were stored for more than four

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[3]A specially constructed all glass gas generator was constructed to aid in the preparation of dry HCl (gas). A stopcock allowed the addition of concentrated H_2SO_4 into a reaction flask containing the oven dried reagent grade NaCl. The HCl gas escaped by way of the side arm and bubbled through the super dry methanol. The percent composition of HCl was determined by titration of an aliquot of the methanol against a solution of NaOH of known concentration.

weeks before analysis; most were analyzed within three days.

A Beckman GC-2 gas chromatograph with a thermal conductivity detector was used for the analysis of all of the samples in this study. A 15% by weight liquid phase of diethylene glycol succinate was adsorbed on chromasorb W (40/60). This was packed in nine foot aluminum columns (3/16" i. d.). The column was operated at 190°C and at helium gas pressures of about 30 to 40 psi.

The identification of the peaks obtained from the GLC of plasma samples was based on the comparison of their relative retention times to that of known standards[4] and on their separation by thin layer chromatography (TLC). The relative retention times reported in this study were calculated by dividing the distance from the start of the record to the middle of the peak in question into the distance from the start of the record to the middle of the methyl stearate peak.

The plasma samples were spotted on TLC plates (Mangold, 1961) and separated according to the degree of unsaturation (Morris, 1962; Privett, 1963) next to a standard solution of known fatty acids. Comparisons were made between the Rf values obtained from four different runs on the plasma samples and a standards solution containing stearic (18:0[5]), oleic (18:1), linoleic (18:2), and linolenic (18:3) acids.

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[4]All fatty acid standards, unless specifically noted, were purchased from the Kensington Scientific Corp., 1165 67th St., Oakland, Calif.

[5]With this method of fatty acid identification the first number indicates the number of carbon atoms and the second number refers to the number of bonds in the carbon chain which are unsaturated.

In addition, bands made by the TLC of rat plasma and a standard solution containing palmitic, stearic, oleic, linoleic, linolenic and arachidonic[6] acids were scraped as quickly as possible off of the TLC plate into hexane. These samples were analyzed by GLC and comparisons were made between the retention times of the standards and the peaks obtained from the rat plasma.

The method of estimating peak area which was chosen was the multiplication of peak height by the width of the peak at half height. Numerous factors such as the interaction between the fatty acids and the column components, nonuniform response of the detector, etc. necessitated the calculation of correction factors for each fatty acid. Correction factors were computed by comparing the response of the instrument to mixtures of gravimetrically determined fatty acid compositions according to the formula:

$$\text{Correction factor} = \frac{\text{Actual \% composition determined gravimetrically}}{\text{Percent composition determined by computing peak area.}}$$

The final percent composition for each fatty acid was obtained by multiplying the area of each peak by its correction factor. This value was then divided by the total corrected area of all of the five fatty acids present in the sample.

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[6]This fatty acid was kindly donated by James Saddler and was the 5, 8, 11, 14-isomer prepared by the Hormmel Institute.

Plasma free fatty acid concentration.

The first method used to determine the effect of estradiol on the level of FFA was that of Itaya and Ui (1965). These authors determined the best conditions for transfer of FFA from an aqueous phase into chloroform and then utilized Duncombes' (1962) micromethod for determining FFA. With this procedure a copper complex is formed with the fatty acid and can be measured colorimetrically. To a glass-stoppered test tube containing 6.0 ml of chloroform and 1.5 ml of phosphate buffer (pH 6.5), 0.2 ml of plasma was added, and the mixture was then shaken for 90 seconds. After a settling period of 15 minutes, the upper layer was aspirated with a fine-tipped pipette. The chloroform phase was then decanted into a second glass-stoppered tube and to it was added 3.0 ml of copper triethanolamine solution.[7] The tube was then shaken 30 times. After 15 minutes, the copper triethanolamine solution was aspirated with a fine-tipped pipette. The residual chloroform layer was filtered, and two drops of sodium diethyldithiocarbamate (0.1% w/v in redistilled butan-2-ol) solution was added. A yellowish brown color developed immediately and was measured without delay at 440 mμ against a reagent blank. The values obtained were then compared to a curve constructed from a

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[7] The copper triethanolamine solution which was used was made up of 9 vol. 1 M triethanolamine, 1 vol. 1 N acetic acid and 10 vol. 6.45% $Cu(NO_3)_2 \cdot 3H_2O$.

series of standard solutions of palmitic acid made up in chloroform and which ranged in concentration from 10 to 80 μeq/liter.

The method of Dole and Meinertz (1960) for determining FFA levels is based on the titration of the fatty acids with dilute alkali after their extraction with a ternary solvent system. With this method 0. 5 ml of fresh plasma was added to 2. 5 ml of an extraction mixture composed of: 1 N H_2SO_4 (0. 1 vol.), heptane (1 vol.), and isopropyl alcohol (4 vol.). The solution was then shaken and allowed to stand at room temperature for about five minutes. One ml of water and 1. 5 ml of heptane were then added and the tube was again shaken. Within five minutes the ternary system separated cleanly into two phases and without delay duplicate 2 ml aliquots of the upper phase were delivered into 15 ml conical bottomed centrifuge tubes.

The duplicate aliquots were titrated against a blank which contained 0. 5 ml of water instead of plasma. The standard solution of palmitic acid which the unknown plasma solutions were compared to was made up in heptane and this solution was substituted for the 1. 5 ml of heptane. The reader is referred to the publication from which this procedure was taken (Dole and Meinertz, 1960) for the details of the titration procedure. Only minor deviations were made from the procedure which they described. A Beckman microburette was used rather than a Gilmont. It was necessary to construct a longer tip for the microburette so that the dilute alkali could be delivered down into

the centrifuge tube. Also carbon dioxide free storage facilities were constructed for storing the indicator solution. This eliminated the necessity of preparing new indicator solution each day.

Hypophysectomy Experiment

The parapharyngeal approach was used in this study to remove the pituitary gland and has been described by Zarrow, _et al._ (1964). All animals were hypophysectomized on either the fifth or sixth day after ovariectomy and were either left as controls or injected with either 2 mg of estradiol, or 10 or 20 IU of adrenocorticotropic (ACTH) hormone. The details relating the exact time course used for each group of hypophysectomized animals are included in Table 18 of the Results section. The autopsy procedure was the same as that used in the estradiol experiments.

The rather small number of animals reported is attributed to a high mortality rate associated with the operation. Numerous efforts were made to remedy this situation, including administering ascorbic acid (50 mg) subcutaneously; however, only limited improvement was realized. It was generally felt that improved facilities, particularly including more rigidly controlled environmental temperature would be needed.

RESULTS

Gas-Liquid Chromatography

Identification of peaks.

The positive identification of the five major peaks obtained from the GLC of the blood plasma would depend upon the collection and direct chemical analysis of the responsible substances. However, on the basis of several kinds of indirect evidence it is possible to conclude with reasonable assurance what substances are present. Preliminary identification of the peaks was made by referring their relative retention times (Table 1) to the tables of relative retention times published by Farquhar, *et al.* (1959). Considering the slightly different temperature and the difference in liquid phases the evidence suggested that the five peaks were caused by the following fatty acids: palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and arachidonic (20:4). An illustration of the peaks produced by these five fatty acids when analyzed by GLC may be seen in Figure 1.

On polar columns increased carbon chain length increases the length of time they are retained in the column and unsaturated fatty acids are eluted after their saturated homologs. A straight line relationship exists when the logarithm of the retention time is plotted against the number of carbon atoms for a series of fatty acid

Table 1. Distance traveled, relative retention time and logarithm of retention time of fatty acid standards and blood plasma samples.

	Distance from start of gas-liquid chromatograph to peak (cm)										
	Myristic 14:0	Pentadecanoic 15:0	Palmitic 16:0	Palmitoleic 16:1	Margaric 17:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Nonadecanoic 19:0	Arachidonic[1] 20:4
Applied Sciences											
Standards											
K-102	4.60		8.25	9.20		15.25	17.17				
K-108			8.45			15.00	16.90	20.90	27.40		52.20
L-203		6.20	8.30		11.18	15.20				20.40	
L-205						15.00	16.90	20.80	27.20		
Blood Plasma											
Samples											
10			8.20			14.80	16.7	20.60			53.10
11			8.37			14.95	16.91	20.92			53.50
12			8.20			14.80	16.75	20.75			53.50
14			8.25			14.85	16.75	20.80			53.80
19			8.25			14.90	16.80	20.80			53.10
20			8.22			14.80	16.70	20.68			53.40
Average			8.25			14.85	16.77	20.76			53.40
Applied Sciences											
Standards					Relative retention time.						
K-102	.3016		0.5410	.6033		1.0	1.1259				
K-108			0.5630			1.0	1.1250	1.3950	1.8250		3.4800
L-203		0.4079	0.5460		0.7355	1.0				1.3421	
L-205						1.0	1.1250	1.3880	1.8120		
Blood Plasma											
Samples											
10			0.5540			1.0	1.1284	1.3919			3.5878
11			0.5598			1.0	1.1311	1.4013			3.5786
12			0.5540			1.0	1.1318	1.4020			2.6149
14			0.5555			1.0	1.1279	1.4007			3.6223
19			0.5537			1.0	1.1275	1.3960			3.5637
20			0.5554			1.0	1.1284	1.3973			3.6081
Average			0.5554			1.0	1.1292	1.3982			3.5959

Table 1. Continued.

	Logarithm of retention time										
	Myristic 14:0	Pentadecanoic 15:0	Palmitic 16:0	Palmitoleic 16:1	Margaric 17:0	Stearic 18:0	Oleic 18:1	Linoleic 18:2	Linolenic 18:3	Nonadecanoic 19:0	Arachidonic[1] 20:4
Applied Sciences											
Standards											
K-102	0.6627		0.9164	0.9638		1.1833	1.2348				
K-108			0.9269			1.1761	1.2279	1.3201	1.4377		1.7177
L-203		0.7924	0.9191		1.0484	1.1818				1.3096	
L-205						1.1761	1.2279	1.3181	1.4346		
Blood Plasma											
Samples											
10			0.9138			1.1703	1.2227	1.3139			1.7251
11			0.9227			1.1746	1.2281	1.3212			1.7283
12			0.9138			1.1703	1.2240	1.3170			1.7283
14			0.9164			1.1717	1.2240	1.3181			1.7308
19			0.9164			1.1732	1.2253	1.3181			1.7251
20			0.9149			1.1703	1.2227	1.3155			1.7275
Average			0.9163			1.1717	1.2245	1.3173			1.7275

[1] This fatty acid was added to Applied Sciences Standard K-108 and was donated by James Saddler. It was purchased from the Hormmel Institute and has double bonds at the following positions: 5, 8, 11 and 14.

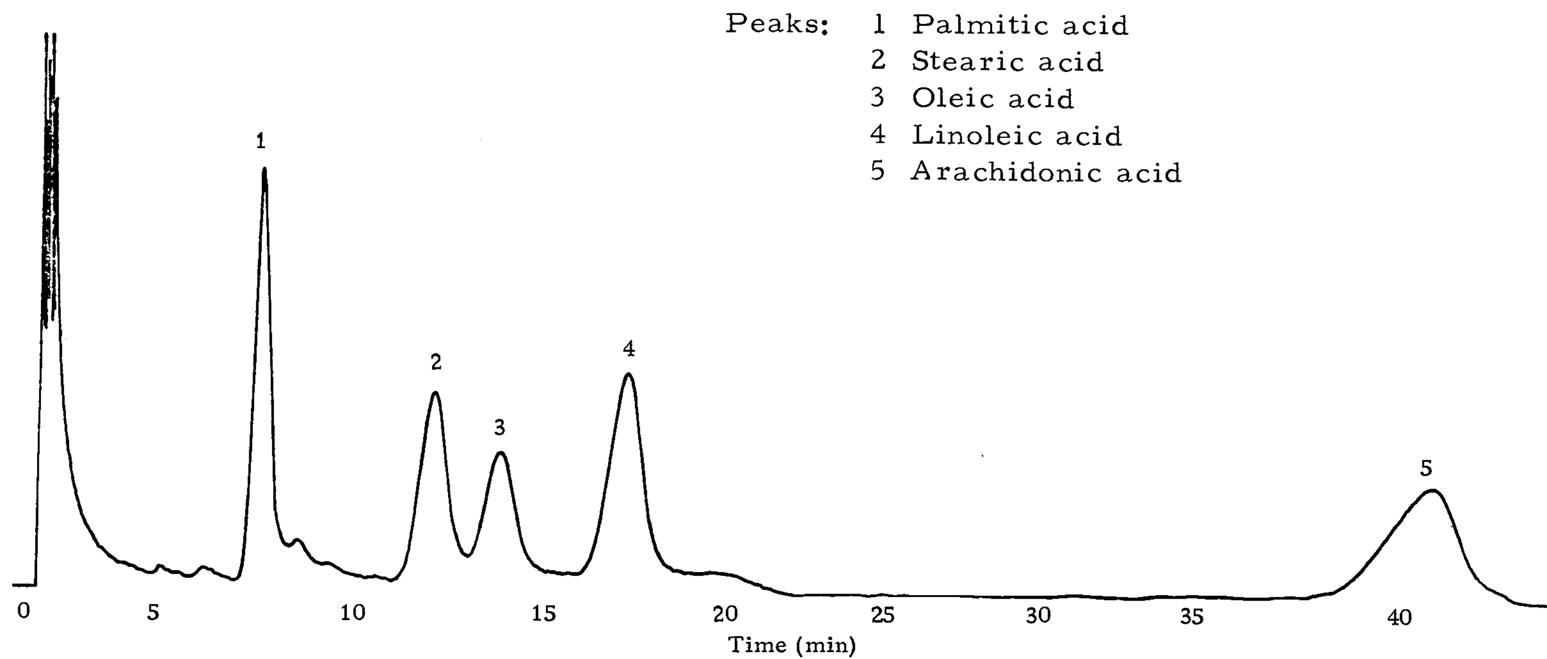

Figure 1. Gas-liquid chromatograph of rat plasma fatty acids.

standards with similar degrees of unsaturation (Figure 1). The data obtained when comparisons were made between the logarithm of the retention times of these peaks obtained from the plasma samples (Table 1) and such a series of standards further confirmed the presence of the above five fatty acids. These comparisons have been illustrated graphically in Figure 2.

Lipid extracts of plasma were also chromatographed on thin layers of silica gel G and the fatty acids present were separated according to the degree of their unsaturation. A standard solution containing stearic, oleic, linoleic and linolenic acids was also spotted along with the plasma samples. Good agreement was noted between the Rf values for the stearic, oleic and linoleic acids, but not for the linolenic acid. These data are included in Table 2.

In addition, preparative TLC plates were prepared using rat plasma and a standard solution containing the following fatty acids: palmitic, stearic, oleic, linoleic, linolenic and arachidonic. The bands of fatty acids which were produced were analyzed by GLC and comparisons were made between the retention times of the standards and the peaks that were produced by the plasma. The bands were arbitrarily assigned numbers running consecutively from one to five. Band number one represented the fatty acids which traveled the greatest distance on the TLC plate and band number five represented the fatty acid which did not migrate from where it was spotted. The

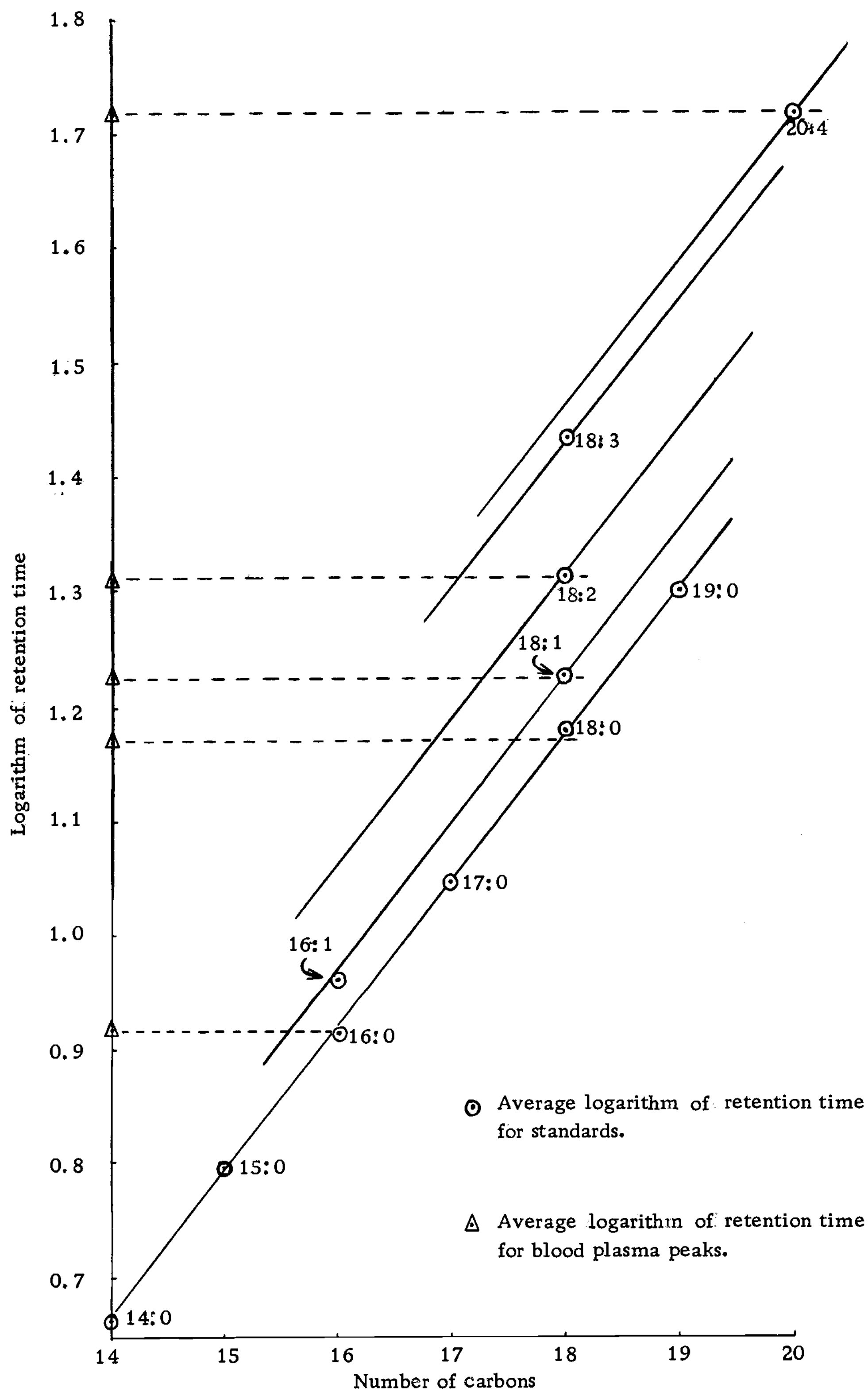

Figure 2. Logarithm of retention time vs. carbon chain length for fatty acid standards and rat plasma samples.

