LIPID METABOLISM IN FEMALE *ASCARIS LUMBRICOIDES* DURING STARVATION

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LUMBRICOIDES DURING STARVATION

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ABREVIATIONS USED IN THESIS

NAD - nicotinamide adenine dinucleotide

NADH - reduced nicotinamide adenine dinucleotide

ATP - adenosine triphosphate

CoA - coenzyme A

μMole- micromole

mM - millimolar

gm - gram

<u>g</u> - gravity

mg% - milligrams per 100 gm. of body weight

C - centigrade

CHAPTER 1

INTRODUCTION

Ascaris lumbricoides is a large parasitic nematode found in the small intestine of hogs and is of universal distribution. A physiologic variety of this parasite also infects man and is a serious health problem in some parts of the world. Young pigs are more susceptible to infection by Ascaris than mature hogs and it has been found that experimentally infected animals weighed at slaughter 45 to 54 pounds less than worm-free litter mates (139). In the United States it is estimated that 7 of every 10 pigs and 1 of every 3 hogs of breeding age are infected with this worm (139). It is estimated that economic loss due to unthriftiness and death of young pigs costs hog raisers in the United States 50 million dollars each year (139). Proper herd management can reduce the economic loss due to Ascaris by reducing the incidence of infection. Infection is difficult to prevent entirely, however, since the eggs in the soil remain infective for several years. It is therefore desirable to use medication to reduce loss from established infections.

The basis of treatment of many infections is interference with the metabolism of the disease organism without interfering with the metabolism of the host. Such methods of approach should be based on a thorough knowledge of the metabolism of both the host and the infectious organism. While considerable knowledge of mammalian metabolism has

been accumulated, little is known about the metabolism of nematodes.

Reviews of nematode metabolism indicate that carbohydrates are probably the primary source of metabolic energy in the adults (19, 23, 26, 27, 55, 121). While *Ascaris* contains relatively large amounts of lipids, little is known about its metabolism. It is not known if the lipids in the adult can be catabolized for energy or to what extent they are transferred from one tissue to another. Most of the lipid in the adult female is in the reproductive tract where it is incorporated into eggs. As the embryo develops from the egg some of the lipid is converted to carbohydrate, however, this has not been demonstrated in the adult. Oxidation of fatty acids has not been demonstrated in either the embryo or adult worm.

Studies of lipid metabolism of mammalian tissues indicate that triglyceride fatty acids can be oxidized for energy production more readily than other lipid fractions. This may be true for *Ascaris*, however, the adult worms are essentially anaerobic organisms and may not be capable of oxidizing fatty acids by the same pathway that is known for mammalian tissue. Adult *Ascaris* does not have a complete tricarboxylic acid cycle or cytochrome system. This is probably related to the worms' anaerobic environment. The absence of a complete cycle would prevent the oxidation of acetate as it is known to occur in aerobic organisms. The accumulation of volatile fatty acids in the medium and in the triglycerides of *Ascaris* tissues suggests that the volatile fatty acids are not oxidized by this parasite.

This investigation was designed to determine the ability of *Ascaris* to metabolize lipids during starvation under aerobic and anaerobic conditions with particular attention to the fatty acids of the saponifi-

able neutral lipids. Total lipid and various lipid fractions were measured after various periods of starvation to determine if the animal used a measurable amount of lipid during starvation. Changes in proportion of the fatty acids of the saponifiable neutral lipid were measured by gas-liquid chromatography. Oxidation of several fatty acids by mitochrondria which were isolated from the muscle tissue of the body wall was investigated.

CHAPTER II

LITERATURE REVIEW

The chemical makeup of *Ascaris* has been well determined, and pathways of carbohydrate metabolism have been studied intensively. Lipid metabolism, on the other hand, has not been well elaborated in this organism. This chapter is a review of what is known of the techniques of *in vitro* culture of *Ascaris* and the interrelationship of carbohydrate and lipid metabolism in *Ascaris* and in mammals.

Culture

The normal habitat of *Ascaris lumbricoides*, the small intestine of the hog, contains a complex solution of organic and inorganic substances maintained slightly above 37°C. The mixture varies according to the diet of the host and is so complex that few investigators have attempted to duplicate it in an artificial medium. Since the present study involved only starvation experiments, only non-nutrient media are discussed in detail.

Early investigators used physiologic saline solutions for the culture of *Ascaris*, but more recent workers have developed media that are based on analysis of inorganic components of the worm's body fluid (3) and pig intestinal contents (74, 75, 80, 81). The pH of *Ascaris* body fluid is near 7 (79), so most media have been adjusted to neutrality. The presence of CO_2 and bicarbonate in the medium seems to have bene-

ficial effects on Ascaris, possibly by aiding in the regulation of intracellular pH (20). The beneficial effects of CO_2 seems to be enhanced at high pH values (76) which indicates that CO_2 may be utilized for some function other than for pH regulation. CO_2 fixation reactions, such as the formation of propionate and succinate (14, 130, 142) may be important reactions in Ascaris metabolism.

The small intestine of the hog is always found to contain gas at the time of slaughter. Analyses of samples of the intestinal gas reveal that it is composed of 7.6-29.6 per cent CO_2, 0.4-8.8 per cent O_2 and the remainder N_2 (21, 103). Studies with other animals indicate that oxygen levels are higher near the mucosa than in the center of the lumen of the intestine (126). It has not been established whether Ascaris is dependent upon the small amount of oxygen available *in vivo* or if it normally exists as a completely anaerobic organism. The use of hydrogen acceptors other than oxygen is widespread in intestinal parasites, however, all parasites will take up oxygen when it is available (121). Ascaris can survive *in vitro* for several days in the absence of oxygen (33, 144) but it is capable of less muscular activity than when it is in air (138). There is some evidence that high oxygen tensions are toxic to Ascaris (96). Oxygen consumption by Ascaris is related to oxygen tension (96, 118), however, there is no increase in glycogen consumption in the absence of oxygen (15, 19, 107). When CO_2 production is used as an index of metabolism, Ascaris has a higher rate of metabolism when oxygen is absent and when the medium contains CO_2, plus potassium, ammonium, sulfate, phosphate and bicarbonate ions (74).

Baldwin and Moyle (3) found that worms exposed to temperatures below 30°C. underwent an irreversible change so that they were unsatis-

factory for measuring the effect of drugs, however, the nature of these deleterious changes is unknown. Since the body temperature of hogs is near 37°C. it is common practice to incubate the worms at this temperature.

Carbohydrates

Content: *Ascaris lumbricoides* as well as most other parasitic worms, contains large quantities of carbohydrates compared to vertebrates (121). The glycogen content of *Ascaris* is reported to be 5.3 per cent to 8.7 per cent of the fresh body weight (19, 143). Trehalose is the most common disaccharide (57). Reducing sugars amount to less than 0.1 per cent of the fresh body weight (35). Fairbairn and Passey (57) reported the following concentrations of glycogen and trehalose in the various tissues of *Ascaris* females:

TOTAL ALKALI-STABLE CARBOHYDRATE IN ASCARIS TISSUE

	% Tissue Weight		
Tissue	Total	Glycogen	Trehalose
hemolymph	1.17	0.40	0.77
intestine	0.84	0.70	0.14
muscle	17.2	15.4	1.8
integument	0.75	0.61	0.14
ovaries	8.4	7.6	0.80
uteri	3.5	2.0	1.5

Most of the carbohydrate reserve of the animals is stored in the muscle tissue, however, the ovaries also contain relatively large amounts of carbohydrate.

Synthesis of Glycogen and Trehalose: The body wall of *Ascaris* is

impermeable to glucose (109), however, glucose, fructose, sorbose, maltose and sucrose are absorbed through the worm's intestine and may be used in glycogen synthesis (37, 51, 79, 87, 109, 145). Lactose, mannose and galactose added to the medium did not stimulate glycogen synthesis (37). Trehalose synthesis has been demonstrated by tissue minces and homogenates under 95% N_2/5% CO_2 (59).

Glycolysis: The Embden-Meyerhof pathway is the major route of breakdown of carbohydrates in *Ascaris* (51, 119, 127) as well as in the tapeworms (120). Some carbohydrate breakdown is by way of the pentose-phosphate pathway (45, 50). In some parasitic worms lactic acid is the major end product of anaerobic glycolysis just as it is in mammals (24, 69, 97, 122), however, in *Ascaris* lactic acid is a relatively minor end product and volatile fatty acids are produced in large quantities (19). When *Ascaris* is returned to air, following a period of 20 hours under anaerobic conditions, it is able to resynthesize only 1/20 to 1/10 of the glycogen used compared to vertebrates which are able to resynthesize 4/5 to 5/6 of the glycogen used (16, 17). This may be the result of the formation of volatile fatty acids by *Ascaris* instead of lactic acid as in the vertebrates.

Tricarboxylic Acid Cycle: The high production of volatile fatty acids by *Ascaris* may be related to the lack of a complete tricarboxylic acid cycle. The complete tricarboxylic acid cycle has been demonstrated in *Ascaris* larvae but attempts to do so in adult *Ascaris* have been unsuccessful (43). Certain of the intermediates in the tricarboxylic acid cycle such as, 2-oxoglutarate, fumarate, and l-malate stimulate oxidation, but citrate, cis-aconitate, oxalacetate and pyruvate with or

without added oxalacetate do not stimulate oxidation (118). Kmetec and Bueding (92) demonstrated that Ascaris succinic dehydrogenase catalyzes the reduction of fumarate at a much higher rate than the oxidation of succinate. They postulated that fumarate is an important hydrogen acceptor in the electron transfer system. The presence of 8.4 mM. of succinate in the perienteric fluid suggests that succinate formation plays an important role in Ascaris metabolism (30). Evidence has been presented for the formation of succinate by CO_2 fixation with both pyruvate and propionate (130). Succinate formation from malate may be important as a source of energy since it has been shown that the dismutation of malate resulting in the formation of equi-molar amounts of pyruvate and succinate does not require oxygen and results in the production of high energy phosphate (137). The association of energy production with succinate formation was also suggested by the finding that the concentration of piperazine which paralyzed the worm also inhibited the formation of succinate (31). Ascaris does produce high energy phosphate, however, Rogers and Lazarus (127) were unable to find any of the usual phosphogens.