Table 2. Thin-layer chromatography of fatty acid standards and lipid extract of rat blood plasma.

	Fatty Acid	Standard[1] D[2]	Standard[1] Rf[3]	Blood Plasma D	Blood Plasma Rf
Run No. 1* (Solvent front 13.6 cm)	Stearic 18:0	12.4	0.912	12.2	0.897
	Oleic 18:1	10.8	0.794	10.6	0.779
	Linoleic 18:2	7.5	0.551	7.4	0.544
	Linolenic 18:3	4.8	0.353	4.3	0.316
Run No. 2 (Solvent front 15.4 cm)	18:0	13.5	0.876	13.2	0,857
	18:1	10.6	0.688	10.5	0.682
	18:2	7.6	0.494	7.3	0.474
	18:3	4.4	0.286	3.9	0.253
Run No. 3 (Solvent front 13.6 cm)	18:0	11.6	0.853	11.4	0.838
	18:1	9.5	0.699	9.4	0.693
	18:2	6.0	0.442	5.8	0.426
	18:3	3.6	0.265	3.2	0.235
Run No. 4 (Solvent front 14.6)	18:0	12.7	0.870	12.5	0.856
	18:1	10.5	0.719	10.5	0.719
	18:2	7.4	0.507	7.1	0.486
	18:3	4.3	0.294	3.9	0.247

[1] Applied Sciences Standard Number L-205.

[2] D = Distance (cm) from origin to most concentrated area of spot.

[3] Rf = Distance from origin to most concentrated area of spot divided by distance from origin to solvent front.

* Solvent system used was 20 percent ether in Skelly F.

retention times for the bands were: band no. 1, standard palmitic acid 8. 45, stearic acid 15. 00, plasma 8. 35 and 14. 78; band no. 2, standard oleic acid 16. 90, plasma 16. 39; band no. 3, standard linoleic acid 20. 90, plasma 20. 51; band no. 4, standard linolenic acid 27. 40, plasma no peak present; band no. 5, standard arachidonic acid 47. 20, plasma 48. 15. These data are included in tabular form in Table 3.

Table 3. Retention times for a standard solution of fatty acids and rat plasma after separation by thin-layer chromatography.

Arbitrarily assigned band no.	Standard fatty acid methyl esters[1]	Retention time(cm) for standard fatty acids	Retention time(cm) for peaks obtained from plasma samples
1	Palmitate	8. 45	8. 35
1	Stearate	15. 00	14. 78
2	Oleate	16. 90	16. 39
3	Linoleate	20. 90	20. 51
4	Linolenate	27. 40	----
5	Arachidonate	47. 20	48. 15

[1]The methyl arachidonate (5, 8, 11, 14 isomer) was kindly donated by James Saddler and was prepared by the Hormmel Institute.

Calculation of correction factors.

When using a thermal conductivity detector it is necessary to know the relationship between detector response and the structure of the substances detected. When the known percent composition of standard fatty acids (Table 4) were divided by the percent composition obtained from peak area measurements after gas chromatography (Table 4), the following correction factors were obtained: methyl palmitate, 0.9128; methyl stearate, 1.0040; methyl oleate, 0.9539; methyl linoleate, 1.0735 and methyl arachidonate, 1.2020.

Estradiol Experiment

Composition of fatty acids in blood plasma.

When considering the changes in the composition of fatty acids in the blood plasma following estrogen treatment, it must be remembered that the changes in individual fatty acids are based on the percentage they comprise of the total fatty acids present; these must not be thought of as absolute increases or decreases. The results of the changes in the fatty acid composition of the blood plasma of individual animals at various intervals following treatment with single injections of various dosages of estradiol-17β may be found in Tables 5, 6 and 7. A summary of this information is included in Table 8.

Table 4A. Determination of correction factors. (Palmitic, oleic and arachidonic fatty acid methyl esters--Mixture No. 1.)

Applied sciences fatty acid methyl ester standards	Quantity weighed (mg)	Purity	Actual quantity present (mg)	Actual % composition
Palmitoleate (16:1)	38.53	96%	36.99	29.42
Oleate (18:1)	28.55	99%	28.26	22.48
Arachidonate (20:4)	63.00	96%	60.48	48.10

	Methyl palmitoleate		Methyl oleate		Methyl arachidonate	
Run No.	Area under peak	Percent total area	Area under peak	Percent total area	Area under peak	Percent total area
1	16.83	35.55	11.62	24.54	18.89	39.90
2	15.99	36.07	11.14	25.13	17.20	38.80
3	14.31	35.72	9.70	24.21	16.05	40.06
4	12.18	34.25	8.77	24.67	14.61	41.08
5	23.26	35.36	16.24	24.69	26.28	39.95
6	12.23	35.25	8.48	24.44	13.98	40.30
Average percent composition of peak areas		35.37		24.61		40.01

$$\text{Methyl oleate correction factor} = \frac{22.48}{24.61} = 0.9133$$

$$\text{Methyl arachidonate correction factor} = \frac{48.10}{40.01} = 1.2020$$

(continued)

Table 4B. Determination of correction factors. (Applied sciences standard K-108.)[1]

Run No.	Methyl palmitate (16:0)		Methyl stearate (18:0)		Methyl oleate (18:1)		Methyl linoleate (18:2)		Methyl linolenate (18:3)	
	Area under peak	Percent total area	Area under peak	Percent total area	Area under peak	Percent total area	Area under peak	Percent total area	Area under peak	Percent total area
1	8.07	21.78	7.47	20.15	7.54	20.35	6.98	18.83	7.00	18.88
2	10.71	21.44	9.99	19.99	10.29	20.60	9.60	19.21	9.37	18.75
3	12.76	22.07	11.76	20.34	11.50	19.89	10.84	18.75	10.95	18.94
4	11.23	21.78	10.00	19.39	10.24	19.85	9.79	18.98	10.31	19.99
5	8.92	22.48	7.82	19.71	7.89	19.88	6.90	17.39	8.14	20.50
Average percent composition of peak areas		21.91		19.92		20.11		18.63		19.42

[1] Actural composition of each fatty acid methyl ester is 20 percent.

$$\text{Methyl palmitate correction factor} = \frac{20}{21.91} = 0.9128$$

$$\text{Methyl stearate correction factor} = \frac{20}{19.92} = 1.0040$$

$$\text{Methyl oleate correction factor} = \frac{20}{20.11} = 0.9945$$

$$\text{Methyl linoleate correction factor} = \frac{20}{18.63} = 1.0735$$

(continued)

Table 4C. Determination of correction factors. (Summary of correction factors for methyl esters of fatty acids computed from Mixture No. 1 and applied sciences standard K-108.)

	Mixture No. 1	Applied sciences K-108	Correction factors which were used
Methyl palmitate		0.9128	0.9128
Methyl stearate		1.0040	1.0040
Methyl oleate	0.9133	0.9945	0.9539
Methyl linoleate		1.0735	1.0735
Methyl arachidonate	1.2020		1.2020

Table 5. The effect of a single dose of 1.0 μg estradiol at varying time intervals after injection on the percent composition of fatty acids in the plasma of seven day castrate female rats.

Hours after injection	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	Methyl palmitate(16:0) %T[2]	Methyl stearate(18:0) AxCF	Methyl stearate(18:0) %T	Methyl oleate(18:1) AxCF	Methyl oleate(18:1) %T	Methyl linoleate(18:2) AxCF	Methyl linoleate(18:2) %T	Methyl arachidonate(20:4) AxCF	Methyl arachidonate(20:4) %T	Body wts. at Cas-tration	Body wts. at Au-topsy	B.W. diff. between C & A[3]
1	13	98	12.81	13.65	18.51	19.72	11.26	12.00	16.43	17.51	34.85	37.13	230	230	0
		99	21.70	19.34	19.27	17.18	21.34	19.02	22.36	19.93	27.50	24.52	235	232	- 3
		103	2.65	15.50	2.98	17.44	2.21	12.93	2.98	17.44	6.27	36.69	231	225	- 6
		229	8.15	14.79	12.73	23.10	7.04	12.78	10.88	19.74	16.30	29.58	243	243	0
		230	5.90	14.40	8.27	20.18	4.02	9.81	7.79	19.01	15.00	36.60	229	221	- 8
		231	4.72	14.54	6.74	20.76	2.81	8.66	5.40	16.64	12.79	39.40	230	235	5
		232	7.39	15.46	10.13	21.19	5.37	11.23	9.12	19.08	15.79	33.03	232	225	- 7
		233	6.34	15.77	8.54	21.24	4.20	10.44	7.08	17.61	14.05	34.94	230	222	- 8
		Ave.		15.43		20.10		12.11		18.37		33.99			- 3.8
		S.E.[4]		0.61		0.70		1.11		0.43		1.71			
3	15	45	7.98	19.91	5.99	14.95	5.10	12.72	6.85	17.09	14.16	35.33	230	209	-21
		46	9.87	18.51	8.78	16.47	7.36	13.80	10.76	20.18	16.55	31.04	232	238	6
		47	9.69	16.31	9.90	16.66	6.05	10.18	9.64	16.22	24.14	40.63	237	226	-11
		48	6.53	16.86	6.32	16.33	3.93	10.15	6.77	17.48	15.17	39.17	246	240	- 6
		50	10.84	16.79	11.24	17.41	7.81	12.10	12.04	18.65	22.63	35.05	234	236	2
		51	9.19	16.85	8.87	16.27	7.52	13.78	9.44	17.30	19.53	35.80	240	238	- 2
		Ave.		17.54		16.35		12.12		17.82		36.17			- 5.3
		S.E.		0.56		0.33		0.67		0.57		1.38			

[1] AxCF = Area under GLC curve multiplied by correction factor (Table 4).

[2] %T = Percent AxCF is of total area under curves of all fatty acids present in sample.

[3] C & A = Castration and autopsy.

[4] S.E. = Standard error.

Table 5. Continued.

Hours after injection	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	Methyl palmitate(16:0) %T[2]	Methyl stearate(18:0) AxCF	Methyl stearate(18:0) %T	Methyl oleate(18:1) AxCF	Methyl oleate(18:1) %T	Methyl linoleate(18:2) AxCF	Methyl linoleate(18:2) %T	Methyl arachidonate(20:4) AxCF	Methyl arachidonate(20:4) %T	Body wts. at Cas-tration	Body wts. at Au-topsy	B.W. diff. between C & A[3]
4	16	57	4.03	19.06	4.08	19.30	2.70	12.77	3.55	16.79	6.78	32.07	237	216	-21
		60	8.68	14.06	11.21	18.16	5.79	9.38	9.66	15.65	26.38	42.74	252	237	-15
		61	6.71	19.01	5.40	15.30	4.98	14.11	6.12	17.34	12.08	34.23	245	236	- 9
		63	8.51	17.74	7.48	15.59	6.58	13.72	8.29	17.28	17.11	35.67	230	220	-10
		Ave.		17.47		17.09		12.49		16.77		36.18			-13.9
		S.E.[4]		1.18		0.98		1.07		0.39		2.31			
12	12	219	7.06	16.88	7.07	16.90	5.77	13.80	8.53	20.40	13.39	32.02	234	223	-11
		220	9.58	17.15	9.72	17.40	6.72	12.03	9.74	17.44	20.10	35.98	238	228	-10
		221	14.89	19.45	12.28	16.04	12.79	16.71	15.75	20.57	20.84	27.22	230	220	-10
		222	17.75	18.53	15.99	16.69	13.49	14.08	18.43	19.23	30.15	31.47	225	226	1
		223	3.59	16.95	3.58	16.90	2.92	13.79	3.54	16.71	7.55	35.65	225	217	- 8
		Ave.		17.79		16.79		14.08		18.87		32.47			- 7.6
		S.E.		0.51		0.22		0.75		0.78		1.60			
24	12	188	12.06	17.53	12.19	17.72	9.40	13.66	14.65	21.30	20.49	29.79	245	268	23
		189	9.66	17.03	10.47	18.46	8.81	15.53	14.91	26.29	12.87	22.69	231	252	21
		190	10.65	17.41	10.33	16.89	11.13	18.20	15.77	25.78	13.28	21.71	250	275	25
		191	7.53	19.20	6.21	15.83	5.49	14.00	8.87	22.62	11.12	28.35	236	261	25
		192	19.75	17.20	20.39	17.76	18.40	16.03	25.42	22.14	30.84	26.86	226	245	19
		193	12.97	17.95	13.60	18.83	8.94	12.38	17.58	24.33	19.15	26.51	224	252	28
		Ave.		17.72		17.58		14.97		23.74		25.98			23.5
		S.E.		0.33		0.44		0.87		0.83		1.29			

[1] AxCF = Area under GLC curve multiplied by correction factor (Table 4).

[2] %T = Percent AxCF is of total area under curves of all fatty acids present in sample.

[3] C & A = Castration and autopsy.

[4] S.E. = Standard error.

Table 5. Continued.

Hours after injection	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	Methyl palmitate(16:0) %T[2]	Methyl stearate(18:0) AxCF	Methyl stearate(18:0) %T	Methyl oleate(18:1) AxCF	Methyl oleate(18:1) %T	Methyl linoleate(18:2) AxCF	Methyl linoleate(18:2) %T	Methyl arachidonate(20:4) AxCF	Methyl arachidonate(20:4) %T	Body wts. at Castration	Body wts. at Autopsy	B.W. diff. between C & A[3]
48	12	132	3.03	16.24	2.99	16.02	2.06	11.04	3.54	18.97	7.04	37.73	256	255	- 1
		137	8.93	17.12	7.19	13.78	5.78	11.08	11.50	22.04	18.77	35.98	228	221	- 7
		249	7.88	18.09	8.29	19.04	5.54	12.72	8.76	20.11	13.08	30.03	213	220	7
		250	8.43	14.18	11.41	19.20	7.06	11.88	11.80	20.02	20.63	34.71	216	227	11
		251	7.60	15.84	9.53	19.87	6.75	14.07	8.67	18.07	15.42	32.14	221	225	4
		252	6.51	15.28	8.01	18.81	4.78	11.22	8.30	19.49	14.99	35.20	215	230	5
		253	16.43	20.50	15.90	19.84	12.64	15.78	15.14	18.90	20.01	24.98	219	224	5
		Ave.		16.75		18.08		12.54		19.66		32.97			4.9
		S.E.[4]		0.78		0.87		0.68		0.48		1.64			
72	12	181	8.64	16.82	9.79	19.06	7.55	14.70	11.26	21.92	14.13	27.51	239	250	11
		182	6.55	14.87	9.10	20.66	4.70	10.67	8.68	19.71	15.01	34.08	237	252	15
		183	7.54	15.15	9.17	18.43	5.09	10.23	8.72	17.53	19.23	38.65	230	240	10
		185	9.59	18.43	10.20	19.61	7.34	14.11	11.40	21.91	13.49	25.93	241	249	8
		187	10.95	17.19	11.06	17.36	7.10	11.15	12.92	20.28	21.67	34.02	229	242	13
		Ave.		16.49		19.02		12.17		20.27		32.04			13
		S.E.		0.66		0.55		0.93		0.81		2.34			

[1] AxCF = Area under GLC curve multiplied by correction factor (Table 4).

[2] %T = Percent AxCF is of total area under curves of all fatty acids present in sample.

[3] C & A = Castration and autopsy.

[4] S.E. = Standard error.

Table 6. The effect of a single dose of 100 μg estradiol at varying time intervals after injection on the percent composition of fatty acids in the plasma of seven day castrate female rats.

Hours after injection	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	%T[2]	Mehtyl stearate(18:0) AxCF	%T	Methyl oleate(18:1) AxCF	%T	Methyl linoleate(18:2) AxCF	%T	Methyl arachidonate(20:4) AxCF	%T	Body wts. at Cas-tration	Body wts. at Au-topsy	B.W. diff between C & A[3]
1	13	224	6.60	15.71	9.10	21.66	4.19	9.97	7.48	17.80	14.64	34.85	230	230	0
		225	10.65	19.88	8.82	16.46	6.93	12.93	11.15	20.81	16.03	29.92	230	218	-12
		226	8.34	16.12	10.59	20.46	5.99	11.57	8.81	17.02	18.02	34.82	270	260	-10
		227	5.89	14.80	8.67	21.78	4.37	10.98	6.32	15.88	14.55	36.56	230	230	0
		228	7.61	14.04	11.02	20.33	5.45	10.06	10.22	18.86	19.89	36.70	249	238	-11
		Ave.		16.11		20.14		11.10		18.07		34.57			- 6.6
		S.E.[4]		1.01		0.97		0.54		0.84		1.23			
3	15	20	10.09	17.75	10.65	18.74	5.86	10.31	8.08	14.22	22.16	38.99	240	232	- 8
		22	6.39	16.82	5.89	15.51	5.67	14.93	7.77	20.46	12.26	32.28	240	233	- 7
		24	5.90	15.49	6.53	17.15	4.51	11.84	6.42	16.86	14.72	38.65	242	220	-22
		25	7.33	15.59	8.73	18.57	6.35	13.51	6.92	14.72	17.68	37.61	240	223	-17
		26	4.93	17.76	5.43	19.57	4.15	14.95	4.48	16.14	8.76	31.57	244	230	-14
		Ave.		16.68		17.91		13.11		16.48		35.82			-13.6
		S.E.		0.50		0.71		0.90		1.10		1.61			
6	18	254	5.38	18.88	5.96	20.92	2.78	9.76	4.77	16.74	9.60	33.70	218	214	- 4
		255	5.45	19.92	4.92	17.98	3.50	12.79	5.32	19.44	8.17	29.86	216	208	- 8
		256	5.32	16.79	5.40	17.05	4.02	12.69	5.70	18.00	11.23	35.46	230	222	- 8
		257	5.88	18.36	6.32	19.74	3.72	11.62	6.56	20.49	9.54	29.79	214	205	- 9
		258	9.63	18.21	9.37	17.72	7.37	13.93	10.83	20.48	15.69	29.67	215	210	- 5
		Ave.		18.43		18.68		12.16		19.03		31.70			- 6.8
		S.E.		0.51		0.47		0.70		0.73		1.21			
12	12	214	7.57	21.58	4.53	12.92	5.32	15.17	8.63	24.61	9.02	25.72	230	200	-30
		215	8.29	19.72	7.23	17.20	5.32	12.66	7.67	18.25	13.52	32.17	232	215	-17
		216	9.08	19.35	7.95	16.94	7.14	15.22	9.34	19.91	13.41	28.58	254	242	-12
		217	9.37	20.20	7.69	16.58	6.59	14.20	8.82	19.01	13.92	30.01	237	215	-22
		218	15.33	17.91	13.90	16.24	11.36	13.27	16.96	19.81	28.04	32.76	230	212	-18
		Ave.		19.75		15.98		14.10		20.32		29.85			-19.8
		S.E.		0.60		0.78		0.51		1.11		1.28			

Table 6. Continued.

Hours after injection	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	%T[2]	Methyl stearate(18:0) AxCF	%T	Methyl oleate(18:1) AxCF	%T	Methyl linoleate(18:2) AxCF	%T	Methyl arachidonate(20:4) AxCF	%T	Body wts. at Castration	Body wts. at Autopsy	B.W. diff. between C & A[3]
24	12	159	12.54	19.13	10.24	15.62	10.25	15.64	15.26	23.28	17.26	26.33	232	245	13
		160	10.28	19.12	8.17	15.19	7.75	14.41	13.11	24.38	14.46	26.89	234	242	8
		161	12.70	22.08	8.52	14.81	10.45	18.17	14.70	25.56	11.15	19.38	228	231	3
		162	10.73	20.60	7.66	14.71	9.01	17.30	13.53	25.98	11.15	21.41	238	243	5
		163	13.47	21.03	8.32	12.99	11.45	17.88	16.09	25.12	14.72	22.98	240	240	9
		164	12.76	20.47	8.55	13.72	11.73	18.82	16.55	26.55	12.74	20.44	238	252	14
		Ave.		20.40		14.51		17.04		25.14		22.90			7.5
		S.E.[4]		0.47		0.40		0.68		0.48		1.27			
48	12	153	8.53	17.70	8.18	16.98	7.46	15.48	10.45	21.70	13.56	28.14	238	250	20
		154	14.31	22.79	8.82	14.06	12.05	19.19	14.47	23.05	13.12	20.91	243	255	12
		155	12.26	20.57	8.95	15.02	8.86	14.87	13.47	22.60	16.06	26.95	245	245	0
		156	10.36	16.59	11.15	17.85	8.22	13.16	12.49	20.00	20.23	32.39	230	235	5
		157	10.64	21.96	6.82	14.07	7.04	14.53	9.74	20.10	14.22	29.34	240	251	11
		158	6.14	17.08	6.03	16.78	5.05	14.05	8.14	22.65	10.58	29.44	248	251	3
		Ave.		19.45		15.79		15.21		21.68		27.86			8.3
		S.E.		1.09		0.66		0.86		0.55		1.57			
72	12	234	6.86	13.66	9.98	19.87	6.70	13.34	8.01	15.95	18.67	37.18	230	234	4
		235	7.28	14.76	9.60	19.46	6.29	12.75	8.61	17.45	17.55	35.58	246	256	10
		236	6.84	15.29	7.88	17.62	4.81	10.75	9.49	21.22	15.70	35.11	223	230	7
		237	9.04	15.33	12.24	20.76	6.31	10.70	9.18	15.57	22.18	37.62	226	218	-8
		238	6.70	16.73	8.90	22.22	4.32	10.79	7.16	17.88	12.97	32.38	228	234	6
		Ave.		15.15		19.99		11.67		17.61		35.57			7.0
		S.E.		0.51		0.76		0.57		1.00		0.93			

[1] AxCF = Area under GLC curve multiplied by correction factor (Table 4).

[2] %T = Percent AxCF is of total area under curves of all fatty acids present in sample.

[3] C & A = Castration and autopsy.

[4] S.E. = Standard error.

Table 7. The effect of a single dose of two milligrams estradiol at varying time intervals after injection on the percent composition of fatty acids in the plasma of seven day castrate female rats.

Hours after injection	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	Methyl palmitate(16:0) %T[2]	Methyl stearate(18:0) AxCF	Methyl stearate(18:0) %T	Methyl oleate(18:1) AxCF	Methyl oleate(18:1) %T	Methyl linoleate(18:2) AxCF	Methyl linoleate(18:2) %T	Methyl arachidonate(20:4) AxCF	Methyl arachidonate(20:4) %T	Body wts. at Castration	Body wts. at Autopsy	B.W. diff. between C & A[3]
1	13	38	6.63	14.86	7.08	15.87	5.67	12.71	8.06	18.07	17.17	38.49	253	238	-15
		39	11.83	16.89	11.66	16.65	9.99	14.26	12.77	18.23	23.79	33.97	240	215	-25
		42	7.75	17.27	7.68	17.12	5.52	12.30	7.56	16.85	16.36	36.46	242	230	-12
		44	5.70	16.46	5.80	16.73	3.67	10.59	5.96	17.19	13.53	39.03	250	235	-15
		Ave.		16.37		16.59		12.46		17.59		36.99			-16.7
		S.E.[4]		0.53		0.26		0.75		0.33		1.15			
3	15	2	2.99	16.24	3.45	18.74	2.73	14.83	3.42	18.54	5.82	31.61	255	248	- 7
		3	6.14	18.19	6.01	17.81	5.13	15.20	5.99	17.75	10.48	31.05	244	232	-12
		17	6.99	15.43	7.83	17.28	7.05	15.56	7.09	15.66	16.33	36.06	242	230	-12
		18	4.67	15.67	5.08	16.64	3.43	11.23	5.07	16.59	12.30	40.25	247	225	-22
		19	6.14	15.35	6.83	17.07	6.25	15.62	7.55	18.87	13.70	34.25	248	234	-14
		Ave.		16.18		17.51		14.48		17.48		34.64			-13.7
		S.E.		0.53		0.36		0.83		0.60		1.67			
6	18	7	5.15	15.31	6.80	20.21	4.18	12.43	5.73	17.03	11.78	35.02	258	250	- 8
		72	4.97	16.75	5.40	18.20	3.55	11.96	5.79	19.51	9.96	33.57	245	246	1
		73	8.25	18.53	7.13	16.01	5.51	12.38	9.07	20.37	14.56	32.70	241	232	- 9
		74	3.72	16.97	4.44	20.26	2.57	11.75	3.18	14.51	8.00	36.50	243	228	-15
		76	3.34	15.02	3.00	13.49	1.98	8.91	5.52	24.83	8.39	37.74	246	234	-12
		Ave.		16.52		17.63		11.49		19.25		35.11			- 8.6
		S.E.		0.63		1.30		0.66		1.73		0.92			
12	12	122	12.16	16.54	11.74	15.97	10.20	13.88	15.53	21.13	23.87	32.48	237	234	- 3
		125	9.12	19.21	7.18	15.12	8.53	17.97	10.45	22.02	12.19	25.68	243	248	5
		126	6.94	19.67	6.50	18.42	4.09	11.59	7.08	20.08	10.67	30.24	232	227	- 5
		129	4.96	18.36	4.13	15.29	3.51	12.99	5.44	20.14	8.97	33.21	237	240	3
		130	8.34	20.48	5.80	14.24	6.19	15.20	9.33	22.91	11.06	27.16	254	246	- 8
		Ave.		18.85		15.81		14.33		21.26		29.75			- 1.6
		S.E.		0.67		0.71		1.08		0.55		1.46			

Table 7. Continued.

Hours after injection	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	Methyl palmitate(16:0) %T[2]	Methyl stearate(18:0) AxCF	Methyl stearate(18:0) %T	Methyl oleate(18:1) AxCF	Methyl oleate(18:1) %T	Methyl linoleate(18:2) AxCF	Methyl linoleate(18:2) %T	Methyl arachidonate(20:4) AxCF	Methyl arachidonate(20:4) %T	Body wts. at Cas-tration	Body wts. at Au-topsy	B.W. diff. between C & A[3]
24	12	113	5.65	22.57	3.28	13.10	4.80	19.18	6.53	26.09	4.77	19.06	231	231	0
		165	7.55	26.44	3.98	13.94	6.39	22.37	6.82	23.87	3.82	13.38	236	240	4
		166	18.44	23.31	9.17	11.59	17.67	22.35	21.38	27.03	12.43	15.12	236	242	6
		167	14.24	22.30	7.36	11.52	12.90	20.20	17.15	26.85	12.22	19.13	232	246	14
		169	5.56	23.81	3.25	13.92	4.39	18.80	6.14	26.21	4.03	17.26	236	250	14
		Ave.		23.69		12.81		20.58		26.01		16.91			7.6
		S.E.[4]		0.74		0.54		0.76		0.56		1.08			
48	12	120	9.85	21.97	5.48	12.22	9.81	21.89	10.74	23.96	8.95	19.96	246	244	- 2
		121	11.41	22.49	5.83	11.49	12.39	24.42	13.25	26.11	7.86	15.49	238	236	- 2
		177	19.70	24.62	8.78	10.98	19.08	23.85	22.68	28.34	9.77	12.21	254	259	- 5
		178	11.99	22.34	6.32	11.78	11.31	21.07	15.11	28.15	8.94	16.66	238	238	0
		179	5.49	22.19	3.58	14.47	5.96	24.09	5.38	21.75	4.33	17.50	228	232	4
		180	4.00	24.49	2.16	13.23	2.59	15.86	4.54	27.78	3.05	18.67	240	235	- 5
		Ave.		23.02		12.36		21.86		26.01		16.75			- 1.6
		S.E.		0.49		0.52		1.32		1.09		1.11			
72	12	171	4.21	25.26	2.03	12.18	3.00	18.03	4.07	24.42	3.35	20.10	240	236	- 4
		173	7.58	24.12	3.51	11.17	6.66	21.19	7.92	25.20	5.76	18.33	241	249	8
		174	13.13	25.24	5.42	10.42	10.88	20.91	15.48	29.75	7.11	13.68	243	242	- 1
		175	7.40	25.37	3.69	12.65	6.14	21.05	7.72	26.46	4.22	14.47	253	253	0
		176	3.78	24.47	1.71	11.07	3.15	20.39	4.14	26.80	2.67	17.29	242	235	- 7
		Ave.		24.89		11.50		20.31		26.53		16.77			- 0.8
		S.E.		0.25		0.40		0.59		0.91		1.20			

[1]AxCF = Area under GLC curve multiplied by correction factor (Table 4).

[2]%T = Percent AxCF is of total area under curves of all fatty acids present in sample.

[3]C & A = Castration and autopsy.

[4]S.E. = Standard error.

Table 8. Summary of the effects of estradiol on the percent composition of fatty acids in the plasma of seven day castrate female rats.

Amt. of estradiol injected	Hours after injection	Methyl palmitate(16:0) %T[1]	S.E.[2]	Methyl stearate(18:0) %T	S.E.	Methyl oleate(18:1) %T	S.E.	Methyl linoleate(18:2) %T	S.E.	Methyl arachidonate(20:4) %T	S.E.	No. of animals	Animal source
0.1 μg	1	15.43	±0.61	20.10	±0.70	12.11	±1.11	18.37	±0.43	33.99	± 1.71	8	Packard
	3	17.54	±0.56	16.35	±0.33	12.12	±0.67	17.82	±0.57	36.17	± 1.38	6	Packard
	4	17.47	±1.18	17.09	±0.98	12.49	±1.07	16.77	±0.39	36.18	± 2.31	4	Packard
	12	17.79	±0.51	16.79	±0.22	14.08	±0.75	18.87	±0.78	32.47	± 1.60	5	Corvallis
	24	17.72	±0.33	17.58	±0.44	14.97	±0.87	23.74	±0.83	25.98	± 1.29	6	Packard
	48	16.75	±0.78	18.08	±0.87	12.54	±0.68	19.66	±0.48	32.97	± 1.64	7	Packard
	72	16.49	±0.66	19.02	±0.55	12.17	±0.93	20.27	±0.81	32.04	± 2.34	5	Packard
100 μg	1	16.11	±1.01	20.14	±0.97	11.10	±0.54	18.07	±0.84	34.57	± 1.23	5	Packard
	3	16.68	±0.50	17.91	±0.71	13.11	±0.90	16.48	±1.10	35.82	± 1.61	5	Packard
	6	18.43	±0.51	18.68	±0.47	12.16	±0.70	19.03	±0.73	31.70	± 1.21	5	Packard
	12	19.75	±0.60	15.98	±0.78	14.10	±0.51	20.32	±1.11	29.85	± 1.28	5	Corvallis
	24	20.40	±0.47	14.51	±0.40	17.04	±0.68	25.14	±0.48	22.90	± 1.27	6	Packard
	48	19.45	±1.09	15.79	±0.66	15.21	±0.86	21.68	±0.55	27.86	± 1.57	6	Packard
	72	15.15	±0.51	19.99	±0.76	11.67	±0.57	17.61	±1.00	35.57	± 0.93	5	Packard
2 mg	1	16.37	±0.53	16.59	±0.26	12.46	±0.75	17.59	±0.33	36.99	± 1.15	4	Packard
	3	16.18	±0.53	17.51	±0.36	14.48	±0.83	17.48	±0.60	34.64	± 1.67	5	Packard
	6	16.52	±0.63	17.63	±1.30	11.49	±0.66	19.25	±1.73	35.11	± 0.92	5	Packard
	12	18.85	±0.67	15.81	±0.71	14.33	±1.08	21.26	±0.55	29.75	± 1.46	5	Packard
	24	23.69	±0.74	12.81	±0.54	20.58	±0.76	26.01	±0.56	16.91	± 1.08	5	Packard
	48	23.02	±0.49	12.36	±0.52	21.86	±1.32	26.01	±1.09	16.75	± 1.11	6	Packard
	72	24.89	±0.25	11.50	±0.40	20.31	±0.59	26.53	±0.91	16.77	± 1.20	5	Packard

[1] %T = Percent of total.