The association of NAD (nicotinamide adenine dinucleotide), NADP (nicotinamide adenine dinucleotide phosphate) and FAD (flavin adenine dinucleotide) with dehydrogenases found in Ascaris mitochondria has been established (26, 28, 29, 39, 129), however, cytochrome c and cytochrome oxidase are not present in the electron transfer system (26, 28, 38). Some elements of the cytochrome system are present in low amounts in the adult while others such as cytochrome c_1, a and a_3 are not present in the tissues of adult worms (38, 89, 90). The insensitivity of oxygen uptake

by muscle pulp to cyanide and 2,4-dinitrophenol along with oxygen uptake being dependent on oxygen tension suggests that the terminal oxidase is a flavoprotein (73, 92, 96, 137). It has been noted that the sperm and eggs do contain some cytochrome (86) and during embryonation and early development of the larvae there is rapid development of a cytochrome system (42, 44, 91, 113). The tricarboxylic acid cycle has been found to be complete in the developing eggs (43, 114).

Volatile Fatty Acids: Bunge (33) in 1890 was probably the first to attribute the odor of a culture of *Ascaris* to the production of volatile fatty aicds. Since that time many investigators have identified steam-volatile fatty acids present in the medium in which *Ascaris* had been cultured and in extracts of *Ascaris* tissues. Most investigators have agreed that the 5-carbon and 6-carbon acids are the most abundant volatile fatty acids with smaller amounts of acetic acid, propionic acid and butyric acid also being present (6, 25, 32, 48, 52, 62, 76, 95, 108, 111, 141, 144). The major portion of the 5-carbon acid is 2-methylbutyric acid (6, 108, 132) and most of the 6-carbon acid is 2-methylvaleric acid (6, 128, 133, 146). Two unsaturated volatile fatty acids, acrylic acid and tiglic acid, have been identified as minor components of the volatile fatty acids (25, 62).

In 1901, Weinland (144) linked volatile fatty acid production to carbohydrate metabolism. The production of volatile fatty acids was found to be 1.16-1.82 meq. per 100 grams of body weight per 24 hours of fasting (52) and 2.5-4.0 meq. per 100 grams of body weight per 24 hours in the presence of glucose (32). Further evidence of carbohydrates being the source of fatty acids was provided when it was shown

that succinate is directly decarboxylated to form propionate and CO_2 (130). It has also been shown that 2-methylbutyrate and 2-methylvalerate are synthesized by the combination of a molecule of acetate and a molecule of propionate or two molecules of propionate (132, 133). Similar condensation has been shown with bacteria in the formation of the 21-carbon branched chain lactone erythromycin (85) and in the formation of corynomycolic acid by Corynebacteria (65). The possibility that volatile fatty acids may be produced from amino acids by oxidative deamination followed by oxidative decarboxylation has been postulated, however, there is no experimental evidence to support this postulate (108).

A qualitative difference in the volatile fatty acids produced in the presence and absence of oxygen has been reported (76). Under 93% N_2/7% CO_2 the 4-carbon and 5-carbon acids increased to a greater extent than the others; in air the 2-carbon and 3-carbon acids increased relative to the other volatile fatty acids.

Lipids

The lipid content of Ascaris lumbricoides has been extensively studied, however, little is known concerning the worm's ability to metabolize such compounds.

Lipid Content: Adult female Ascaris lumbricoides contain a relatively large amount of lipid estimated to be from 1.46% to 1.75% of the wet body weight (18, 53, 62, 144). The variation in determinations is due to differences in methods used to extract the lipids. The reproductive tract represents a much smaller proportion of the body weight than the body wall but contains 66% of the total lipid whereas the body wall

contains only 20% (53). On a dry weight basis, the body wall is 2.8% lipid and the reproductive tract is 27.2% lipid (53).

Analysis of lipids of <u>Ascaris</u> shows that phospholipids account for a larger proportion of the lipid in the body wall than in the reproductive system (54). The fatty acids of the triglycerides accounted for 46.8% of the lipid in the body wall (54) and 64.8% in the reproductive system (53). The most abundant esterified volatile fatty acids are 2-methyl-valeric acid, and acetic acid (6). The most abundant esterified non-volatile fatty acids are hexadecanoic, octadecadienoic, octadecaenoic, octadecanoic acids and an unknown high molecular weight fatty acid (6). The unsaponifiable material includes sterols (36, 53, 54, 65) and ascaryl alcohols which are particularly abundant in the reproductive system (53, 54, 58, 62, 64, 98, 136).

<u>Lipid Metabolism</u>: Evidence of lipid metabolism in <u>Ascaris</u> is meager. Weinland (144) and von Brand (15, 18) found no change in total lipid following starvation of the worm for as long as 5 days. Mueller (109) reported that small pieces of lateral line tissue cultured in hanging drops for as long as 8 days were apparently able to catabolize lipids since histological examination showed a decrease in lipid content following starvation. Hirsch and Bretschneider (78) found a decline in lipid droplets and some evidence of redistribution of fat in the cells of the intestinal wall following starvation of the worms in physiologic salt solutions. Schulte (135) measured total lipid following starvation of the worm and found an increase of 0.1 gm./100 gm. body weight after 24 hours. Esterases have been found in several tissues from <u>Ascaris</u> (34, 99, 125). Experiments with developing <u>Ascaris</u>

embryos and larvae have shown that some of the stored lipid is converted to glycogen during development (57, 115), however, this has not been demonstrated in adult worms.

The above reports indicate that the lipids in some tissues of *Ascaris* are in a dynamic state, however, the results obtained with whole worms indicate that there is no change in total lipid. It is important that studies be made to establish the capability of *Ascaris* to synthesize and catabolize lipids.

Fatty Acid Oxidation: Fatty acid oxidation has not been examined in *Ascaris lumbricoides*. Most of what is known of fatty acid oxidation has been gained from work with mammalian tissues. Oxidation of fatty acids by mammalian tissue has been shown to be a function of the mitochondria (2, 100, 101, 102, 104, 110, 117, 134). It has been shown that an extract of the acetone powder of mitochondria contains the enzymes required for fatty acid oxidation (70).

Oxidation of fatty acids has been shown to require ATP (adenosine triphosphate) (100, 117) and CoA (coenzyme A) (46) although Green and Wakil (72) report that CoA generally had no stimulating effect on mitochondrial preparations. Oxidation of fatty acids with carbon skeletons containing 12 carbons or more was stimulated by the addition of tricarboxylic acid cycle intermediates such as fumarate or succinate (88, 94), however, the oxidation of fatty acids with shorter carbon skeletons was not affected by the presence of these intermediates. It has been postulated that the effect of tricarboxylic acid cycle intermediates was due to an extra-tricarboxylic acid cycle action (105). The amount of fatty acid broken down by liver slices in the presence

of malonate was not changed, but less was oxidized to CO_2 and more was converted to acetocetate than in the absence of malonate (67).

Oxidation of most fatty acids proceeds through beta oxidation, the successive cleavage of two carbon units from the carboxyl end of the molecule (2, 72, 134). There are indications that propionic acid, isocaproic acid (70) and 2-methyl acids (40) are not oxidized by beta oxidation. In mammals the branched chain 2-methylbutyric acid is cleaved to form a molecule of acetate and a molecule of propionate in a manner similar to beta oxidation by a series of reversible reactions (41, 124). Propionyl CoA is converted to pyruvate by carboxylation forming first methylmalonyl CoA (8, 60, 61) which is then converted to succinyl CoA by a transcarboxylation reaction (9). Giovalelli and Stumpf (68) proposed on the basis of their work with peanut cotyledons that propionate is oxidized to acetate and carbon dioxide. Saz and co-workers (128, 129, 130, 131, 132, 133) have shown that in *Ascaris* the formation of 2-methylbutyrate, 2-methylvalerate, propionate and succinate follow steps that are essentially the reverse of those discussed above.

In view of the large decrease in the glycogen content in *Ascaris* during starvation it seems reasonable to assume that the bulk of the energy produced in this parasite is the result of carbohydrate catabolism. The presence of large quantities of neutral lipid fatty acids and the presence of a lipid catabolizing system in the embryos leads one to postulate that adult *Ascaris* is capable of lipid catabolism. It was the purpose of this study to determine whether *Ascaris* uses a measurable amount of lipid when starving, and to determine if the mitochondria are capable of oxidizing fatty acids.

CHAPTER III

MATERIALS AND METHODS

This study was divided into three experiments. In the first experiment, the total lipid in the body wall and reproductive system of individual worms was measured following starvation of the worms. The worms were incubated in a non-nutrient medium under N_2, 95% N_2/5% CO_2, air and 95% air/5% CO_2 for specified periods prior to the extraction of lipids from the tissues. In the second experiment, qualitative and quantitative measurements were made of the fatty acids of the saponifiable neutral lipids of the body wall, ovary-oviduct tissue and the uterus plus eggs following starvation. The worms used in this experiment were incubated in a non-nutrient medium under 95% N_2/5% CO_2 and 95% air/5% CO_2. Although the worms were incubated individually the tissues from a number of worms were pooled prior to the extraction of the lipids to enable more accurate measurement of the quantity of individual fatty acids in the various tissues. The fatty acids of the saponifiable neutral lipids of each of the pooled samples were isolated and analyzed by gas-liquid chromatography. In the third experiment, the rate of fatty acid oxidation by mitochondria from Ascaris muscle tissue was determined by measuring the rate of reduction of 2,6-dichlorophenolindophenol which was added to the incubation system.

All worms used in this study were collected at the Wilson Packing Company plant in Oklahoma City. They were washed in warm tap water at

the time of collection and transported to the laboratory in a salt solution maintained between 34°C. and 39°C. The salt solution used was modified after Harpur (75) and contained:

NaCl	61 mM.
$NaHCO_3$	60 mM.
KCl	24 mM.
$CaCl_2$	1 mM.
$MgSO_4$	5 mM.
KH_2PO_4	0.5 mM.
NH_4Cl	14 mM.

The amount of bicarbonate included in this medium was increased over that used by Harpur (75) to maintain the pH near 7.3.