[2] S.E. = Standard error.

The control animals for the estradiol treated rats may be found in Table 9. It can be noted that there are two separate groups of animals which were non-injected and without food for 12 hours prior to autopsy and also two separate groups which were previously treated with a single injection of propylene glycol 72 hours prior to autopsy. All of the animals with numbers below 300 were done at about the same time the estradiol-injected animals were treated. These experiments were done during the spring and summer of 1965. The percent composition of the plasma fatty acids of all of the control animals in these experiments were quite similar except those animals treated with propylene glycol 72 hours prior to autopsy. In order to determine whether or not this discrepancy in fatty acid composition was due to the propylene glycol, two additional groups of animals were run during the summer of 1966. One of the groups of animals did not receive any injection (animals no. 366 to 370) and the other group of animals (no. 359 to 364) received the same amount of propylene glycol taken from the same bottle as in the 1965 animals which resulted in a discrepancy in the plasma fatty acid composition. Although the composition of fatty acids in the 1966 animals are somewhat different from the 1965 control animals (due to difference in diet) the excellent agreement between the two groups indicates that the propylene glycol has no effect on the composition of fatty acids of the plasma. The percent composition of the plasma fatty acids

Table 9. Percent composition of fatty acids in the plasma of seven day castrate, noninjected and propylene glycol control female rats.

Treatment	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	Methyl palmitate(16:0) %T[2]	Methyl stearate(18:0) AxCF	Methyl stearate(18:0) %T	Methyl oleate(18:1) AxCF	Methyl oleate(18:1) %T	Methyl linoleate(18:2) AxCF	Methyl linoleate(18:2) %T	Methyl arachidonate(20:4) AxCF	Methyl arachidonate(20:4) %T	Body wts. at Castration	Body wts. at Autopsy	B.W. diff. between C & A[3]
Non-injected (Packard Rats)	12	52	6.54	22.74	4.09	14.22	3.26	11.33	4.91	17.07	9.96	34.63	238	192	-46
		54	9.86	14.94	9.49	14.38	10.28	15.58	12.54	19.00	18.81	28.50	246	230	-16
		56	6.12	16.46	6.04	16.25	5.89	15.86	7.35	19.77	11.77	31.66	241	225	-16
		64	7.61	16.32	8.60	18.44	5.77	12.37	8.88	19.04	15.77	33.82	239	239	0
		65	3.56	18.79	3.26	17.20	2.44	12.88	3.81	20.10	5.88	31.03	245	224	-21
		66	11.26	16.12	9.75	13.95	9.94	14.23	13.11	18.76	25.81	36.94	244	234	-10
		77	3.23	13.17	4.64	18.92	2.94	11.99	4.57	18.64	9.14	37.28	234	233	- 1
		78	9.38	17.16	8.81	16.13	9.04	16.54	11.59	21.20	15.83	28.96	243	256	13
		80	8.76	15.35	8.49	14.88	7.27	12.74	9.39	16.45	23.16	40.58	236	223	- 1
		83	3.58	14.46	4.01	16.20	3.18	12.85	5.06	20.44	8.92	36.04	226	230	4
		84	5.29	16.45	5.61	17.44	4.63	14.40	5.22	16.23	11.41	35.48	239	240	1
		86	5.33	17.55	4.59	15.11	4.52	14.88	6.43	21.17	9.50	31.28	230	246	16
		140	10.99	16.25	11.97	17.70	8.59	12.70	13.14	19.43	22.92	33.90	231	245	14
		141	11.10	15.80	13.13	18.69	9.66	13.75	14.94	21.26	21.43	30.50	254	256	2
		142	11.78	17.57	11.48	17.12	8.54	12.74	15.89	23.70	19.36	28.87	237	247	10
		Ave.		16.61		16.44		13.66		19.48		33.30			- 3.5
		S.E.[4]		0.56		0.43		0.39		0.51		0.92			
(Berkeley Rats)	12	366	18.35	18.58	17.31	17.52	18.83	19.06	21.20	21.46	23.09	23.37	128	150	22
		367	11.88	18.65	10.44	16.39	10.61	16.66	11.59	18.20	19.17	30.10	143	149	5
		368	19.12	18.37	17.95	17.25	19.51	18.75	21.31	20.48	26.17	25.15	150	159	9
		369	6.32	19.79	5.27	16.50	5.21	16.32	7.16	22.42	7.97	24.96	141	143	2
		370	6.95	16.95	6.98	17.02	6.59	16.07	8.40	20.49	12.08	29.46	146	161	15
		Ave.		18.47		16.94		17.37		20.61		26.61			10.6
		S.E.		0.45		0.22		0.63		0.70		1.34			
(Packard Rats)	18	94	6.91	17.13	6.56	16.25	4.33	10.73	7.14	17.69	15.42	38.20	254	213	-41
	18-1/4	95	10.65	16.55	11.71	18.20	6.69	10.39	12.10	18.80	23.20	36.05	235	228	- 7
	18-1/2	96	5.95	16.25	6.59	17.99	4.22	11.52	6.64	18.13	13.22	36.10	233	214	-19
	18-3/4	97	8.21	16.17	8.91	17.53	7.01	13.79	9.43	18.56	17.25	33.95	231	215	-16
		Ave.		16.52		17.49		11.61		18.29		36.07			-20.7
		S.E.		0.22		0.44		0.76		0.24		0.87			

Table 9. Continued.

Treatment	Hours without food	Animal number	Methyl palmitate(16:0) AxCF[1]	Methyl palmitate(16:0) %T[2]	Methyl stearate(18:0) AxCF	Methyl stearate(18:0) %T	Methyl oleate(18:1) AxCF	Methyl oleate(18:1) %T	Methyl linoleate(18:2) AxCF	Methyl linoleate(18:2) %T	Methyl arachidonate(20:4) AxCF	Methyl arachidonate(20:4) %T	Body wts. at Castration	Body wts. at Autopsy	B. W. diff. between C & A[3]
Hours after .1cc propylene glycol 1 (Packard Rats)	13	145	7.06	15.00	8.18	17.38	5.91	12.56	9.06	19.26	16.84	35.79	238	248	10
		147	6.57	16.90	6.40	16.46	4.77	12.27	8.71	22.40	12.43	31.97	230	253	23
		Ave.		15.95		16.92		12.41		20.83		33.88			16.0
		S.E.[4]		0.95		0.46		0.15		1.57		1.91			
48 (Packard Rats)	12	244	8.38	17.97	8.83	18.93	7.09	15.20	6.67	14.30	15.67	33.60	216	200	-16
		245	10.50	17.38	10.07	16.67	8.29	13.72	12.66	20.96	18.89	31.27	222	227	5
		247	7.73	18.87	6.26	15.28	6.33	15.45	8.62	21.04	12.02	29.35	221	220	- 1
		248	7.26	15.42	7.76	16.48	5.42	11.51	9.34	19.83	17.31	36.76	216	217	1
		Ave.		17.41		16.84		13.97		19.03		32.74			- 2.8
		S.E.		0.73		0.76		0.90		1.60		1.60			
72 (Packard Rats)	12	239	6.45	16.83	6.32	16.50	5.13	13.38	8.42	21.97	12.00	31.31	217	228	11
		240	8.73	19.25	7.59	16.74	5.88	12.97	9.40	20.73	13.75	30.32	214	207	- 7
		241	11.50	17.37	11.18	16.89	10.28	15.53	15.08	22.78	18.25	27.57	229	235	6
		242	4.83	18.28	4.36	16.51	3.28	12.42	6.00	22.71	7.94	30.08	221	227	6
		243	10.20	16.28	10.39	16.58	10.57	16.87	14.31	22.84	17.19	27.43	218	235	17
		Ave.		17.60		16.64		14.23		22.21		29.34			9.4
		S.E.		0.53		0.07		0.84		0.82		0.78			
72 (Berkeley Rats)	12	359	13.50	16.85	13.24	16.53	13.48	16.83	15.82	19.75	24.06	30.16	150	161	11
		360	7.85	19.67	6.57	16.46	5.81	14.56	7.78	19.49	11.90	29.82	140	145	5
		361	9.54	21.72	8.14	18.53	8.37	19.06	8.68	19.76	9.19	20.92	130	146	16
		362	6.10	19.07	5.64	17.64	5.12	16.01	6.01	18.79	9.11	28.49	144	148	4
		364	14.24	16.27	17.52	20.02	14.01	16.01	16.05	18.34	25.68	29.36	144	153	9
		Ave.		18.72		17.84		16.49		19.23		27.75			9.0
		S.E.		0.99		0.67		0.21		0.28		1.73			

[1] AxCF = Area under GLC curve multiplied by correction factor (Table 4).

[2] %T = Percent AxCF is of total area under curves of all fatty acids present in sample.

[3] C & A = Castration and autopsy.

[4] S.E. = Standard error.

of the non-injected animals was as follows: palmitic acid, 18. 47 ± 0. 45; stearic acid, 16. 94 ± 0. 22; oleic acid, 17. 37 ± 0. 63; linoleic acid, 20. 61 ± 0. 70; and arachidonic acid, 26. 61 ± 1. 34. The percent composition of the propylene glycol-injected animals was: palmitic acid, 18. 72 ± 0. 99; stearic acid, 17. 84 ± 0. 64; oleic acid, 16. 49 ± 0. 21; linoleic acid, 19. 23 ± 0. 28 and arachidonic acid, 27. 75 ± 1. 73.

At all three dosages of estradiol which were checked (0. 1 μg, 100 μg, 2 mg), the fatty acid composition did not change for six hours following injection. The first changes which were observed occurred at the 100 μg and 2 mg dosages 12 hours after injection. The changes included a slight increase in the percent composition of the palmitic, oleic and linoleic methyl esters and a slight decrease in the stearic and arachidonic methyl esters. Twelve hours later (24 hours after injection) similar but more marked changes had occurred at the high dosage. Comparing the percent composition of the individual fatty acids in the non-injected control and 2 mg estradiol treated animals 24 hours after injection the following changes were observed: palmitic acid changed from 16. 61 to 12. 81; oleic acid changed from 13. 66 to 20. 58; linoleic acid changed from 19. 48 to 26. 01 and archidonic changed from 33. 20 to 16. 91. At both 48 hours and 72 hours after injection the composition of fatty acids did not change from the level which was established by 24 hours. These

changes in the percent composition of plasma fatty acids after injection of 2 mg estradiol may be seen in Figure 3.

For the two lower dosages the same kinds of changes in fatty acid composition had also occurred by 24 hours after injection. However, these changes were not as pronounced. The changes which occurred in the percent composition of plasma fatty acids in rats treated with 100 μg estradiol 24 hours after injection as compared to non-injected animals were as follows: palmitic acid, non-injected, 16.61, 24 hours after injection, 20.40; stearic acid, non-injected, 16.44, 24 hours after injection 14.51; oleic acid non-injected, 13.66, 24 hours after injection 17.04; linoleic acid, non-injected, 19.48, 24 hours after injection 25.15 and archidonic, non-injected, 33.30, 24 hours after injection, 22.90. By 48 hours after injection this change in composition had begun to disappear and by 72 hours after injection the return to the composition in the non-injected controls was complete.

Twenty-four hours after injection of 0.1 μg estradiol the change in percent composition of fatty acids was as follows: palmitic acid, no change; stearic acid, no change; oleic acid, non-injected, 13.66, 24 hours after injection, 14.97; linoleic acid, non-injected, 19.48, 24 hours after injection, 23.74; arachidonic acid, non-injected, 33.30, 24 hours after injection, 25.98. By 48 hours after injection the composition of the fatty acids had completely returned to that

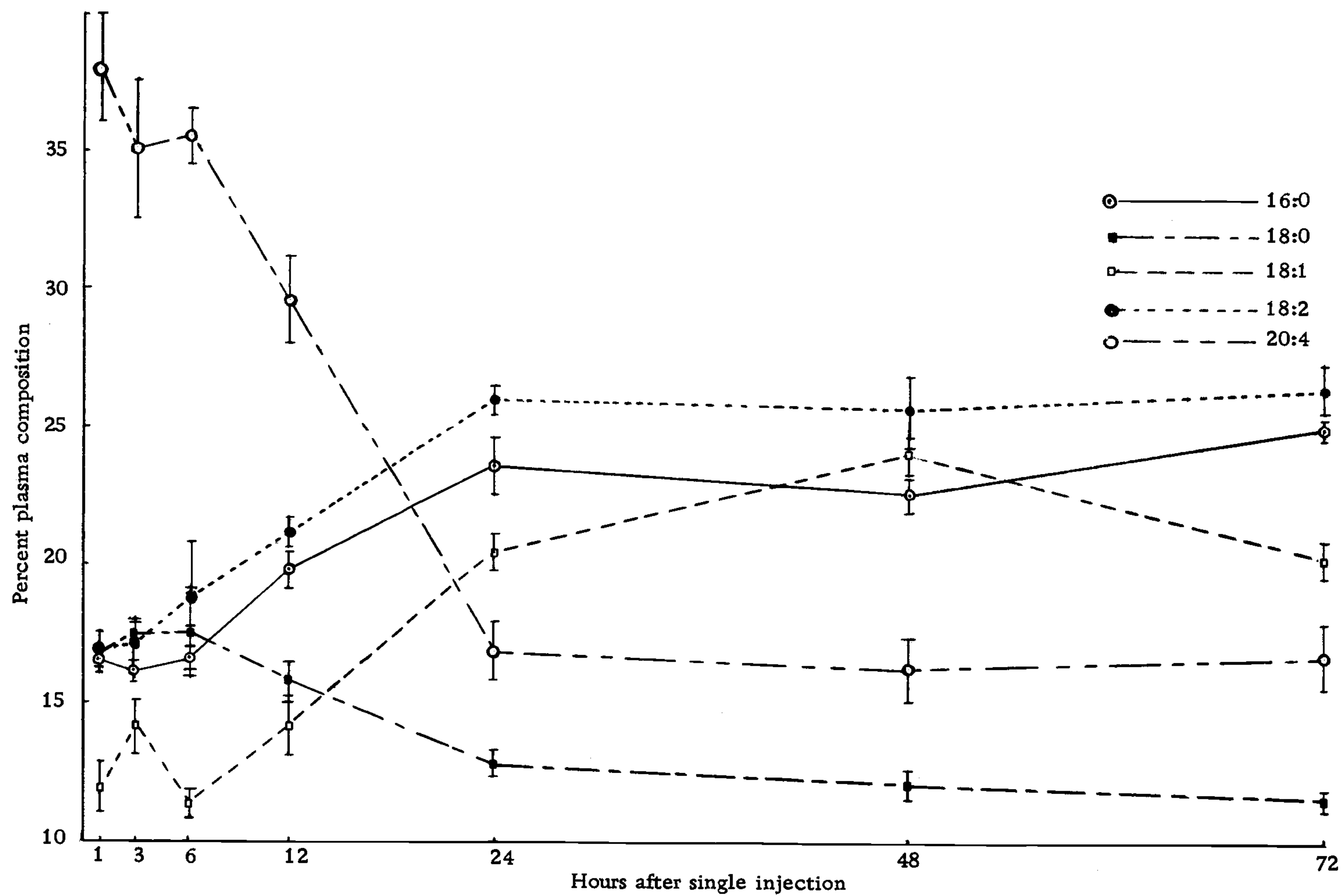

Figure 3. Effect of two milligrams estradiol on plasma fatty acid composition.

found in non-injected control animals. These effects of 0.1 μg estradiol on the unsaturated fatty acid composition are similar to the first observable changes (12 hours after injection) which took place at the two higher dosages. The changes which took place in the plasma fatty acids following 0.1 μg and 100 μg estradiol may be seen in Figures 4 and 5.

Composition of fatty acids in adipose tissue.

The fatty acid composition of the dorso-lumbar fat pad of non-injected control rats and those treated with various dosages of estradiol at various time intervals after injection may be found in Table 10. The average fatty acid composition of the fat pads of six non-injected control animals was: palmitic acid, 25.83%; stearic acid, 6.56%; oleic acid, 42.38%; and linoleic acid, 25.22%. The fatty acid composition of the fat pad of those animals treated with estradiol did not differ from that of the non-injected animals.

In an effort to determine a relationship between fatty acids in the fat tissue and the fatty acids present in the blood plasma after treatment with estradiol, Table 11 was constructed. This table includes the composition of fatty acids of the plasma diluted with various amounts of the fatty acid composition of adipose tissue. The relationships which may be seen between the fatty acids of plasma diluted by one-half with the fatty acids in fat and the fatty acid

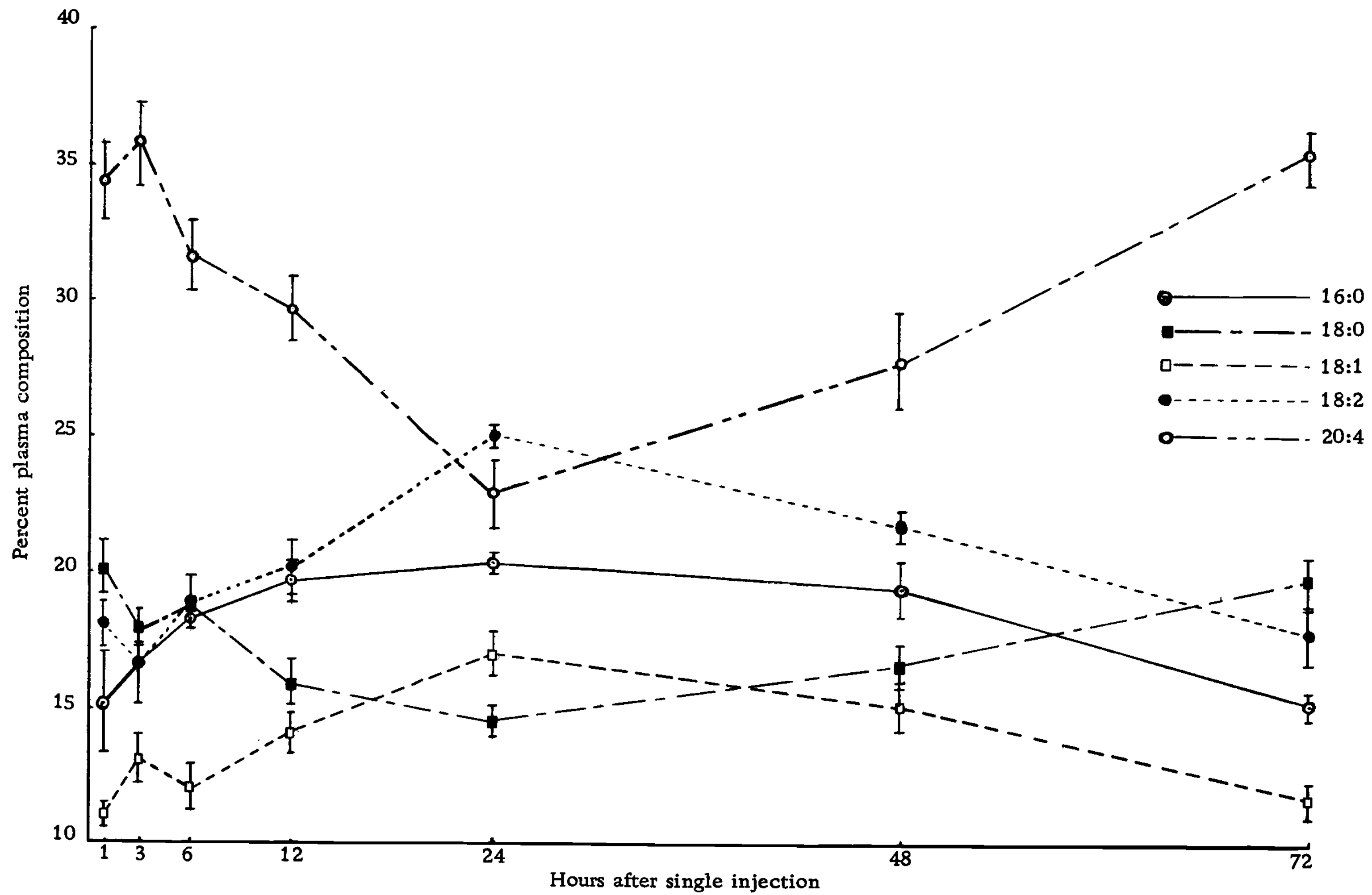

Figure 4. Effect of 100 μg estradiol on plasma fatty acid composition.

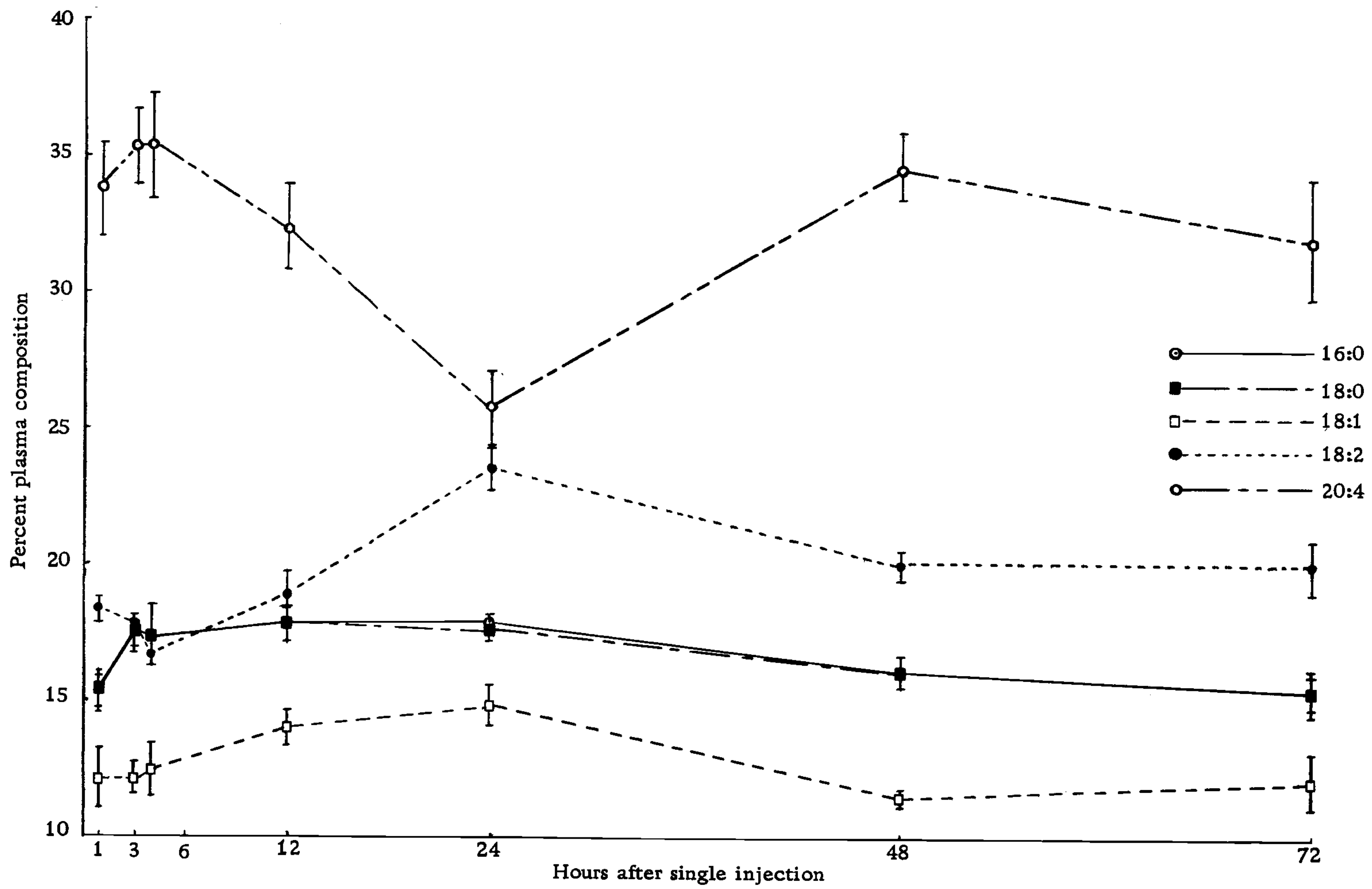

Figure 5. Effect of 0.1 μg estradiol on plasma fatty acid composition.

Table 10. Percent composition of fatty acids in dorso-lateral fat pads from seven day ovariectomized noninjected control and estradiol injected rats.

Treatment		Hours without food	Animal number	Methyl palmitate (16:0)		Methyl stearate (18:0)		Methyl oleate (18:1)		Methyl linoleate (18:2)	
				AxCF[1]	%T[2]	AxCF	%T	AxCF	%T	AxCF	%T
Noninjected		12	66	8.79	27.63	2.07	6.51	14.05	44.16	6.90	21.69
			77	8.54	25.57	1.71	5.13	13.55	40.57	9.60	28.74
			78	16.34	26.86	3.53	5.80	25.30	41.59	15.66	25.74
			80	12.26	24.33	4.24	8.41	22.06	43.77	11.84	23.49
			83	22.68	24.56	6.70	7.25	39.61	42.89	23.37	25.30
			84	14.08	26.04	3.40	6.29	22.32	41.28	14.27	26.39
			Ave.		25.83		6.56		42.38		25.22
			S.E.[3]		0.53		0.51		0.57		0.99
Amt. of estradiol	Hr. after injection										
100 μg	24	12	163	21.39	25.61	5.25	6.28	35.77	42.82	21.12	25.28
			164	13.93	25.04	2.92	5.25	21.99	39.53	16.79	30.18
2 mg	3	15	4	8.89	23.10	2.17	5.64	16.50	42.88	10.92	28.38
2 mg	24	12	166	12.85	25.67	2.04	4.08	21.44	42.84	13.72	27.41
			167	13.44	23.27	4.09	7.08	23.53	40.74	16.70	28.91
2 mg	72	12	175	12.09	26.49	2.74	6.00	19.42	42.55	11.39	24.96
			176	6.80	22.19	1.82	5.94	13.07	42.65	8.95	29.21

[1] AxCF = Area under GLC curve multiplied by correction factor (Table 4).

[2] %T = Percent AxCF is of total area under curves of all fatty acids present in sample.

[3] S.E. = Standard error.

Table 11. Theoretical percent composition of plasma fatty acids after dilution with various amounts of adipose tissue fatty acids.

Fractional amt. of:						
Plasma fatty acids	Adipose tissue fatty acids	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Archidonic acid
8/8	0/8	16. 61	16. 44	13. 66	19. 48	33. 30
7/8	1/8	17. 65	15. 21	17. 25	20. 20	29. 14
3/4	1/4	18. 91	13. 97	20. 84	20. 92	24. 98
5/8	3/8	20. 07	12. 74	24. 43	21. 63	20. 81
1/2	1/2	21. 21	11. 50	28. 02	22. 35	16. 65
3/8	5/8	22. 37	10. 26	31. 61	23. 07	12. 49
1/4	3/4	23. 53	9. 03	35. 20	23. 79	8. 33
1/8	7/8	24. 68	7. 79	38. 79	24. 50	4. 16
0/8	8/8	25. 83	6. 56	42. 38	25. 22	----

composition of plasma from rats which have been treated 24 hours previously with a single injection of 2 mg estradiol are as follows: palmitic acid plasma-fat mixture, 21. 21%; estradiol-treated, 24. 89%; stearic acid plasma-fat mixture, 11. 50%, estradiol treated, 11. 50%; oleic acid plasma-fat mixture, 28. 02%, estradiol treated, 20. 31%; linoleic acid plasma-fat mixture, 22. 35%, estradiol treated, 26. 53%; archidonic acid plasma-fat mixture, 16. 65%, estradiol treated, 16. 77%.

Concentration of the plasma free fatty acids.

On the basis of the evidence which suggested a rather close relationship between the plasma fatty acid composition of both estradiol treated rats and plasma diluted by the fatty acids present in fat, it was decided to examine the concentration of free fatty acids (FFA) after estradiol treatment. It was assumed that if the fat tissue were releasing fatty acids into the circulatory system this could be detected as an increase in the concentration of FFA.

The concentration of FFA in the plasma at various time intervals following the injection of a single dose of 2 mg estradiol as determined by the procedure of Itaya and Ui (1965) are in Table 12, Figure 6. It was anticipated that an increase would occur at 12 to 24 hours after injection; however, this did not occur. There was, instead, a decrease in FFA of about 85 μeq per liter between 12 and 24 hours after injection of estradiol. The decrease in FFA between the non-injected animal (0 hours after injection) and those exposed to 2 mg estradiol for 72 hours was 125 μeq per liter. At 0, 6, 12, 24, 48 and 72 hours after injection, the respective concentrations (μeq per liter plasma) of FFA were observed: 360 ± 46; 334 ± 31; 380 ± 17; 295 ± 33; 258 ± 33; 235 ± 22.

The values obtained for the concentration of FFA using the Itaya and Ui colorimetric procedure were about one third lower than

Table 12. Plasma free fatty acid concentrations (μeq/liter) determined by the method of Itaya and Ui at various time intervals after injection of a single dose of two milligrams estradiol.

Animal number	Body wt. at castration	Hours after injection					
		0	6	12	24	48	72
287	232	345			255		
288	262	285			270		
289	240	525	345		360		
290	242	210	396				
291	254	450	345				
292	260	345	249				
293	252			435		273	264
294	260			378		---	---
295	282			330		240	255
296	235			381		339	246
297	264			378		180	174
Ave.	253	360	334	380	295	258	235
S. E.[1]	4	46	31	17	33	33	21

[1]Standard error.

the values normally quoted for rat plasma. The method was checked on starved rats and increases in the concentration of FFA of two- to threefold were observed 48 to 72 hours after starvation. It was decided to check these results by using a different procedure. Accordingly, the titrimetric method of Dole and Meinertz (1960) was chosen. In their extraction procedure a ternary mixture was used and it was felt this might account for a more complete extraction of the fatty acids.

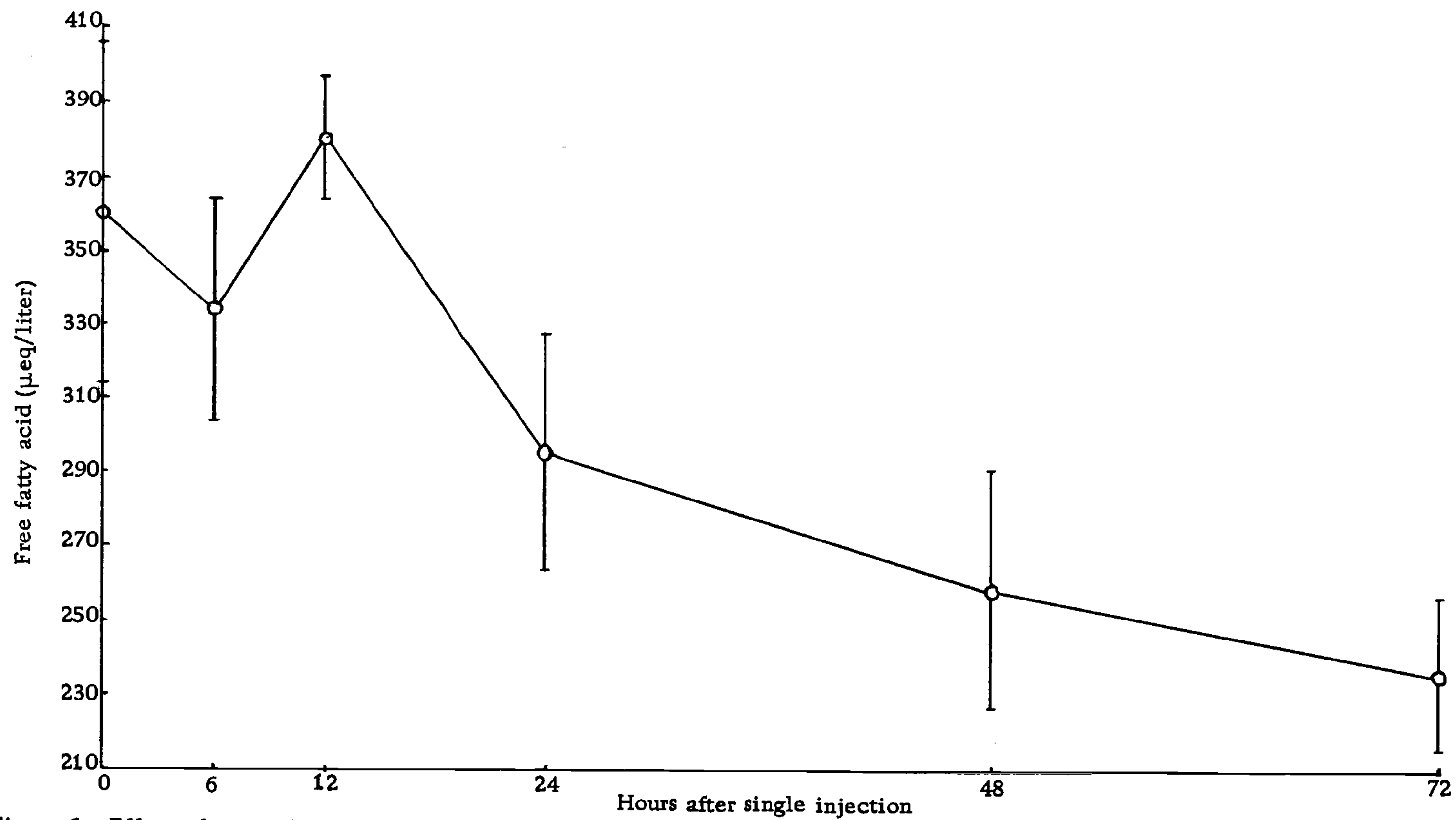

Figure 6. Effect of two milligrams estradiol on plasma free fatty acid concentration using the method of Itaya and Ui.