First Experiment: Total Lipid

Culture: In the first set of experiments the total lipid in the body wall and reproductive tract of individual worms was determined after starvation under air, 95% air/5% CO_2, N_2 and 95% N_2/5% CO_2 for periods of 1 to 8 days.

Individual worms were weighed to the nearest 0.05 gm. and placed separately into large test tubes containing 40 ml. of the salt solution. The tubes were connected in series of 5 by glass and rubber tubing so that the appropriate gas could be bubbled through the salt solution. The tubes were placed in a water bath at 37°C. and the salt solution was changed every 24 hours. Worms that failed to survive were removed each day. The eggs from each worm were collected daily and stored separately for lipid analysis.

Lipid Extraction: At the end of the specified period of starvation worms were removed individually from the test tubes and a longitudinal incision was made in the body wall. The digestive tract was carefully

removed and discarded. The reproductive system was removed, blotted on paper toweling and weighed to the nearest 0.001 gm. The body wall was also blotted on paper toweling and then weighed to the nearest 0.001 gm. Lipids were extracted from the separate tissues according to the technique of Folch et al. (63). Lipids from the eggs produced by each worm were extracted with two 5 ml. portions of chloroform:methanol (2:1, v/v) and added to the lipids extracted from the reproductive system. The lipid extract was quantitatively transferred to a tared 100 ml. beaker and the solvent was evaporated from the lipids by directing a stream of warm air over the beakers. The weight of the total lipids was determined to the nearest 0.1 mg. by difference.

Statistical Analysis: The total lipid determinations from the first experiment were carried through the following statistical analysis. The mean value for the lipid content of the body wall and reproductive system plus eggs for each experimental group and for the control group of worms was calculated and the standard error was determined for each mean. The mean values of the lipid content of the body wall and reproductive system plus eggs of the worms in each experimental group were compared with the mean values of the lipid content of the comparable tissues of the worms in the control group. The mean values of the total lipid in each of the tissues of worms starved under aerobic conditions were compared with the mean values of the total lipid in the comparable tissue of worms starved under anaerobic conditions. The mean values of the total lipid of each of the tissues of worms starved in atmospheres containing CO_2 were compared with the mean values of the total lipid of the comparable tissues of worms starved in atmos-

pheres not containing CO_2. The mean value of the total lipid in each of the tissues following 2 days starvation was compared with the mean values of the total lipid content of the comparable tissues from worms starved for different periods of time under the same atmosphere. The change in total lipid during starvation did not appear to follow the same pattern in the two tissues. In an effort to determine the "overall" lipid change in starving *Ascaris* the total lipid values for the body wall and reproductive system plus eggs from each worm were summed. These "combined lipid values" were treated to the above statistical analysis.

Differences between mean values were evaluated for significance at the .1, .05, and .01 levels by the *lsd* (least significant difference) method. Three series of *lsd* values were calculated; one for the body wall values, one for the reproductive system plus eggs, and one for the "combined lipid values." The *lsd* values for each series were calculated as described below. The variance (s^2) for all the values of lipid content was calculated. Since the number of worms (n) represented by each of the mean values was not the same, the standard deviation of the mean values ($s_{\bar{d}}$) for each possible comparison was calculated by the following formula:

$$s_{\bar{d}} = \sqrt{s^2 \frac{n_1 + n_2}{n_1 n_2}}$$

The *lsd* for each possible comparison was calculated by the formula

$$ts_{\bar{d}} = \underline{lsd}.$$

Table A.3 in Steele and Torrie (140) was used to determine the appropriate t values. The degrees of freedom of t was 159, since there was

a total of 177 observations and 18 means were calculated. Appendix B includes the calculated lsd values for all possible comparisons.

Second Experiment: Fatty Acids

The second set of experiments was designed to determine the changes that occurred in the fatty acids of the neutral lipid esters during starvation of adult female Ascaris lumbricoides in 95% N_2/5% CO_2 and 95% air/5% CO_2. The tissues analyzed were the body wall, ovary-oviduct and uterus plus eggs. Tissues from a number of worms were pooled to accumulate enough lipid so that minor components might be more accurately measured.

Culture: The worms were cultured as in the first set of experiments.

Lipid Extraction and Purification: The lipids were extracted from the tissues as described by Folch, et al. (63). Silicic acid column chromatography was used to separate the neutral lipids from the phospholipids (6, 12). Recovery of lipids from the silicic acid column as determined by gravimetric means was 96.3%. Separation of neutral lipids from the phospholipids was checked by assaying for total phosphate before and after column chromatography using the method described by Bartlett (5) as modified by Bottcher, et al. (13). The phospholipids were discarded without further analysis.

The neutral lipids were saponified and the soaps were removed from the unsaponifiable lipids by means of the method by Schulz and Becker (136) as modified by Fairbairn (53). The soaps were acidified with 10 N sulfuric acid and steam distilled to separate the volatile fatty

acids from the mixture. The quantity of volatile fatty acids was determined by titration of the distillate with 0.01 N NaOH assuming the average molecular weight of the volatile fatty acids to be 100. The volatile fatty acids were stored as dry sodium salts until prepared for gas-liquid chromatography. The non-volatile fatty acids were extracted from the saponification mixture by four successive washings in a separatory funnel with equal volumes of ethyl ether. After drying over Na_2SO_4 the ether was evaporated and the non-volatile fatty acids were weighed to the nearest 0.1 mg. The non-volatile fatty acids were stored in petroleum ether under nitrogen at -20°C.

Gas-Liquid Chromatography

Fatty acids were analyzed on a Barber Colman model 15 gas chromatograph using a 6 ft. (4 mm. I. D.) U shaped column. Argon was the carrier gas and the instrument was equipped with a beta ionization detector.

Volatile Fatty Acids: Free volatile fatty acids were prepared by the method of Gehrke and Lamkin (66) and stored in ethyl ether under nitrogen at -20°C. until analyzed. The free volatile fatty acids were analyzed on a column packed with Gas Chrom CLP* (100-120 mesh) coated with 50% DC 550 with 15% stearic acid. The column temperature was 100°C., the detector temperature was 145°C. and the flash heater was operated at 178°C. The gas flow rate was 190.2 ml. per minute as measured by a soap film flow meter.

Non-volatile Fatty Acids: Methyl esters of the non-volatile acids were made using the BF_3 reagent and following the procedure

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outlined by Metcalf and Schmitz (106). The methyl esters were stored in hexane under nitrogen at -20°C. until analyzed. A column packed with Gas Chrom CLH* (100-120 mesh) coated with ethylene glycol succinate (14.5%) was used to analyze the non-volatile fatty acid methyl esters. The column temperature was 178°C., the detector temperature was 215°C. and the flash heater was operated at 240°C. The gas flow rate was 91.2 ml. per minute. For aid in identification of some of the methyl esters of the non-volatile fatty acids, a few samples were also run on a column packed with Gas Chrom CLH* (100-120 mesh) coated with Apiezon L (20%). The column temperature was 200°C., the detector temperature was 225°C. and the flash heater was operated at 240°C. The gas flow rate was 90 ml. per minute. The methyl esters were identified by comparison with known standards and by semilog plots of the relative retention times versus the number of carbon atoms in the acid (78, 83, 84, 85). Percentage composition of the fatty acids in each sample was determined by measuring the area of each elution peak by the triangulation method.

Third Experiment: Fatty Acid Oxidation

This series of experiments was conducted with unstarved *Ascaris* and was designed to determine the ability of mitochondria isolated from muscle tissue to oxidize fatty acids. An attempt was made to identify volatile fatty acid end products of the oxidation.

Mitochondria Preparation: Mitochondria were isolated in a manner similar to that used by Kmetec, *et al*. (93). Muscle strips were pre-

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pared as described by Laser (96), weighed, cut into small pieces, and homogenized with either an all-glass Ten Broeck homogenizer or a Potter-Elvejehm homogenizer with a motor driven Teflon pestle. All samples were prepared in 0.25 M sucrose using 4 ml. of sucrose per gram of tissue. The homogenate was centrifuged at 500-550 g (gravity) for 15 minutes. The sediment was discarded and the supernatant centrifuged at 12,000-12,500 g for 30 minutes. The pellet was resuspended in 1 ml. of 0.25 M sucrose per gm. of muscle tissue and centrifuged at 12,000-12,500 g for 30 minutes. The last step was repeated once and the final pellet was suspended in 1 ml. of 0.25 M sucrose per gm. of muscle tissue.

Incubation and Assay: Fatty acid oxidation was determined by measuring the reduction of 2,6-dichlorophenolindophenol based on the technique described by Ells (47). A similar system has been used to measure butyryl coenzyme A dehydrogenase activity (71). Various preparations of Ascaris muscle were used including, homogenate, cell debri sedimented at 500 g, supernatant after removal of mitochondria and the mitchondria. A few samples of homogenate and mitochondria were placed in the refrigerator for as long as 48 hours prior to use and others were placed in a deep freeze at -20°C. for 24 hours.

The reaction was started by adding an aliquot of one of the muscle preparations to a test tube containing 60 μMoles phosphate buffer pH 7.3, 10 μMoles $MgCl_2$, 10 μMoles KCN, 1.53×10^{-7} M 2,6-dichlorophenol-indophenol, 1.15 mg. of phenazine methosulfate, substrate and distilled water to make a final volume of 6 ml. Dye reduction was measured at 600 millimicrons in a Colman, Jr. spectrophotometer. Tests were run to determine the optimum pH of the incubation mixture, the optimum enzyme

concentration, the appropriate substrate concentration and the effect of various substances including, ATP, CoA, cytochrome c, succinate, fumarate, malonate, and bicarbonate. The rate of oxidation of the sodium salts of acetic, propionic, butyric, 2-methylbutyric, 2-methylvaleric, heptanoic, palmitic and oleic acids were measured in the course of the experiment.