The concentration of FFA in the plasma at various time intervals following estrogen treatment using the Dole and Meinertz procedure may be found in Table 13, Figure 7. The FFA values obtained with this procedure were quite close to those reported in the literature for rat plasma. In spite of the gross difference in values between the two methods the changes which took place in the concentration of FFA following estrogen treatment were similar for both methods. The decrease which took place with the method of Itaya and Ui between 12 and 24 hours after injection occurred between 6 and 12 hours with the Dole and Meinertz technique. The magnitude of the decrease was about the same, i. e. 100 μeq per liter plasma compared to 85 μeq per liter for the Itaya and Ui method. The decrease in FFA between the non-injected animals (0 hours after injection) and those which received 2 mg estradiol for 72 hours was also similar for the two methods. That is 121 μeq per liter plasma for the Dole and Meinertz method compared to 125 μeq per liter plasma for the Itaya and Ui method. With the Dole and Meinertz method the concentrations in μeq per liter plasma of the FFA at 0, 6, 12, 24, 48 and 72 hours after injection were respectively: 734 ± 98; 759 ± 24; 658 ± 48; 629 ± 76; 565 ± 73; 636 ± 40.

Both the non-injected controls and the propylene glycol controls showed similar decreases in the concentration of FFA. Seven days after castration the concentration of FFA in the non-injected controls

Table 13. Body weight (g) and free fatty acid concentrations (μeq/liter) determined after the method of Dole and Meinertz in noninjected controls, propylene glycol control and estradiol treated seven day ovariectomized rats.

Treat-ment	Animal number	B.W.[1] at cas-tration	FFA[2] at injec-tion	B.W. at injec-tion	FFA 6 hrs. after inj.	B.W. 6 hrs. after inj.	FFA 12 hrs. after inj.	B.W. 12 hrs. after inj.	FFA 24 hrs. after inj.	B.W. 24 hrs. after inj.	FFA 48 hrs. after inj.	B.W. 48 hrs. after inj.	FFA 72 hrs. after inj.	B.W. 72 hrs. after in.	B.W. diff. between C & A[3]
2 mg estradiol	329	253	1034	229					476	229			951	200	-53
	330	240	585	225					532	225			728	214	-26
	331	230	838	222					717	219					
	333	242	891	230					887	224			866	212	-30
	334	230	709	212					531	211			757	205	-25
	Ave. ± S.E.								629 ± 76						
	335	226	846	208	1000	203					831	202	634	200	-26
	336	226	561	211	623	206					557	208	405	200	-26
	337	226	726	225	874	216					730	211			
	338	250	658	243	677	232					505	198	339	230	-20
	340	224	736	215	619	210					659	203			
	324	230	822	244							648	230	443	227	- 3
	325	248	708	245							613	238	580	235	-13
	326	250	684	263							492	236	566	232	-18
	328	238	817	246							557	236	444	232	- 6
	Ave. ± S.E.				759 ± 24										
	341	195	915	205			644	198			844	195			
	342	196	329	196			679	195			476	186	628	186	-10
	344	200	611	210			792	209			864	200	786	194	- 6
	345	194	591	195			495	190			581	176	950	171	-23
	346	190		189			681	185			830	168	678	166	-24
	Ave. ± S.E.						658 ± 48				656 ± 37				-20.6
	347	202	685	202											
	348	202	726	209									567	204	2
	349	193	859	185									506	182	-11
	351	200	681	189									589	197	- 3
	352	208	865	203									662	196	-12
	Ave. ± S.E.		734 ± 98										636 ± 40		- 6.0

Table 13. Continued.

Treatment	Animal number	B.W.[1] at castration	FFA[2] at injection	B.W. at injection	FFA 6 hrs. after inj.	B.W. 6 hrs. after inj.	FFA 12 hrs. after inj.	B.W. 12 hrs. after inj.	FFA 24 hrs. after inj.	B.W. 24 hrs. after inj.	FFA 48 hrs. after inj.	B.W. 48 hrs. after inj.	FFA 72 hrs. after inj.	B.W. 72 hrs. after inj.	B.W. diff. between C & A[3]
Non-injected controls	353	209	829	224									728	235	26
	354	202	779	214									539	230	28
	355	208		218									714	232	24
	356	207	493	216									476	224	17
	357	200	614	204									698	218	18
	358	195	751	200									546	214	19
	377	223		210									677	214	-9
	378	222		230									478	233	11
	379	218		220									534	223	5
	380	214		220									662	222	8
	381	216		216									555	218	2
	282	226		218									502	222	-4
	Ave. ± S.E.		713 ± 61										592 ± 87		12.1
Propylene glycol controls	18	220	541	218									538	227	7
	21	220	690	212									602	228	8
	28	205	699	200									791	208	3
	29	209	774	216									481	228	19
	30	198	746	201									665	206	8
	31	206	797	216									604	230	24
	38	216	937	216									740	231	15
	371	213		208									635	216	3
	372	205		204									540	211	-2
	373	210		209									617	215	10
	375	226		224									435	231	5
	Ave. ± S.E.		741 ± 45										595 ± 92		8.4

[1]B.W. = Body weight.

[2]FFA = Free fatty acid.

[3]C & A = Castration and autopsy.

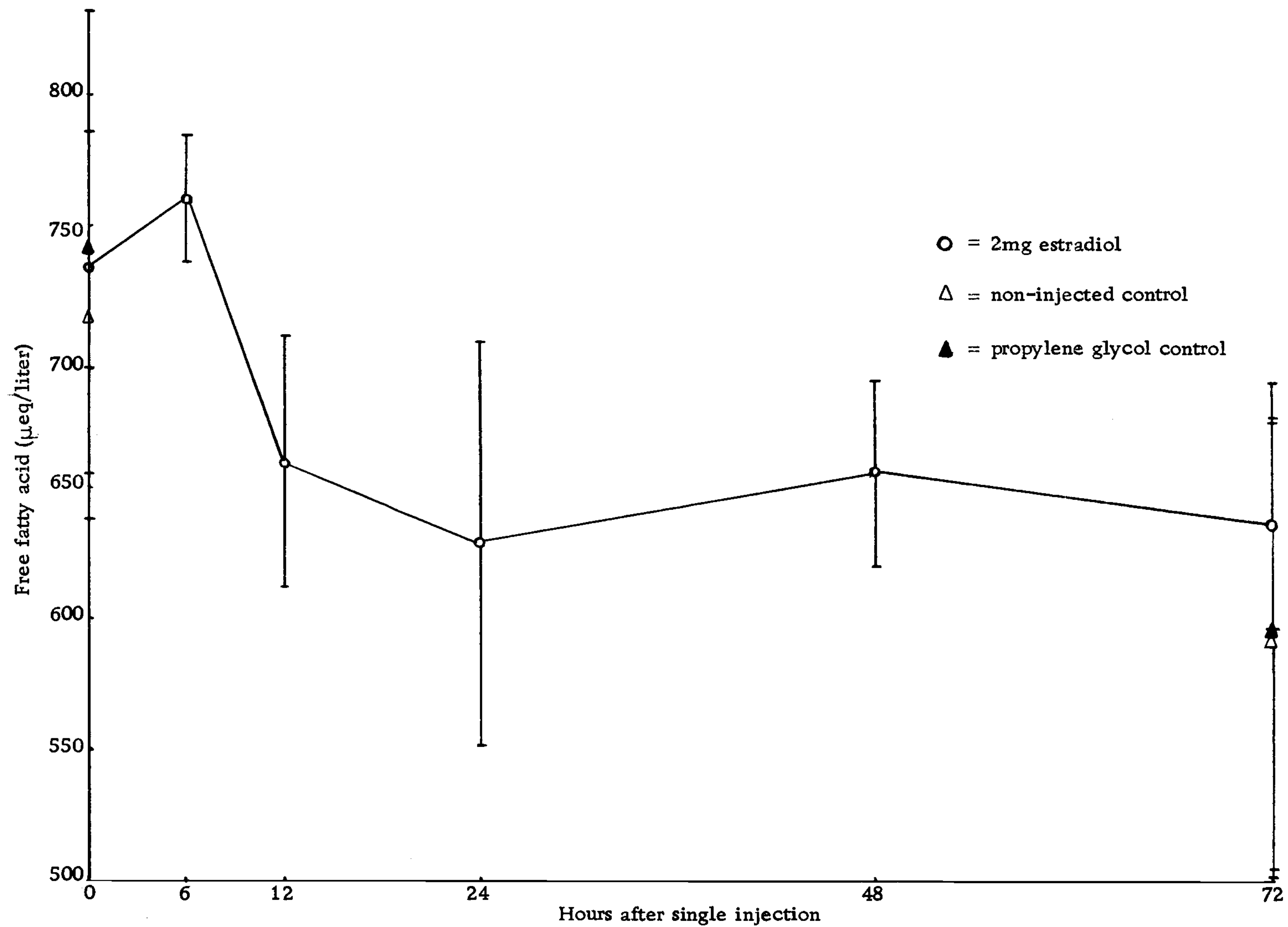

Figure 7. Effect of two milligrams estradiol on plasma free fatty acid concentration using the method of Dole and Meinertz.

was 713 ± 61 μeq per liter plasma whereas 72 hours later (ten days after castration) the concentration of FFA was 592 ± 87 μeq per liter of plasma. The propylene glycol controls changed from 741 ± 45 μeq per liter plasma at seven days after castration to 595 ± 92 μeq per liter plasma 72 hours later (i. e., ten days after castration).

Body weight and dorso-lumbar fat pad weight.

Both body weight and dorso-lumbar fat pad weight data may be found in Tables 14, 15, 16 and 17, and Figure 8. Table 14 includes the data for non-injected and propylene glycol control animals and Tables 15, 16 and 17 include these data for the 0. 1 μg, 100 μg and 2 mg estradiol-treated animals.

The criteria used to assess the effect of estradiol on body weight was the amount of weight gained or lost during the seven to ten day period from castration to autopsy. These weight changes do not accurately reflect true gains or losses in body weight. This is because the animals had access to food prior to taking their body weight at castration, whereas, the body weights taken at autopsy were on animals which were without food for 12 to 18 hours before being killed.

The non-injected control animals showed an apparent decrease in body weight of 5. 4 g after seven days castration. This most likely is a difference due to *ad libitum* castrate body weights as opposed to

Table 14. Body and dorso-lumbar fat pad weights of propylene glycol and noninjected castrate female rats.

Treatment	Hours without food	Animal number	Body wt. at castration (g)	Body wt. at autopsy (g)	Body wt. diff. between C & A[1]	Fat pad wt. (mg)	% Fat pad of body wt. at autopsy $X10^{-1}$
Non-injected 7 days after castration.	12	52	238	192	-46	219	1.14
		54	246	230	-16	559	2.43
		55	238	230	- 8	389	1.69
		56	241	225	-16	515	2.29
		58	240	194	-45	283	1.46
		64	239	239	0	264	1.10
		65	245	224	-21	275	1.23
		66	244	234	-10	443	1.89
		68	242	245	3	380	1.55
		77	234	233	- 1	437	1.87
		78	243	256	13	534	2.09
		79	240	238	- 2	525	2.20
		80	236	223	-13	394	1.77
		81	253	240	-13	383	1.59
		82	230	229	- 1	202	0.88
		83	226	230	4	407	1.77
		84	239	240	1	300	1.25
		85	238	231	- 7	377	1.63
		86	230	246	16	403	1.64
		87	233	232	- 1	494	2.13
		88	231	230	- 1	287	1.25
		139	246	260	14	598	2.30
		140	231	245	14	352	1.44
		141	254	256	2	654	2.55
		142	237	247	10	491	1.99
		Ave.			- 5.4	407	1.72
		S.E.[2]				24	0.09
	15	89	232	223	- 9	521	2.34
	15-1/4	90	240	222	-18	564	2.54
	15-1/2	91	245	225	-20	408	1.81
	16	93	240	240	0	341	1.42
		Ave.			-11.8	458	2.03
		S.E.				51	0.25
	18	94	254	213	-41	160	0.75
	18-1/2	95	234	228	- 7	543	2.38
	18-1/2	96	233	214	-19	255	1.19
	18-3/4	97	231	215	-16	212	0.99
		Ave.			-20.7	293	1.33
		S.E.				86	0.36

[1] C & A = Castration and autopsy.

[2] S.E. = Standard error.

Table 14. Continued.

Treatment	Hours without food	Animal number	Body wt. at castration (g)	Body wt. at autopsy (g)	Body wt. diff. between C & A	Fat pad wt. (mg)	% Fat pad of body wt. at autopsy $X10^{-1}$
Non-injected 10 days after castration.	12	353	209	235	26		
		354	202	230	28		
		355	208	232	24		
		354	207	224	17		
		357	200	218	18		
		358	195	214	19		
		377	223	214	- 9		
		378	222	233	11		
		379	218	223	5		
		380	214	222	8		
		381	216	218	2		
		382	226	222	- 4		
		Ave.			12.1		
Hours after inj.							
0.1 cc propylene glycol 1	13	144	230	245	15	363	1.48
		145	238	248	10	470	1.89
		146	228	242	14	405	1.67
		147	230	253	23	540	2.13
		Ave.			15.5	444	1.79
		S.E.				38	0.14
48	12	244	216	200	-16	173	0.87
		245	222	227	5	259	1.14
		247	221	220	- 1	366	1.66
		248	216	217	1	285	1.31
		Ave.			- 2.9	271	1.24
		S.E.				40	0.16
72	12	239	217	228	1	324	1.42
		240	214	207	- 7	323	1.56
		241	229	235	6	442	1.88
		242	221	227	6	448	1.97
		243	218	235	17	492	2.09
		18	220	227	7		
		21	220	228	8		
		28	205	208	3		
		29	209	228	19		
		30	198	206	8		
		31	206	230	24		
		38	216	231	15		
		371	213	216	3		
		372	205	211	- 2		
		375	226	231	5		
		376	228	226	- 2		
		Ave.			7.1	406 ± 35	1.78 ± 0.13

Table 15. The effect of a single dose of 0.1 μg estradiol at varying time intervals after injection on body and dorso-lumbar fat pad weights of seven day castrate female rats.

Hours after injection	Hours without food	Animal number	Body wt. at castration (g)	Body wt. at autopsy (g)	Body wt. diff. between C & A[1]	Wt. (mg) of left dorso-lumbar fat pad	% Wt. fat pad of body wt. at autopsy X10^{-1}
1	13	98	230	230	0	233	1.01
		99	235	232	- 3	591	2.55
		102	230	230	0	379	1.65
		103	231	225	- 6	510	2.27
		229	243	243	0	683	2.81
		230	229	221	- 8	555	2.51
		231	230	235	5	547	2.33
		232	232	225	- 7	745	3.31
		233	230	222	- 7	436	1.96
		Ave.			- 2.9	520	2.27
		S.E.[2]				52	0.22
3	15	45	230	209	-21	247	1.18
		46	232	238	6	587	2.47
		47	237	226	-11	279	1.23
		48	246	240	- 6	646	2.69
		49	230	209	-21	434	2.07
		50	234	236	2	470	1.99
		51	240	238	- 2	345	1.45
		Ave.			- 7.6	429	1.87
		S.E.				57	0.23
4	16	57	237	216	-21	387	1.79
		59	240	236	- 4	314	1.33
		60	252	237	-15	344	1.45
		61	245	236	- 9	655	2.77
		62	253	250	- 3	405	1.62
		63	230	220	-10	234	1.06
		Ave.			-10.3	390	1.67
		S.E.				58	0.24
12	12	219	234	223	-11	202	0.91
		220	238	228	-10	735	3.22
		221	230	220	-10	383	1.74
		222	225	226	1	368	1.63
		223	225	217	- 8	386	1.78
		Ave.			- 7.6	414	1.86
		S.E.				87	0.38
24	12	188	245	268	23	505	1.88
		189	231	252	21	372	1.48
		190	250	275	25	408	1.48
		191	236	261	25	307	1.18
		192	226	245	19	423	1.73
		193	224	252	28	411	1.63
		Ave.			23.5	404	1.56
		S.E.				26	0.03

Table 15. Continued.

Hours after injection	Hours without food	Animal number	Body wt. at castration (g)	Body wt. at autopsy (g)	Body wt. diff. between C & A[1]	Wt. (mg) of left dorso-lumbar fat pad	% Wt. fat pad of body wt. at autopsy X10^{-1}
48	12	131	230	240	10	525	2.19
		132	256	255	- 1	466	1.83
		135	248	254	6	445	1.75
		136	238	245	7	513	2.09
		137	228	221	- 7	361	1.63
		249	213	220	7	343	1.56
		250	216	227	11	264	1.16
		251	221	225	4	347	1.54
		252	215	230	15	460	2.00
		253	219	224	5	225	1.00
		Ave.			5.7	395	1.67
		S.E.[2]				32	0.24
72	12	181	239	250	11	387	1.55
		182	237	252	15	493	1.96
		183	230	240	10	419	1.75
		184	238	256	18	533	2.08
		185	241	249	8	382	1.53
		187	229	242	13	411	1.70
		Ave.			13	437	1.76
		S.E.				79	0.08

[1] Castration and autopsy.

[2] Standard error.

Table 16. The effect of single dose of 100 μg estradiol at varying time intervals after injection on body and dorso-lumbar fat pad weights of seven day castrate female rats.

Hours after injection	Hours without food	Animal number	Body wt. at castration (g)	Body wt. at autopsy (g)	Body wt. diff. between C & A[1]	Wt. (mg) of left dorso-lumbar fat pad	% Wt. fat pad of body wt. at autopsy X10^{-1}
1	13	224	230	230	0	487	2.12
		225	230	218	-12	519	2.38
		226	270	260	-10	490	1.88
		227	230	230	0	405	1.76
		228	249	238	-11	534	2.24
		Ave.			- 6.6	487	2.08
		S.E.[2]				22	0.11
3	15	20	240	232	- 8		
		22	240	233	- 7		
		24	242	220	-22		
		25	240	223	-17		
		26	244	230	-14		
		Ave.			-13.6		
6	18	254	218	214	- 4	253	1.18
		255	216	208	- 8	345	1.66
		256	230	222	- 8	378	1.70
		257	214	205	- 9	242	1.18
		258	215	210	- 5	213	1.01
		Ave.			- 6.8	286	1.35
		S.E.				32	0.14
12	12	214	230	200	-30	121	0.61
		215	232	215	-17	421	1.96
		216	254	242	-12	300	1.24
		217	237	215	-22	426	1.98
		218	230	212	-18	285	1.34
		Ave.			-19.8	311	1.43
		S.E.				56	0.25
24	12	159	232	245	13	453	1.85
		160	234	242	8	252	1.04
		161	228	231	3	407	1.76
		162	238	243	5	648	2.67
		163	240	249	9	564	2.27
		164	238	252	14	560	2.22
		Ave.			7.5	481	1.97
		S.E.				58	0.23

Table 16. Continued.

Hours after injection	Hours without food	Animal number	Body wt. at castration (g)	Body wt. at autopsy (g)	Body wt. diff. between C & A[1]	Wt. (mg) of left dorso-lumbar fat pad	% Wt. fat pad of body wt. at autopsy $X10^{-1}$
48	12	153	238	250	20	658	2.63
		154	243	255	12	797	3.12
		155	245	245	0	532	2.17
		156	230	235	5	370	1.57
		157	240	251	11	635	2.53
		158	248	251	3	626	2.49
		Ave.			8.3	603	2.42
		S.E.[2]				58	0.21
72	12	234	230	234	14	391	1.67
		235	246	256	10	586	2.29
		236	223	230	7	556	2.42
		237	226	218	- 8	280	1.28
		238	228	234	6	206	0.88
		Ave.			9.0	404	1.71
		S.E.				74	0.29

[1] Castration and autopsy.

[2] Standard error.

Table 17. The effect of a single dose of two milligrams estradiol at varying time intervals after injection on body and dorso-lumbar fat pad weights of seven day castrate female rats.

Hours after injection	Hours without food	Animal number	Body wt. at castration (g)	Body wt. at autopsy (g)	Body wt. diff. between C & A[1]	Wt. (mg) of left dorso-lumbar fat pad	% Wt. fat pad of body wt. at autopsy $X10^{-1}$
1	13	38	253	238	-15	538	2.26
		39	240	215	-25	393	1.83
		41	254	228	-26	290	1.27
		42	242	230	-12	353	1.53
		44	250	235	-15	416	1.77
		Ave.			-18.6	398	1.73
		S.E.[2]				41	0.16
3	15	2	255	248	- 7		
		3	244	232	-12		
		4	253	215	-38		
		17	242	230	-12		
		18	247	225	-22		
		19	248	234	-14		
		Ave.			-17.5		
6	18	6	245	241	- 4		
		7	258	250	- 8		
		72	245	245	1	653	2.65
		73	241	232	- 9	520	2.24
		74	243	228	-15	458	2.01
		75	253	250	- 3	635	2.54
		76	246	234	-12	604	2.58
		Ave.			- 7.1	574	2.40
		S.E.				37	0.12
12	12	122	237	234	- 3	490	2.09
		124	241	245	4	370	1.51
		125	243	248	5	432	1.74
		126	232	227	- 5	276	1.21
		127	236	238	2	333	1.40
		128	238	238	0	537	2.26
		129	237	240	3	477	1.99
		130	254	246	- 8	442	1.80
		Ave.			- 0.3	420	1.75
		S.E.				31	0.13

Table 17. Continued.

Hours after injection	Hours without food	Animal number	Body wt. at castration (g)	Body wt. at autopsy (g)	Body wt. diff. between C & A[1]	Wt. (mg) of left dorso-lumbar fat pad	% Wt. fat pad of body wt. at autopsy $X10^{-1}$
24	12	110	247	244	- 3	474	1.94
		111	238	252	14	382	1.52
		112	237	232	- 5	381	1.64
		113	231	231	0	292	1.26
		114	242	250	8	305	1.22
		115	247	260	13	312	1.20
		165	236	240	4	387	1.61
		166	236	242	6	568	2.35
		167	232	246	14	522	2.12
		168	235	248	13	469	1.89
		169	236	250	14	173	0.69
		170	239	257	18	479	1.86
		Ave.			8.0	395	1.61
		S.E.[2]				33	0.13
48	12	116	240	230	-10	277	1.20
		117	239	226	-13	520	2.30
		118	242	231	-11	238	1.03
		119	236	231	- 5	628	2.72
		120	246	244	- 2	306	1.25
		121	238	236	- 2	482	2.04
		171	254	259	5	650	2.51
		178	238	238	0	345	1.45
		179	228	232	4	451	1.94
		180	240	235	- 5	353	1.50
		Ave.			- 3.9	425	1.79
		S.E.				46	0.19
72	12	171	240	236	- 4	351	1.49
		173	241	249	8	602	2.42
		174	243	242	- 1	379	1.57
		175	255	253	0	568	2.24
		176	242	235	- 7	293	1.25
		348	202	204	2		
		349	193	182	-11		
		351	200	197	- 3		
		352	208	196	-12		
		Ave.			- 3.1	439	1.79
		S.E.				100	0.23

[1] Castration and autopsy.

[2] Standard error.

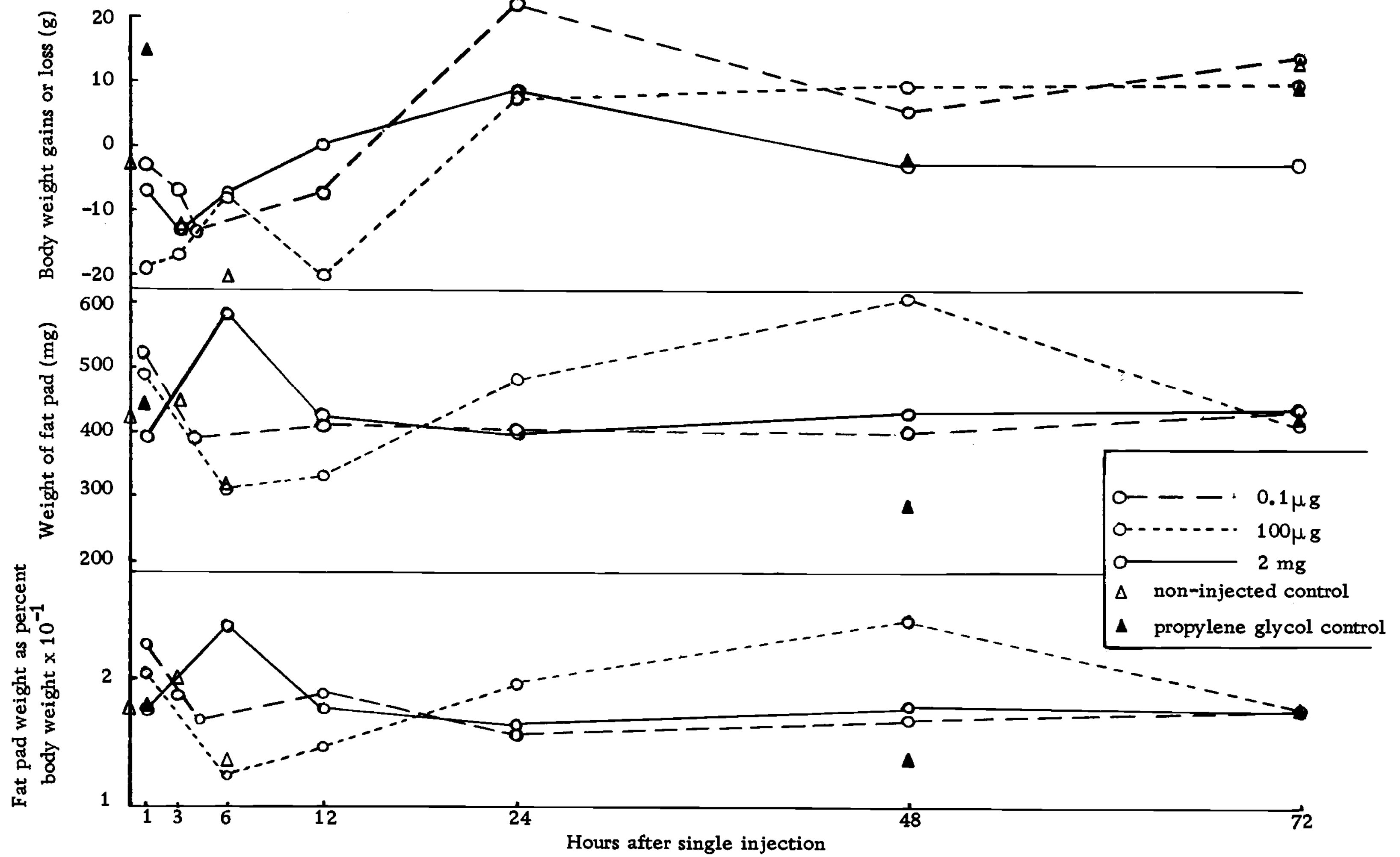

Figure 8. Body and dorso-lumbar fat pad weights following estradiol treatment.

12-hour post absorptive autopsy body weights. Non-injected control animals which were without food for 18 hours prior to autopsy exhibited a 20. 7 g decrease in body weight. By ten days after castration (comparable to 72 hours after injection for the treated animals) the animals increased in body weight by 12. 1 g. The propylene glycol control animals were similar to the non-injected control animals with one exception. The one hour propylene glycol-injected animals showed an average increase in body weight of 15. 5 g. This increase, however, is based on only four animals.

The changes in body weight of animals treated with the lowest dosage of estradiol (0. 1 μg) were quite similar to the changes which occurred in the control animals. After 13 hours without food (one hour after injection) they exhibited a 2. 9 g decrease in body weight. This compares favorably with the 12 hour post-absorbtive non-injected controls which had a 5. 4 g decrease in body weight. After 16 hours without food (four hours after injection) their body weight had decreased by an average of 10. 3 g as compared to a decrease of 11. 8 g in the non-injected animals. By the tenth day after castration (72 hours after injection) the 0. 1 μg estradiol-treated animals had gained 13 g as compared to 12. 1 g for the non-injected controls and 7. 1 g for the propylene glycol controls.

At the two higher dosages of estradiol the decrease in body weight after 18 hours without food was not as great as that in the control animals. Whereas the control animals lost an average of 20. 7 g after 18 hours without food, the animals treated with both

100 μg and 2 mg estradiol decreased in body weight by only 6.8 g and 7.1 g respectively. By the tenth day after castration (72 hours after injection) there was a difference between the animals treated with 2 mg estradiol and both the control and the animals treated with the lower doses of estradiol. By this time, whereas both the control and 0.1 μg and 100 μg estradiol-treated animals gained weight (i.e. non-injected control, 12.1 ± g; propylene glycol control, 7.1 ± g; 0.1 μg estradiol, 13 ± g; and 100 μg estradiol, 9.0 ± g) the 2 mg estradiol-treated animals lost weight (i.e., 3.1 g).

As may be seen in Figure 8, the results of the weight of dorso-lumbar fat pads have been plotted both as the absolute weight of the organ and also as a percent of the total body weight. There are only very minor differences in the data when plotted in the two different ways. The fluctuations which occur in the fat pad weights at various intervals after treatment with estradiol are slight and there does not appear to be any difference between the control animals and those treated with estradiol. Further, no difference exists in the fat pad weights of animals treated with the three dosages of estradiol used for the duration of time (three days) studied.

Hypophysectomy Experiment

The effect of hypophysectomy on the composition of fatty acids in the blood plasma is similar to the effects brought about by large

dosages (2 mg) of estradiol. After 24 hours hypophysectomy the percent composition of the five major fatty acids, palmitic, stearic, oleic, linoleic, and arachidonic was 23.65 ± 1.76, 12.46 ± 0.40, 17.38 ± 0.10, 25.69 ± 0.78 and 20.81 ± 2.47 respectively (Table 18). The percent composition of these acids 24 hours after injection of 2 mg estradiol was 23.69 ± 0.73, 12.81 ± 0.54, 20.58 ± 0.76, 26.01 ± 0.56 and 16.91 ± 1.08. Although there are only two animals in this study which were hypophysectomized for 48 hours prior to autopsy, they both had fatty acid compositions quite similar to those which had been hypophysectomized for 24 hours. The average percent fatty acid composition for these two animals was 22.44, 12.74, 17.93, 28.11 and 18.77. This similarity between the 24 and 48 hour hypophysectomized animals is found also in the animals treated for 24 and 48 hours with 2 mg estradiol.

In an attempt to better elucidate the relationship between the effects of hypophysectomy and the effects of estradiol on the fatty acids in the blood plasma, several animals were both hypophysectomized and given an injection of 2 mg estradiol. The results of this treatment may be found in Table 18. One might expect that by combining these two treatments an additive effect on the plasma fatty acids would result. This, however, did not occur. The average composition for two-day hypophysectomized animals given 2 mg estradiol 24 hours prior to autopsy was palmitic 25.44 ± 1.46%,

Table 18. Percent composition of plasma fatty acids in ovariectomized, hypophysectomized rats noninjected and injected with either estradiol or adrenocorticotropic hormone (ACTH).

Treatment	Animal number	Methyl palmitate(16:0) AxCF	Methyl palmitate(16:0) %T	Methyl stearate(18:0) AxCF	Methyl stearate(18:0) %T	Methyl oleate(18:1) AxCF	Methyl oleate(18:1) %T	Methyl linoleate(18:2) AxCF	Methyl linoleate(18:2) %T	Methyl arachidonate(20:4) AxCF	Methyl arachidonate(20:4) %T	Hypophysectomy	Animal source
Ovariectomized 5 days	212	9.47	27.71	4.37	12.79	6.71	19.64	9.01	26.37	4.61	13.49	I[1]	Packard
then hypophysectomized	208	5.02	20.13	2.97	11.91	4.18	16.76	6.56	26.30	6.21	24.90	C	Packard
for 1 day.	209	12.24	25.45	6.36	13.23	8.44	17.55	11.61	24.15	9.43	19.62	C	Packard
	210	13.68	25.37	6.60	12.24	9.61	17.83	14.36	26.63	9.66	17.92	C	Packard
Ave. of complete animals ± S.E.[2]			23.65 ± 1.76		12.46 ± 0.40		17.38 ± 0.32		25.69 ± 0.78		20.81 ± 2.10		
	283	17.04	21.35	12.02	15.06	12.86	16.11	18.07	22.64	19.82	24.83	I	Berkeley
	285	11.87	19.71	8.35	13.86	8.32	13.82	14.05	23.33	17.63	29.27	C	Berkeley
	279	12.11	20.29	9.42	15.78	10.41	17.44	12.32	20.64	15.42	25.84	C	Berkeley
Ovariectomized 6 days	298	19.54	22.55	10.85	12.52	16.05	18.52	24.13	27.84	16.09	18.57	C	Berkeley
then hypophysectomized	303	11.98	22.34	6.95	12.96	9.30	17.34	15.22	28.38	10.17	18.97	C	Berkeley
for 2 days.													
Ovariectomized 6 days	205	11.12	27.15	4.68	11.43	6.48	15.82	10.47	25.57	8.20	20.02	C	Packard
then hypophysectomized	201	7.98	22.54	5.63	15.90	5.60	15.81	10.29	29.07	5.90	16.66	PI	Packard
for 2 days. 2mg estradiol	203	7.50	26.64	3.43	12.18	5.02	17.83	7.87	27.96	4.33	15.38	C	Packard
injected 24 hrs. prior to autopsy.													
Ave. of above 3 animals ± S.E.			25.44 ± 1.46		13.17 ± 1.42		16.49 ± 0.67		27.53 ± 1.03		17.35 ± 1.38		
Ovariectomized 6 days	260	8.98	20.70	9.69	22.34	6.38	14.71	8.54	19.69	9.78	22.55	C	Packard
then hypophysectomized	261	10.73	21.07	9.02	17.71	6.99	13.72	10.43	20.48	13.76	27.02	C	Packard
and injected with 10 IU ACTH for 24 hrs.													
Ovariectomized 6 days	259	14.52	20.86	11.27	16.19	10.75	15.45	15.67	22.52	17.38	24.97	C	Packard
then hypophysectomized	262	9.26	19.22	10.38	21.54	7.71	16.00	11.00	22.83	9.83	20.40	C	Packard
and injected with 20 IU ACTH for 24 hrs.													