End Product Analysis: The determination of end products was done with larger scale incubations. Mitochondria isolated from one to thirty grams of muscle tissue were added to flasks containing up to 60 ml. final volume including the acid substrate, phosphate buffer and potassium cyanide. Incubations were carried out in 125 ml. flasks in a water bath maintained at 37°C. Nitrogen was flushed through some of the flasks during incubation and compressed air was flushed through other flasks to determine the effect of oxygen on the amount of dye reduced. The dye 2,6-dichlorophenolindophenol was added as it was reduced since Drysdale and Lardy (46) reported that large quantities of the oxidized dye are inhibitory. When dye reduction stopped, the reaction mixture was centrifuged at 12,000-12,500 g for 30 minutes to remove the mitochondria which were discarded. One ml. of 4.75 N NaOH was added to the supernatant and it was evaporated to a volume of 0.5-1.0 ml. Fatty acids were extracted as described by Barker (4) and identified by silicic acid chromatography (49) and paper chromatography (123). The paper chromatograms were exposed to ultraviolet light to detect unsaturated compounds. Acid spots on the paper chromatograms were detected by a modification of the method used by Osteux, et al. (112) using 0.2 gm. of phenol red in 15 ml. H_2O plus 70 ml. of ethyl

alcohol and 0.3 ml. of 1.0 N NaOH. The indicator was mixed 5:1 (v/v) with 0.125 M tris buffer pH 9.0 in 2 parts H_2O and 1 part ethyl alcohol before use. The spots were stable for over 48 hours.

TABLE I

TOTAL LIPID OF THE BODY WALL AND REPRODUCTIVE SYSTEM OF FEMALE <u>ASCARIS LUMBRICOIDES</u> FOLLOWING STARVATION IN AIR, 95% AIR/5% CO_2, N_2 AND 95% N_2/5% CO_2***

Group	Number of Worms in Each Group	Body Wall		Reproductive Tract		Combined	
		Mean mg %*	S.E.**	Mean mg %	S.E.	Mean mg %	S.E.
CONTROL	20	335.44	11.27	1160.40	59.32	1498.56	58.56
NITROGEN							
Day 2	9	570.03	38.08	1533.47	79.26	2102.49	82.65
Day 4	10	442.90	15.83	1588.63	85.99	1961.54	93.88
Day 6	3	417.62	35.52	1420.58	143.47	1838.22	136.21
N_2/CO_2							
Day 1	10	358.86	25.97	1496.59	53.86	1855.46	54.48
Day 2	9	331.80	14.44	1683.29	72.95	2015.09	81.79
Day 3	10	312.01	8.03	1280.45	75.71	1592.38	70.96
Day 4	10	356.26	17.15	1456.28	74.87	1812.55	62.62
Day 6	10	338.11	14.85	1480.38	98.53	1818.83	90.05
Day 8	6	356.14	32.43	1372.34	105.18	1728.47	105.59
AIR							
Day 2	10	309.11	8.76	1516.49	100.44	1825.60	100.10
Day 4	10	320.84	17.01	1446.59	102.08	1767.43	104.48
Day 6	10	391.40	17.85	1347.18	73.83	1737.52	78.29
AIR/CO_2							
Day 1	10	334.99	21.50	1500.35	96.18	1835.34	105.75
Day 2	10	375.18	18.03	1454.49	103.74	1829.68	101.64
Day 3	10	337.54	10.43	1528.21	62.84	1865.75	61.42
Day 4	10	337.22	15.29	1332.27	105.52	1679.50	93.37
Day 6	10	306.18	17.85	1535.49	114.09	1841.67	115.33

*mg%-mg lipid/100g body weight **S.E.-standard error of mean

***Values calculated from data listed in Appendix A.

TABLE II

LEVEL OF SIGNIFICANCE OF THE DIFFERENCE BETWEEN MEANS BASED ON THE lsd

	Control Vs. all other means			Day 2 Vs. other days in same treatment			Air Vs. N_2			CO_2 Vs. No CO_2		
	Body Wall	Reprod. Tract	Comb.	Body Wall	Reprod. Tract	Comb.	Body Wall	Reprod. Tract	Comb.	Body Wall	Reprod. Tract	Comb.
N_2												
2	.01	.01	.01	-	-	-						
4	.01	.01	.01	.01	ns	ns						
6	.05	ns	.05	.01	ns	.05						
N_2/5% CO_2												
1	ns	.01	.01	ns	ns	ns						
2	ns	.01	.01	-	-	-				.01	ns	ns
3	ns	ns	ns	ns	.01	.01						
4	ns	.01	.01	ns	.1	ns				.01	ns	ns
6	ns	.01	.01	ns	ns	ns				.05	ns	ns
8	ns	.1	.1	ns	.05	.05						
AIR												
2	ns	.01	.01	-	-	-	.01	ns	.05			
4	ns	.01	.01	ns	ns	ns	.01	ns	ns			
6	.05	.1	.01	.01	ns	ns	ns	ns	ns			
AIR/5% CO_2												
1	ns	.01	.01	ns	ns	ns	ns	ns	ns			
2	.1	.01	.01	-	-	-	ns	.1	ns	.05	ns	ns
3	ns	.01	.01	ns	ns	ns	ns	.05	.05			
4	ns	ns	.1	ns	ns	ns	ns	ns	ns	ns	ns	ns
6	ns	.01	.01	.01	ns	ns	ns	ns	ns	.01	ns	ns

ns-not significant

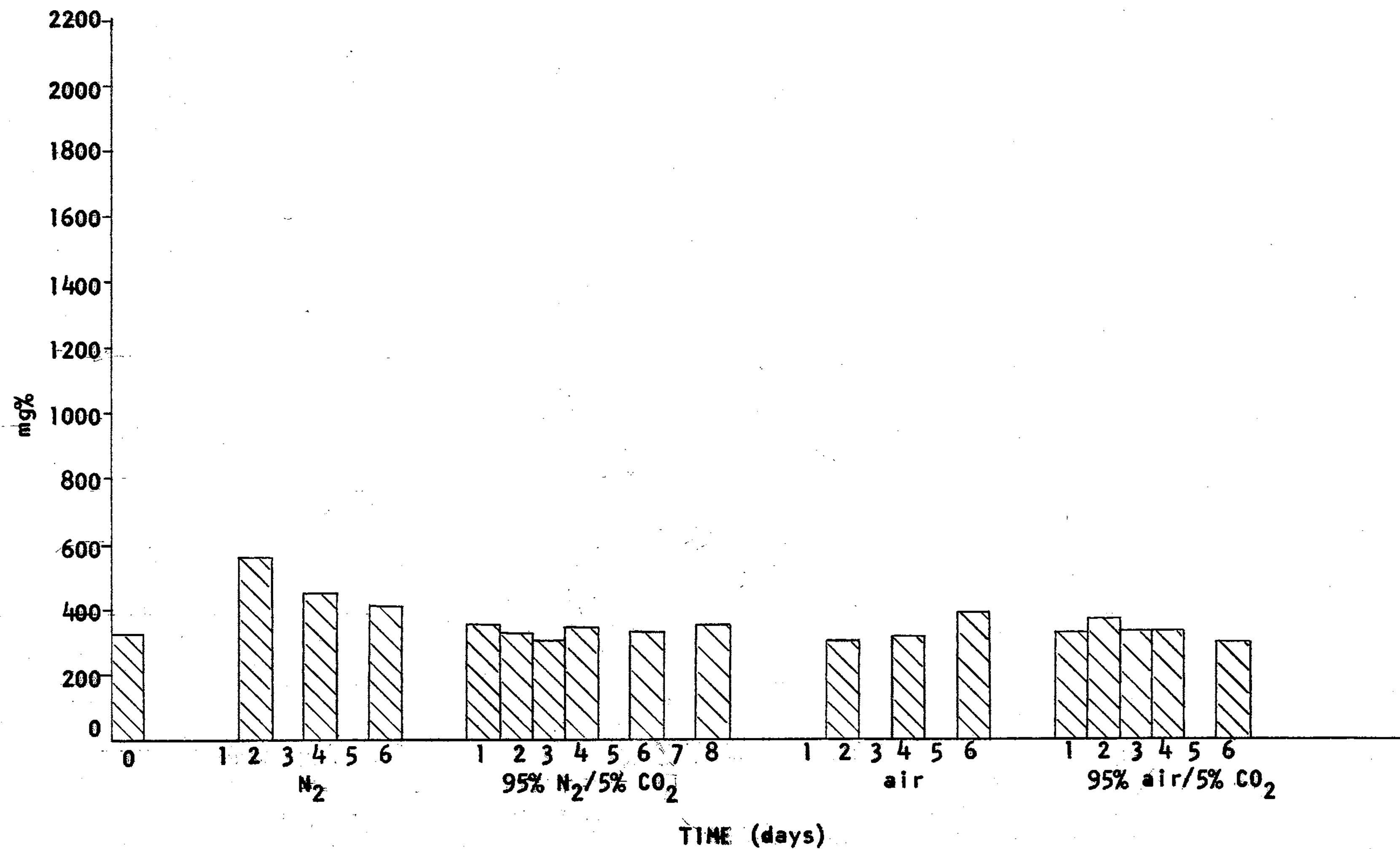


Figure 1. Total Lipid in the Body Wall Following Starvation Under N_2, 95% N_2/5% CO_2, Air and 95% Air/5% CO_2.

lipid content of the body wall of worms starved under an atmosphere of air was significantly higher on day 6 than that of the control group, but on days 2 and 4 did not vary significantly from that of the control group. The total lipid in the body wall on day 2 when the worms were starved under 95% air/5% CO_2 was significantly higher than the value in the control worms. The values for days 1, 3, 4 and 6 were not significantly different from the control group.

A comparison was made between the lipid content of the body wall of worms starved under aerobic and anaerobic conditions. When CO_2 was not included in the atmosphere under which the worms were starved, the lipid content of the body wall under anaerobic conditions on days 2 and 4 was significantly higher than under aerobic conditions. On day 6 there was no significant difference. When CO_2 was included in the atmosphere under which the worms were starved, there was no significant difference in the lipid content of the body wall under aerobic and anaerobic conditions.