[1] I = Incomplete; C = Complete; PI = Possibly incomplete.

[2] Standard error.

stearic 13. 17 ± 1. 42%, oleic 16. 49 ± 0. 67%, linoleic 27. 53 ± 1. 03% and arachidonic 17. 35 ± 1. 38%. Clearly, the percent fatty acid composition when the treatments were combined was similar to that of the two treatments when given separately.

Adrenocorticotropin (ACTH) has been implicated in the mobilization of fatty acids (Rudman, Brown and Malkin, 1963). It is for this reason that an attempt was made to cause the plasma fatty acid composition in hypophysectomized animals to return to the composition found in control animals by injecting ACTH. At the dosages studied (10 and 20 IU) for a 24 hour interval between injection and autopsy, ACTH appears to be ineffective in preventing the alteration of the plasma fatty acid composition which occurs after hypophysectomy (Table 18). There is no difference between the two dosages of ACTH studied and although the number of animals is quite small (two animals to each dosage) the percent composition of stearic acid is much greater than in non-injected castrate hypophysectomized rats, and the oleic acid appears to be considerably lower.

DISCUSSION

Estrogens participate in the process of reproduction by bringing about various behavioral and morphological changes. They are produced as a result of increasing levels of gonadotropins released from the pituitary. Many of the details of how estrogens are important in integrating the later stages of reproduction such as bringing the two sexes together at the time of ovulation and assuring that the uterus is adequately prepared for implantation of the blastocyst are still unknown.

With the increased activity and diverse morphological changes which occur during the reproductive cycle one can expect that the neural and hormonal control over the metabolism of the various animal organs is complex. Interest in the metabolism of lipids in the past ten years has increased, but there has been little investigation of the relationship between the recently discovered hormonally induced changes in fatty acid metabolism and reproduction.

One of the most pronounced effects of estrogens on the metabolism of lipids has been known since the early 1950's. Adlersberg, in 1957, summarized numerous reports which indicated that the administration of estrogens causes an increase in serum phosphatides, and a lowering of cholesterol and the cholesterol to phosphatide ratio. One could speculate that the increase in phosphatides brought about

by estrogens is a relic adaptive mechanism perhaps acquired by fish and concerned with yolk deposition.

In the present study changes in the relative amounts of the fatty acids present in the blood plasma have been observed after injection of estradiol. Similar changes in the plasma fatty acids have been observed following hypophysectomy. The basic similarity between the effects of these two treatments suggests that they are related and is of value in interrupting the mechanism of this estrogenic effect.

In an attempt to understand the relationship between the effect of estrogen treatment and hypophysectomy, Table 11 was constructed to show the theoretical composition of fatty acids in plasma after dilution with various amounts of the fatty acid composition found in adipose tissue (i. e. 1/8 plasma fatty acids-7/8 adipose tissue fatty acids). When the plasma fatty acid composition of estradiol injected and hypophysectomized animals is compared to these theoretical values, a close correlation may be seen between both sets of experimental values and the theoretical values for plasma fatty acids which have been diluted by 50% with adipose tissue fatty acids. It is suggested that because of the difference between the fatty acid composition of adipose tissue (Table 10) and blood (Table 9) the release of fatty acids from adipose tissue into the blood brings about the altered composition of plasma fatty acids observed in this study following

both estrogen treatment and hypophysectomy.

Only in the domestic fowl (Heald and Rookledge, 1964) has there been any evidence that estrogens increase the level of FFA by acting directly on adipose tissue. McKerns and Clynes (1961) have suggested that estrogens may act directly on adipose tissue by inhibiting glucose-6-phosphate dehydrogenase (G-6-PD), but the mechanism which they propose whereby this enzyme inhibition increases the synthesis of fatty acids is an indirect one. They suggest that the estrogen inhibition of G-6-PD which also occurs in the adrenal cortex brings about a quantitative shift in the balance of adrenal and pituitary hormones and that the increase in pituitary peptides stimulates fatty acid synthesis and release.

Rudman (1963) points out that in the intact animal the mobilization of fatty acids occurs within 30 minutes after subcutaneous injection of adipokinetic peptides and persists for 2 to 12 hours. Considering the lack of evidence in mammals for a direct adipokinetic effect of estrogens and the fact that in this study the fatty acid composition was not affected until 12 hours after subcutaneous injection of estradiol, it is proposed that estradiol is acting indirectly through the stimulation or inhibition of some other endocrine gland.

It is suggested that increased levels of estradiol inhibit the production of adrenocortical steroids secreted by the adrenal gland, thereby inducing greater amounts of adrenocorticotropin (ACTH) to

be released from the pituitary gland. It has been known for some time that estrogen administration is followed by a nonfunctional enlargement of the adrenal gland (Glasser and Leathem, 1955; Vogt, 1955, 1957; Kitay, 1963). This suggests that there is an increased release of ACTH from the pituitary gland. ACTH, when tested in rats and mice or upon isolated adipose tissue from these species, is the most potent lipolytic agent derived from the pituitary gland (Rosenberg, 1953; Astwood, 1955). The stimulus to the fatty tissue, to release fatty acids after estradiol treatment in the intact rat, probably arises from a primary inhibition of the adrenal gland with a secondary "feedback" stimulus from the pituitary gland.

Certainly the possibility of a direct action of estrogens on the fat cell should not be excluded when considering the mechanism where by estradiol alters the plasma fatty acid composition. There are three commonly employed indicators of an animal's response to the adipokinetic action of a hormone. They are: 1. the increase in circulating FFA concentration within the first hour after injection (Girolamo, *et al.*, 1961; Raben, *et al.*, 1961; Bogdonoff, *et al.*, 1961; Friesen, Barrett and Astwood, 1962); 2. increase in hepatic esterified lipids three to six hours after injection (Payne, 1949; Szego and White, 1949; Levin and Farber, 1952; Rosenberg, 1953); and 3. increase in circulating esterified lipids 12-24 hours after injection

(Rudman, Seidman and Reid, 1960; Shafrir, Sussman and Steinberg, 1960; Friesen, Barrett and Astwood, 1962).

It is possible that an animal in a condition of reduced caloric intake could have an increased turn-over rate for the plasma FFA in the absence of an increase in the level of plasma FFA. This, it is suggested, would be due to an increased release of FFA from adipose tissue, but at the same time an increased utilization by the caloricly deficient tissues. Under these conditions no adipokenetic activity for the compound in question would be detected if one of the above three methods were employed.

McCalla, et al. (1957) have shown that when FFA are injected into rats more radioactively labeled carbon is recovered in the expired carbon dioxide from caloricly deficient (fasting) recipients than from carbohydrate fed animals. This would indicate that the oxidation of FFA is "spared" in the fed state and that its oxidation is relied on to a greater extent in the caloricly deficient animal.

The evidence accumulated in this study suggests that estradiol may bring about increased mobilization of FFA in the absence of any increase in the level of plasma FFA. It has been observed that the following conditions are associated with estradiol injection: 1. increased turn-over of plasma fatty acid as suggested by the altered plasma fatty acid composition (Table 8) and presumably due to release of fatty acids from adipose tissue (Table 11); 2. absence of an

increase in the level of circulating FFA (Tables 12 and 13); and 3. reduced caloric intake as evidenced by a decrease in body weight (Table 17). The decrease in body weight immediately following estrogen treatment has been observed before and is suggested by Marques, Mundt and Correa (1964) to be due to reduced food intake. If these assumptions are correct then gas chromatographic analysis of plasma fatty acid changes would appear to be a less falable indication of fatty acid mobilization than the methods most commonly employed.

The mechanism whereby the plasma fatty acid composition is altered in rats following hypophysectomy is believed to be related to the stress brought about by surgery. It has been known since the pioneer work of Cannon, _et al._ (1931) that the sympathetic nervous system has an important role in supplying the emergency needs of the organism. Activity of the sympathetic nervous system results in the mobilization of extra energy fuel in the form of fatty acids (Dole, 1956; Gordon and Cherkes, 1956). Havel and Goldfien (1959) suggest that the tonic effect of the sympathetic nervous system on lipid metabolism may be brought about in two ways. First, in responsive species it acts by regulating the level of circulating norepinephrin in the blood, and second, in all species by regulating the blood flow so as to give access for various adipokinetic hormones to the fat cell. The catecholamines are believed to activate an adipose tissue lipase

which results in an accelerated production of FFA (Schotz and Page, 1960; Bjorntorp and Furman, 1962).

The belief that the altered plasma fatty acid composition following hypophysectomy is the result of the activity of the sympathetic nervous system is based largely on the close correlation between the plasma fatty acid composition in the complete and incompletely hypophysectomized animals (Table 18). In addition to the action of catecholamines from the sympathetic nervous system Mezey, Foley and Altszuler (1961) have demonstrated that fat from hypophysectomized rats takes up less glucose than fat from normal rats. This would also cause a greater release of fatty acids from adipose tissue (Annison, 1964) and would alter the composition of the plasma fatty acids.

The action of the catecholamines in the case of the hypophysectomized animals and ACTH in the case of the estradiol-treated animals would generally account for the compositional changes observed in the plasma fatty acids in this study. There are, however, slight differences between the plasma fatty acid composition which resulted from both estrogen treatment and hypophysectomy and that composition which would be expected if the adipose tissue fatty acids were released completely randomly.

Meinertz (1963) has described a selective retention of oleic acid by adipose tissue incubated with epinephrine. That the lipolytic enzyme was hydrolyzing oleic acid to a lesser extent than the other

fatty acids present was confirmed by detecting the accumulation of oleic acid in adipose tissue diglycerides. Furthermore, during fasting and diabetes a progressive fall in the glyceride concentration of palmitic and palmitoleic acids and a progressive rise in the stearic, oleic and linoleic acids has been observed (Jeanrenaud, 1961). This suggests that the specificity of adipose tissue lipases for certain ester linkages is of importance in determining the pattern of FFA which are mobilized.

Certainly other factors may be important in determining the pattern of FFA mobilized. Hollenberg and Douglas (1962) suggest that of equal importance may be an alteration in the rate of synthesis and oxidation of fatty acids. Miller, et al. (1962) reported differences in the rates of release of individual fatty acids by subcutaneous epigastric adipose tissue in the dog and non-uniform uptake of fatty acids by different anatomical sites and Rothlin and Bing (1961) reported a preferential extraction of oleic acid from arterial blood by human and dog heart.

The significance of the estrogenic effects reported in this study may be related to the metabolic alterations which accompany sexual behavior. It is suggested that the altered plasma fatty acid composition in the absence of any increase in FFA reflects an increased turnover rate for the plasma fatty acids. This may be a mechanism for meeting the increased energy requirements which would accompany

the increased activity associated with estrus behavior. The duration of the treatments chosen in this study were intended to be comparable to the length of the estrus cycle in the rat. A more direct approach using radioactively labeled isotopes of fatty acids will be needed before any definitive statement may be made concerning the effect of estrogens on the turnover rate of plasma fatty acids.

There have been no detailed studies on the relationship of estrogens to lipid metabolism in those more primitive chordates which produce a telolecithal egg. With the apparent widespread distribution of estrogens in the animal kingdom (Botticelli, *et al.*, 1960, 1961) and the high concentration of lipid material present in yolk it does not seem unreasonable to believe that estrogens may have become involved in the transport of lipids. The findings of Heald and Rookledge (1964) that in the domestic fowl estrogens act directly on adipose tissue causing the release of FFA would support this view. Finally, with the evolution of the homeothermic condition in mammals and consequently the need for more sophisticated regulatory mechanisms it would seem that estrogens would continue to be implicated in the turnover rate of plasma fatty acids.

SUMMARY

Much interest has been generated, since the first review of the physiology of adipose tissue (Wertheimer and Shapiro, 1948), about the importance of lipid metabolism in meeting the caloric demand during conditions other than starvation. Interest has been centered on adipokinetic hormones which bring about the release of FFA from adipose tissue. The object of this study has been to examine the effect of estradiol-17β on FFA metabolism.

Female Sprague-Dawley rats in most cases weighing 220-240 grams were ovariectomized and seven days later were either treated as controls or received a single injection of either 0. 1 μg, 100 μg or 2 mg of estradiol-17β dissolved in 0. 1 cc propylene glycol. These rats were autopsied either 1, 3, 6, 24, 48 or 72 hours after injection. Some of the ovariectomized animals were also hypophysectomized on either the fifth or sixth day after castration and of these some were injected with a single dose of either 2 mg estradiol or 10 or 20 IU of ACTH.

Chemical techniques employed were the determination of fatty acid composition by gas-liquid chromatography and colorimetric and titrametric determination of fatty acid concentrations. Techniques used in the identification of fatty acids which are present in rat blood plasma included comparing their relative retention times to those

of earlier workers (Farquhar, _et al._, 1959) who had chemically identified them. The fatty acids wере also separated by TLC and compared to known standards. Finally, comparisons were made between the retention times of plasma fatty acids separated by TLC and known standards of fatty acids similarly separated by TLC.

The colorimetric technique of Itaya and Ui (1965) for determining FFA concentrations in blood plasma was tried; however, the values obtained with this method were considerably lower than those reported in the literature. A second titrametric method (Dole and Meinertz, 1960) was used and FFA values similar to those reported by other investigators were obtained. In addition to the chemical techniques employed, both body and dorso-lumbar fat pad weights were recorded.

It was determined that estradiol at all levels tested brings about a change in the composition of the fatty acids present in the plasma 24 hours after injection. This change is more pronounced with increased levels of the hormone. At the lowest dosage tested (0. 1 μg) only slight changes appear at 24 hours after injection. With 100 μg estradiol the fatty acid composition has started to change by 12 hours after injection; it is complete by 24 hours and then it returns to the control level by 72 hours. With 2 mg estradiol the changes are the same as with 100 μg except that by 72 hours there is no indication of a return to the control level of plasma fatty acids.

At 24 hours after injection of 2 mg estradiol palmitic acid changes from 16.61 ± 0.56% in the non-injected controls to 23.69 ± 0.74% in the treated. Stearic acid changes from 16.44 ± 0.43% to 12.81 ± 0.54%, oleic acid changes from 13.66 ± 0.39% to 20.58 ± 0.76%, linoleic acid changes from 19.48 ± 0.51% to 26.01 ± 0.56% and arachidonic acid changes from 33.30 ± 0.92% to 16.91 ± 1.08%.

The fatty acid composition of adipose tissue was determined for a limited number of animals. There were no changes after estradiol treatment at either of the dosages which were checked.

The only change which was noted in the plasma concentration of FFA was a slight decrease over the three day period which was checked. This was true for both methods which were used and occurred in both the treated and control animals. It is believed that this was the result of the technique (heart puncture) used to obtain the blood sample.

The weights of the dorso-lumbar fat pads were not affected by the estradiol treatment, but a slight decrease was seen in the body weight of those animals which received 2 mg estradiol. In the hypophysectomized, and hypophysectomized and estradiol or ACTH treated animals the composition of plasma fatty acids was similar to those noted after estradiol treatment.

On the basis of a close similarity between the altered plasma fatty acid composition following estradiol treatment and

hypophysectomy and theoretically calculated plasma fatty acid compositions which would result from dilution with the fatty acids present in adipose tissue, it has been suggested that these treatments alter the plasma fatty acid composition by causing the release of fatty acids from adipose tissue. The mechanism suggested for this estrogenic effect is a primary inhibition of the adrenal gland with a concomitant secondary feedback stimulus from the pituitary gland (ACTH) to the fatty tissue to release fatty acids. The absence of any increase in FFA is believed to be due to increased utilization by the tissues. Marques, Mundt and Correa (1964) have observed reduced food intake following estrogen treatment and, further, the decrease in body weight observed in this study suggests that the tissues may be subjected to a deficiency in caloric intake.

The mechanism whereby the plasma fatty acid composition is altered in the hypophysectomized animals is thought to be related to the stress associated with surgery. Presumable increased levels of catecholeamines released in response to increased tonic activity of the sympathetic nervous system are responsible for activation of an adipose tissue lipase.

The significance of the estrogenic effect reported in this study is believed to be related to the increased activity associated with estrous behavior. The altered plasma fatty acid composition is

believed to reflect increases in the turnover rate of FFA. These increased amounts of FFA are believed to be the energy source which is utilized.

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