The effect of CO_2 on the lipid content of the body wall was determined by comparing the mean values obtained using worms starved under atmospheres containing CO_2 with those of worms starved in the absence of CO_2. Under anaerobic conditions the lipid content of the body wall was significantly lower when CO_2 was included in the atmosphere than when it was absent. Under aerobic conditions the lipid content of the body wall of worms starved under an atmosphere containing CO_2 was significantly higher on day 2 than the lipid content of worms starved in the absence of CO_2. On day 4 there was no significant difference. On day 6 the body wall of the group starved under aerobic conditions in

the presence of CO_2 contained significantly less lipid than the body wall of the group starved in the absence of CO_2.

Reproductive System Plus Eggs: The reproductive system plus eggs is referred to as the reproductive system throughout the remainder of this discussion. The total lipid in the reproductive systems under the experimental conditions are shown in Figure 2. All values for total lipid in the reproductive system under various experimental conditions are higher than the control value, however, the difference is not significant for 3 values (see Table II). These are: day 6 under N_2, day 3 under 95% N_2/5% CO_2 and day 4 under 95% air/5% CO_2. The graph in Figure 2 shows that under N_2, 95% N_2/5% CO_2 and air there was an increase in lipid in the reproductive system the first few days of starvation followed by a decline. Under 95% air/5% CO_2 the lipid content did not decline after the initial increase.

There was no significant difference between the total lipid of the reproductive system of worms starved under aerobic conditions and the total lipid of the reproductive system of worms starved under anaerobic conditions when CO_2 was not included in the atmosphere under which the worms were starved. When CO_2 was included in the atmosphere, there was a significantly greater amount of lipid in the reproductive system on day 2 under anaerobic conditions than under aerobic conditions. On day 3, when CO_2 was included in the atmosphere, there was a significantly smaller amount of lipid in the reproductive system under anaerobic conditions than under aerobic conditions. There was no significant difference in lipid content of the reproductive system on days 1, 4 and 6 when CO_2 was included in the atmosphere under which the worms were

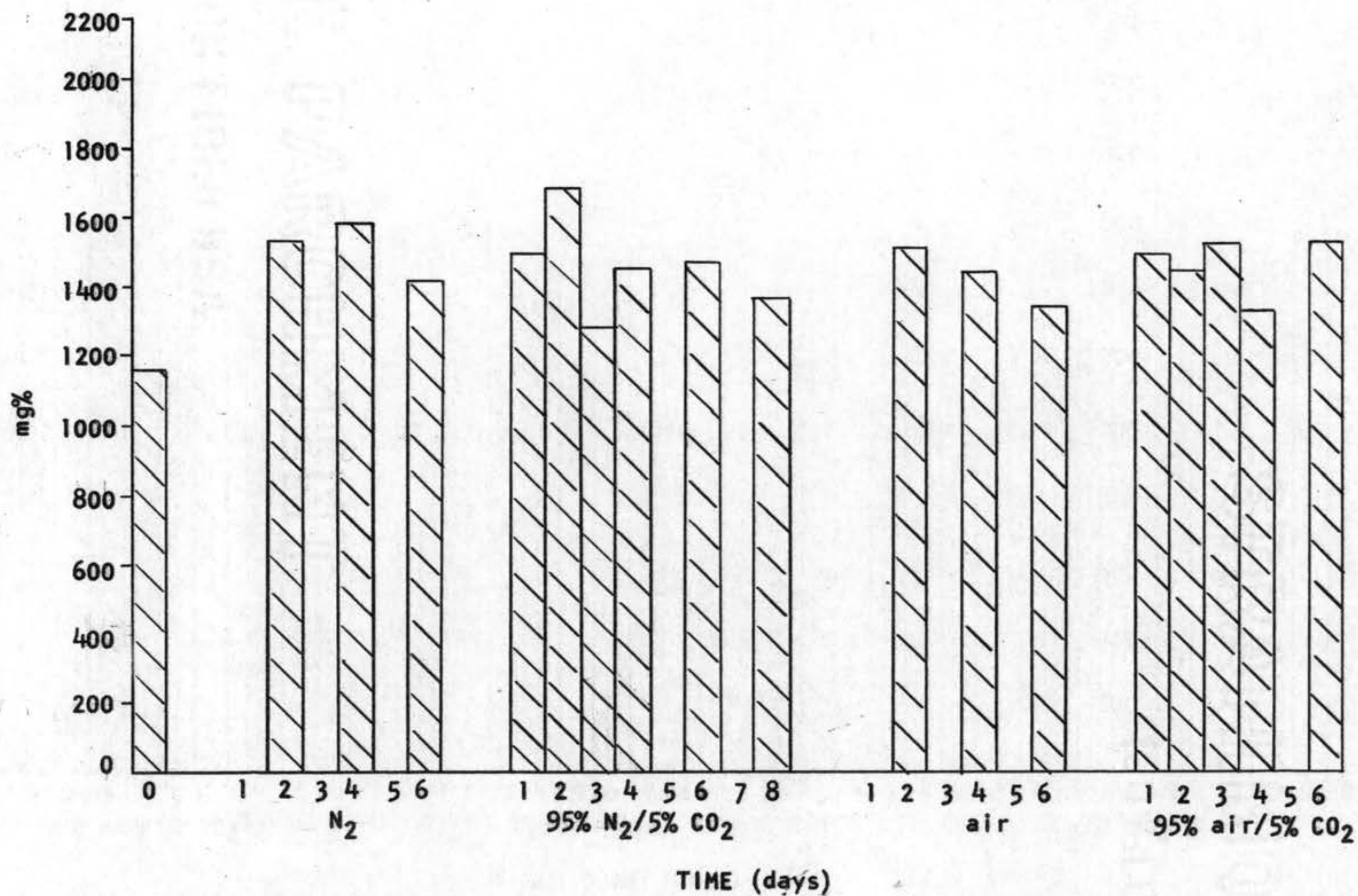


Figure 2. Total Lipid in the Reproductive System plus Eggs Following Starvation Under N_2, 95% N_2/5% CO_2, Air and 95% Air/5% CO_2.

starved. There was no significant difference between the mean values of the total lipid of the reproductive systems of worms starved in the presence and absence of CO_2 under either aerobic or anaerobic conditions.

<u>Combined Results</u>: The total lipid of the body wall and reproductive system combined are illustrated in Figure 3. When the results were combined the total lipid under all experimental conditions except day 3 under 95% N_2/5% CO_2 were significantly higher than that of the control worms.

A comparison of the combined results under aerobic and anaerobic conditions reveals few significant differences. When CO_2 was not included in the atmosphere under which the worms were starved, there was a significantly greater amount of lipid in the combined tissues under anaerobic conditions on day 2 than under aerobic conditions. The difference was not significant on days 4 and 6. When CO_2 was included in the atmosphere under which the worms were incubated, there was significantly less lipid in the combined tissues on day 3 under anaerobic conditions than under aerobic conditions. The difference was not significant on the other days. A comparison of the results in the presence and absence of CO_2 indicated that inclusion of CO_2 in the atmosphere under which the worms were starved had no significant effect on total lipid in the combined body wall and reproductive system.

<u>Discussion</u>: The results of this series of experiments indicate that there was an increase in lipid in the reproductive system during starvation under both aerobic and anaerobic conditions. The results for the body wall are variable, but when the difference from the control value was significant the means of the experimental groups had

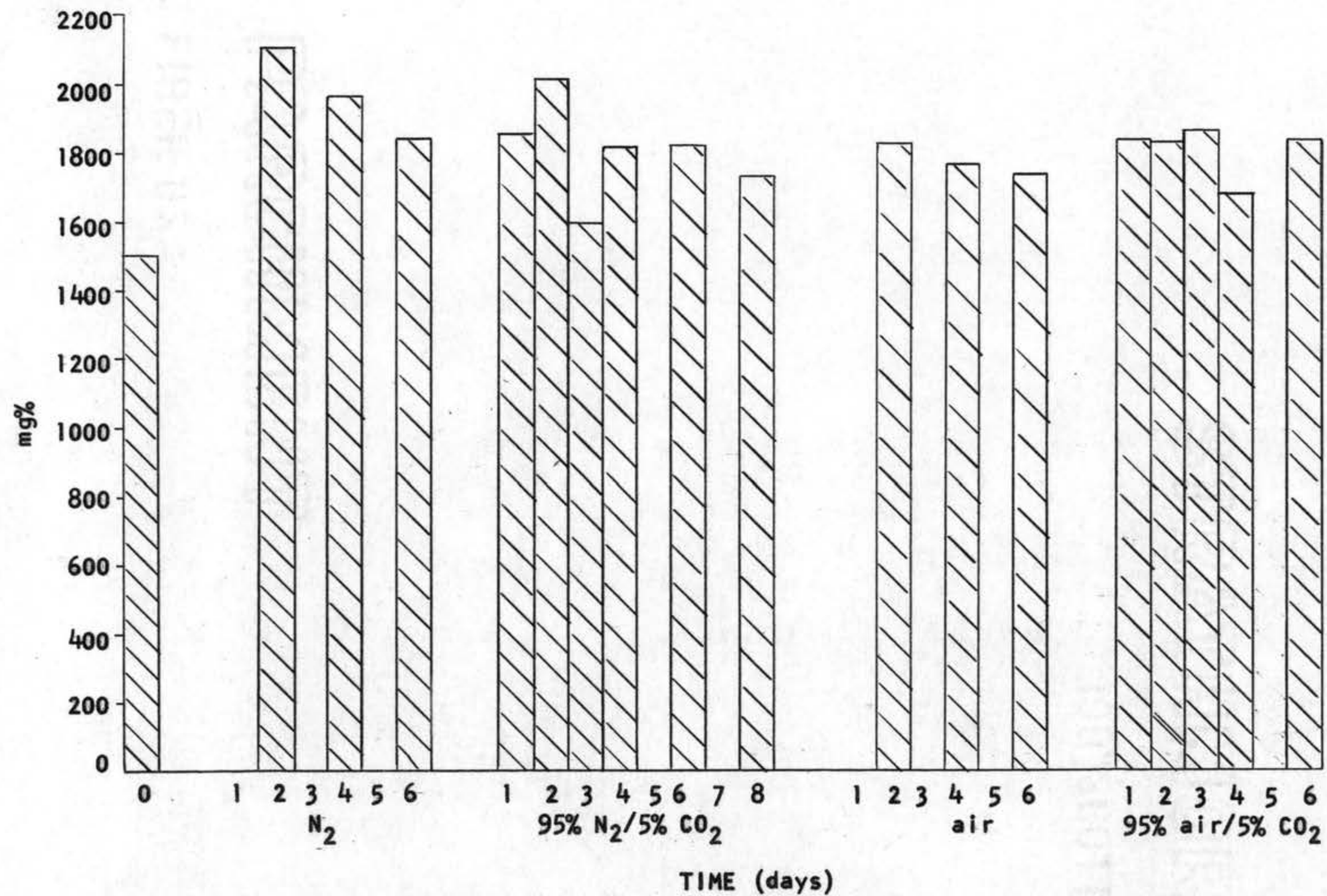


Figure 3. Total Lipid in the Body Wall and Reproductive System Combined Following Starvation Under N_2, 95% N_2/5% CO_2, Air and 95% Air/5% CO_2.

higher values than the mean of the control group. This indicates that lipid was synthesized by Ascaris even during starvation. Previous investigators have found no change in total lipid in the whole worm during starvation under aerobic and anaerobic conditions (15, 18, 144), except Schulte (135) who found an increase in total lipid of 0.1 gm./100 gm. body weight per 24 hours under anaerobic conditions. The glycogen content of Ascaris decreases during starvation (16, 17, 144), so the increase in lipid in the tissues observed in this investigation could have been the result of conversion of carbohydrate to lipid. Most of the increase in lipid during starvation of Ascaris occurred in the reproductive tract. This was probably related to the high rate of incorporation of lipid into eggs. Fairbairn (55) has estimated that in vivo, Ascaris females incorporate approximately 16 mg. of lipid per day into eggs. Production of eggs may continue for as long as 48 hours during starvation.

Some previous investigators have found cytological evidence of lipid mobilization in lateral line tissue and in the intestinal wall during culture of these tissues (78, 109). Mobilization of lipid may have occurred in this experiment, however, the only indication of possible mobilization in the body wall was when the worms were starved under atmospheres of N_2 and 95% air/5% CO_2. Under both atmospheres the lipid content of the body wall was highest on day 2 but was significantly less by day 6. This indicates that under these conditions lipids were synthesized more rapidly than they were mobilized during the first two days of starvation but later they were mobilized more rapidly than they were synthesized. A similar effect was noted in the reproductive

system when the worms were cultured under 95% N_2/5% CO_2 and air.

Second Experiment: Fatty Acids

The purpose of the second series of experiments was to determine if there are changes in the relative amounts of specific lipid fractions in the body wall, ovary-oviduct and uterus plus eggs as the result of starvation of the worms under an atmosphere of either 95% air/5% CO_2 or 95% N_2/5% CO_2. The lipid fractions measured were total lipid, neutral lipid, phospholipid, and the fatty acids of the saponifiable neutral lipids. The fatty acids were subjected to qualitative and quantitative analysis by gas-liquid chromatography.

Fatty acids of the saponifiable neutral lipids were examined in greater detail than were the other lipid fractions since this lipid fraction was thought to be the most likely fraction to be mobilized during starvation of the worm. Results of the quantitative analysis of the lipid fractions of the tissues are tabulated in Appendix C and are graphically illustrated in Figures 4 through 8. Statistical significance of the differences noted could not be determined since there was only one replication of each treatment.

Total Lipid: Total lipid in the body wall, ovary-oviduct and uterus plus eggs of the control group and of the groups subjected to the various experimental treatments is graphically illustrated in Figure 4. The lipid content in the body wall (Figure 4a) and ovary-oviduct tissue (Figure 4b) following starvation under both aerobic and anaerobic conditions was about the same as the control value for these tissues. The lipid content of the uterus plus eggs (Figure 4c)

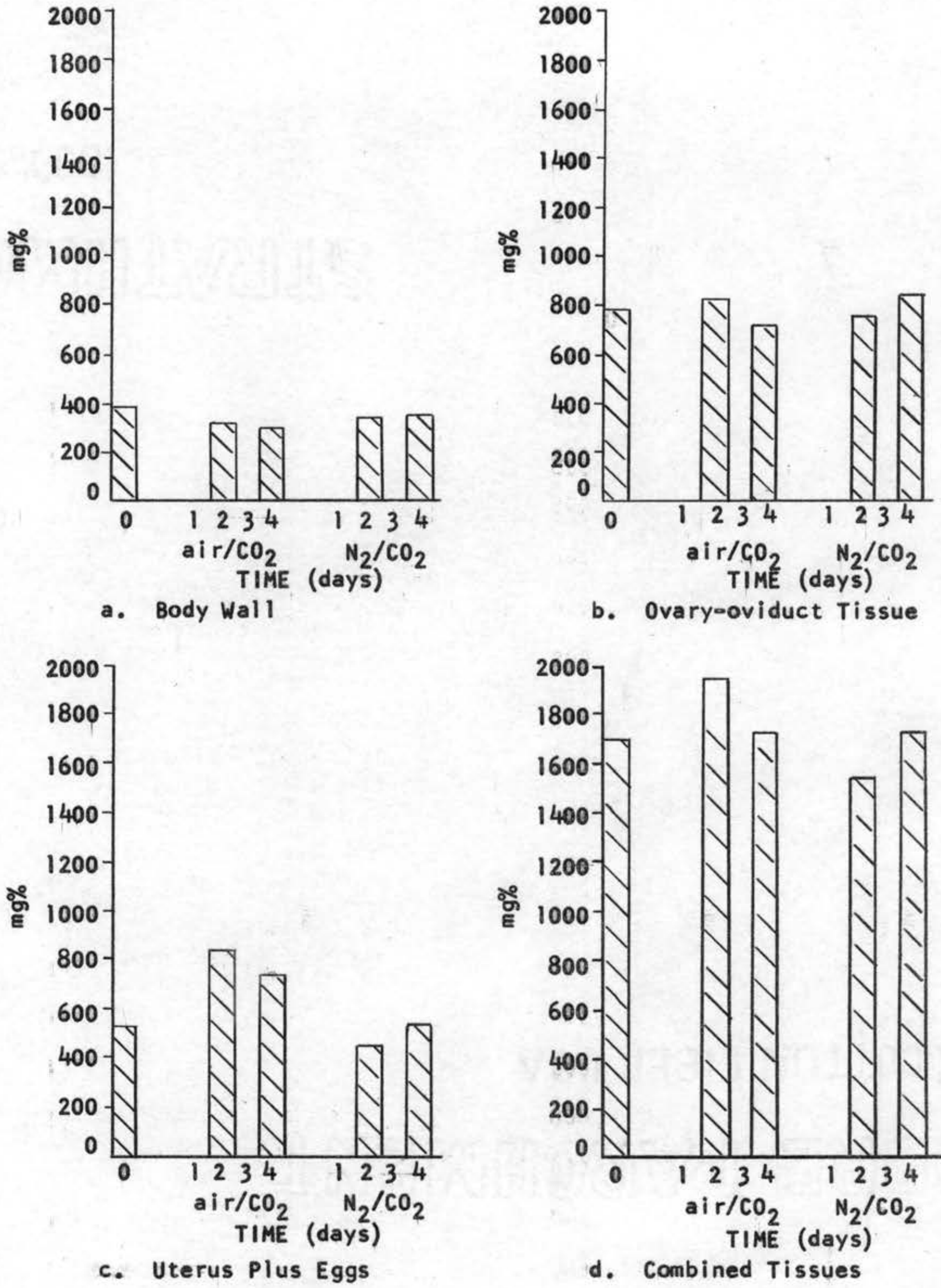


Figure 4. Total Lipid in the Body Wall, Ovary-oviduct Tissue and Uterus Plus Eggs Following Starvation Under 95% Air/5% CO_2 and 95% N_2/5% CO_2.

after 2 days starvation under aerobic conditions was about 50% greater than it was in the control group. After 4 days of aerobic starvation the lipid content of this tissue was 34% higher than it was in the control group. When the worms were starved for periods of 2 and 4 days under anaerobic conditions the lipid content of the uterus plus eggs was near the control value. When the values for aerobic conditions are inspected in Figure 4d, it is obvious that at the end of 2 days starvation the total lipid is 15% higher than the control value. After worms had been starved for 4 days under these environmental conditions the total lipid content of the tissues was similar to that of the control group. Under anaerobic conditions the total lipid of the combined tissues on day 2 was 9% less than in the control group but on day 4 it was about the same as in the control group.

Neutral Lipid: The neutral lipid content of the body wall, ovary-oviduct and uterus plus eggs under the various experimental conditions is graphically illustrated in Figure 5. The neutral lipid in the body wall appeared to decrease with time during starvation under both aerobic and anaerobic conditions. Under aerobic conditions the neutral lipid content of the body wall on day 4 was 84.9 mg% compared to the control value of 133.9 mg%. Under anaerobic conditions the neutral lipid content of the body wall on day 4 was 100.7 mg%. In both portions of the reproductive system the neutral lipids changed during starvation in a pattern similar to the changes in the total lipid. Under aerobic conditions the neutral lipid content of the reproductive tissues was higher on day 2 than on day 4, while under anaerobic conditions the lipid content of the reproductive tissues was lower on day

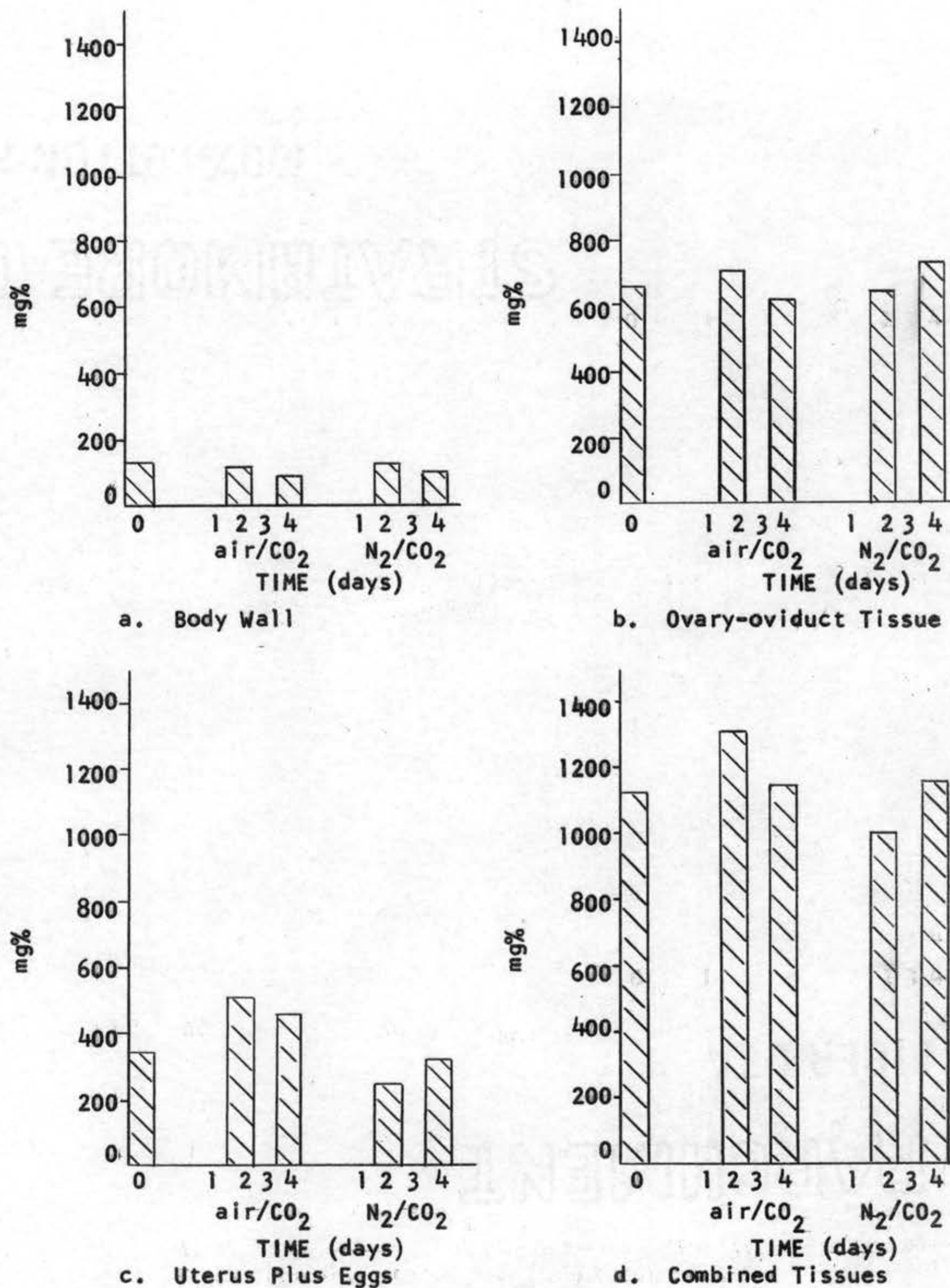


Figure 5. Neutral Lipid Content of the Body Wall, Ovary-oviduct Tissue and Uterus Plus Eggs Following Starvation Under 95% Air/5% CO_2 and 95% N_2/CO_2.

2 than on day 4. Comparison of the difference between the control value and experimental values of the total lipid and neutral lipid in the combined tissues revealed that on day 4 under anaerobic conditions the neutral lipids accounted for 48% of the change in total lipid while under all the other experimental conditions the neutral lipids accounted for 77-79% of the change in the total lipid from the control value.

Phospholipid: The phospholipid content of the body wall (Figure 6a) and ovary-oviduct (Figure 6b) under both aerobic and anaerobic conditions was either a little less than the control value or about the same as the control value. In the uterus plus eggs (Figure 6c) under aerobic conditions the phospholipid content on day 2 was about 50% higher than the control value and on day 4 was about 34% higher than the control value. Under anaerobic conditions the phospholipid content of the uterus plus eggs was only slightly above the control value. Under aerobic conditions the phospholipid content of the combined tissues on day 2 was greater than the control value but on day 4 was near the control value. Under anaerobic conditions the phospholipid content of the combined tissues on day 2 was less than the control value but on day 4 was near the control value.

Non-volatile Fatty Acids: The amounts of non-volatile fatty acids of the saponifiable neutral lipids (hereafter referred to as non-volatile fatty acids) in the tissues under the various experimental conditions are graphically illustrated in Figure 7. In the body wall (Figure 7a) under aerobic conditions there was an increase in non-volatile fatty acids while under anaerobic conditions there was a decrease. In the ovary-oviduct tissue (Figure 7b) under aerobic

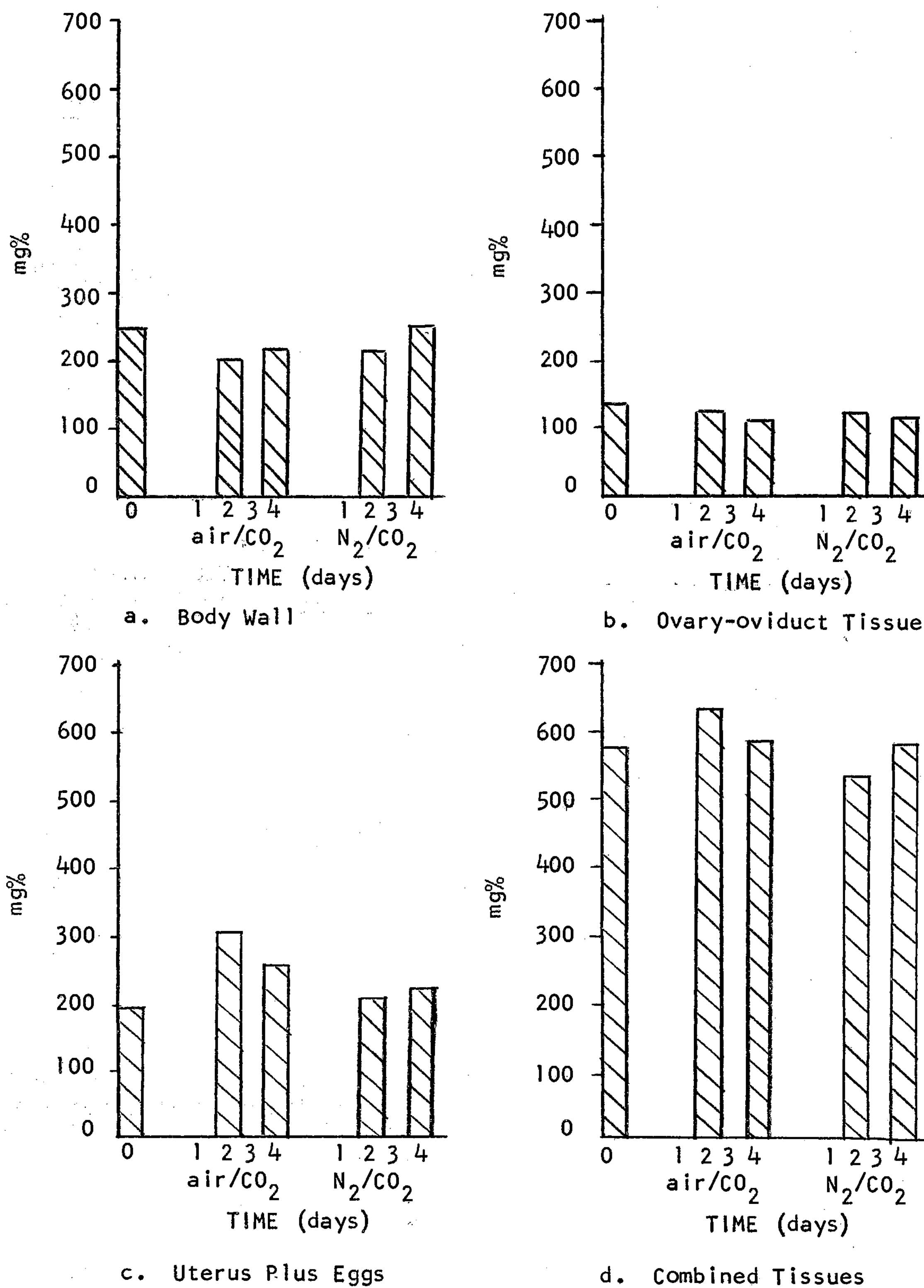


Figure 6. Phospholipid Content of the Body Wall, Ovary-oviduct Tissue and Uterus Plus Eggs Following Starvation Under 95% Air/ 5% CO_2 and 95% N_2/5% CO_2.

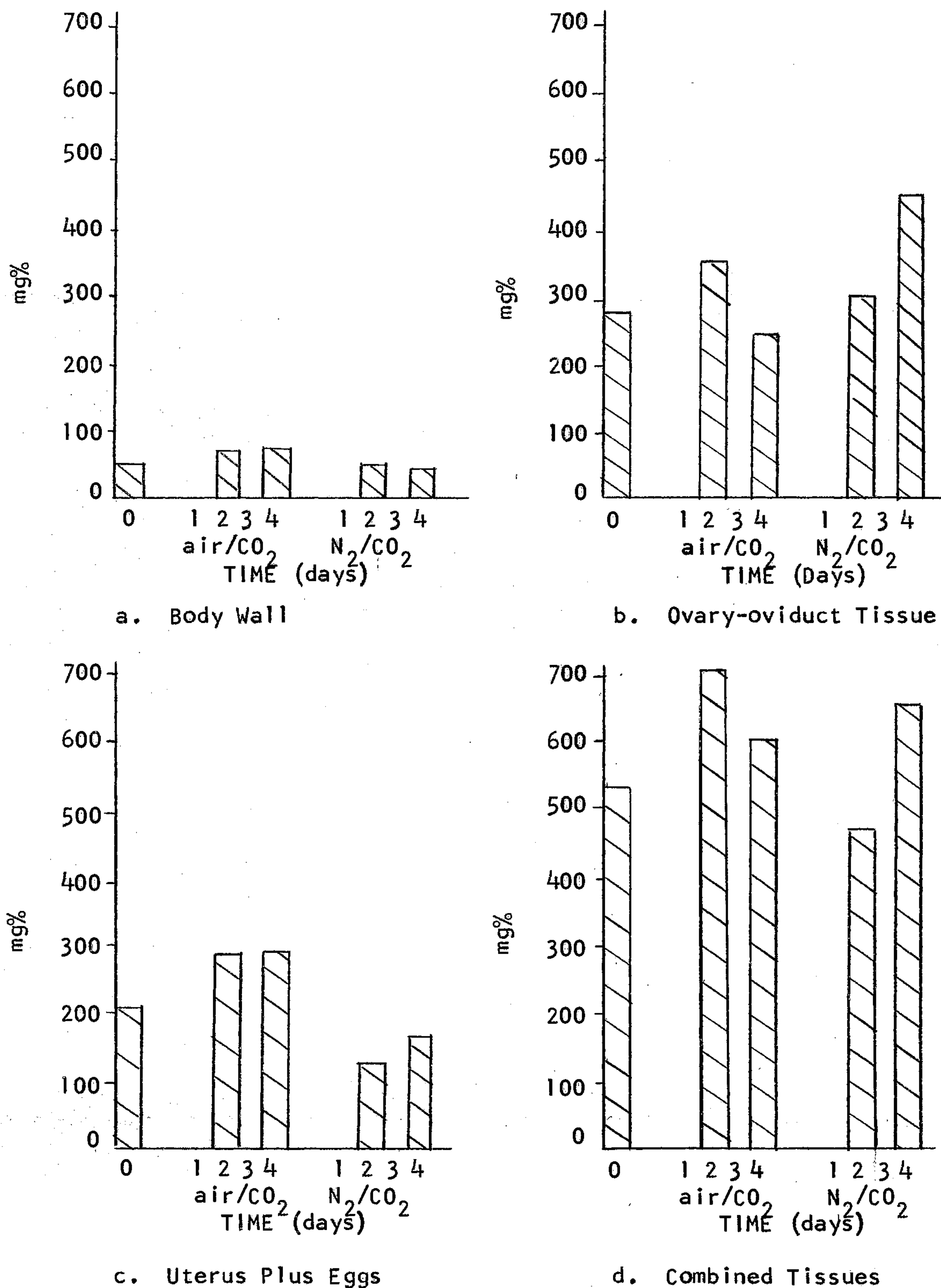
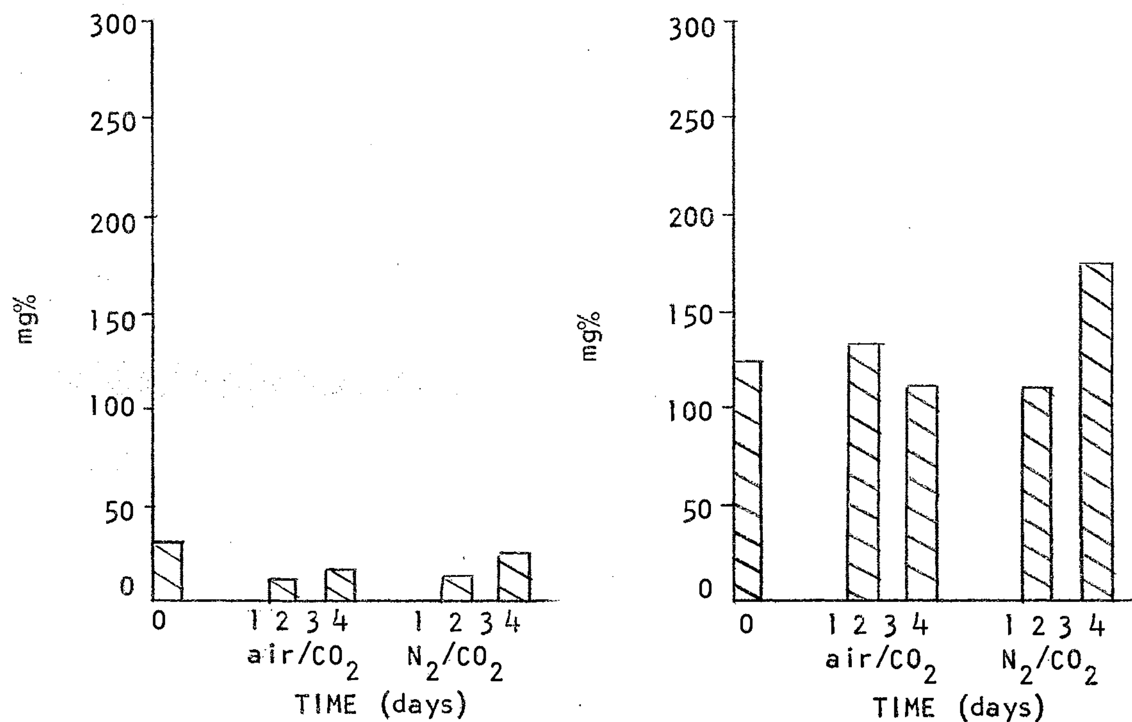


Figure 7. Non-volatile Fatty Acids of the Neutral Lipid of the Body Wall, Ovary-oviduct Tissue and Uterus Plus Eggs Following Starvation Under 95% Air/5% CO_2 and N_2/5% CO_2.

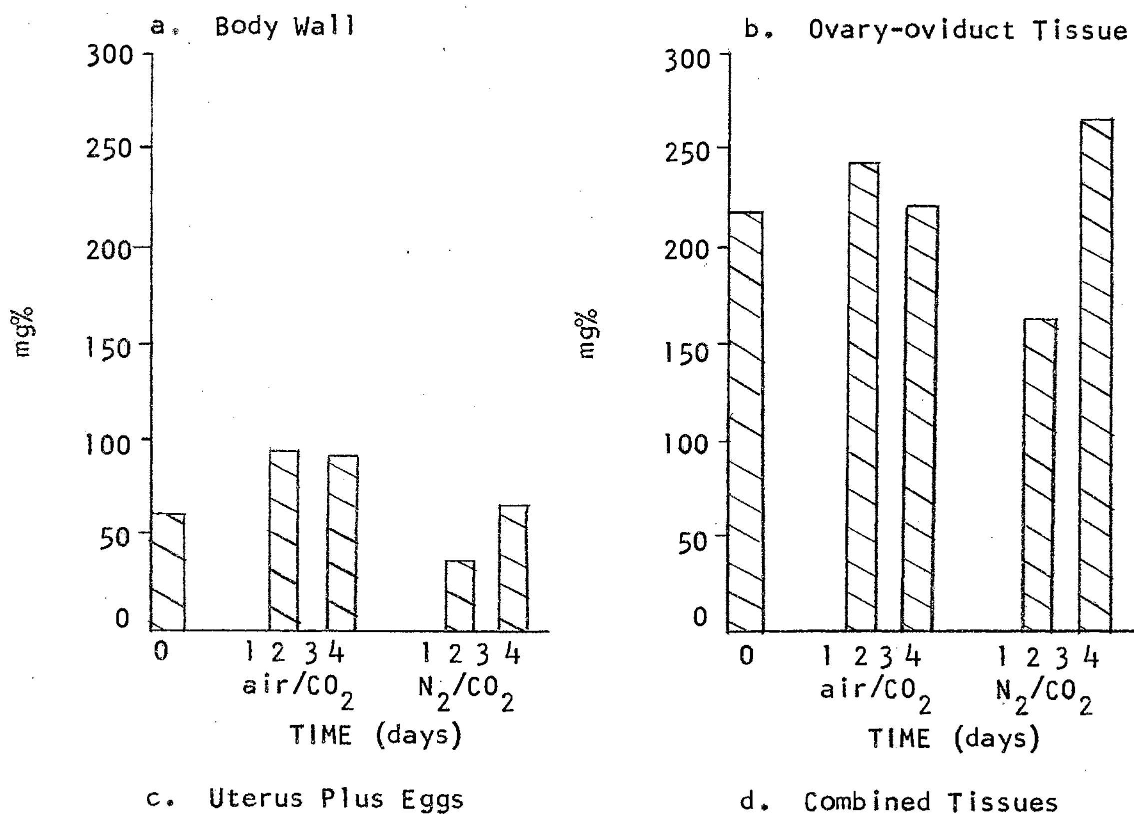
conditions on day 2 the content of non-volatile fatty acids was 27% greater than the control value while on day 4 the amount was 12% less than the control value. Under anaerobic conditions the quantity of non-volatile fatty acids in the ovary-oviduct tissue on day 2 was 9% greater than in the ovary-oviduct tissue of the control worms and on day 4 it was 63% greater than in the ovary-oviduct tissue of the control worms. Under aerobic conditions there was about 37% more non-volatile fatty acids in the uterus plus eggs (Figure 7c) than in the uterus plus eggs of the control worms. Under anaerobic conditions the content of non-volatile fatty acids in the uterus plus eggs on day 2 was 41% less than in the uterus plus eggs of the control worms and on day 4 it was 23% less than in the uterus plus eggs of the control worms. Under aerobic conditions the quantity of non-volatile fatty acids was highest in the three tissues combined on day 2. The non-volatile fatty acid content of the combined tissue was lower on day 4 than on day 2 but was still above the control value on day 4. Under anaerobic conditions the amount of non-volatile fatty acids in the combined tissues on day 2 was less than the control value, while on day 4 it was greater than the control value.

Volatile Fatty Acids: The amount of volatile fatty acids of the saponifiable neutral lipids (referred to as volatile fatty acids in the remainder of this disussion) in the body wall, ovary-oviduct and uterus plus eggs under the various experimental conditions is graphically illustrated in Figure 8. The quantity of volatile fatty acids in the body wall following starvation under aerobic and anaerobic conditions was less than 25 mg%, which is lower than in the body wall of the control worms which contained 32 mg%. The amount of volatile fatty acids



a. Body Wall

b. Ovary-oviduct Tissue



c. Uterus Plus Eggs

d. Combined Tissues

Figure 8. Volatile Fatty Acids of the Neutral Lipid of the Body Wall, Ovary-oviduct Tissue and Uterus Plus Eggs Following Starvation Under 95% Air/5% CO_2 and 95% N_2/5% CO_2.

in the ovary-oviduct tissue (Figure 8b) after starvation under aerobic conditions was about the same as in the ovary-oviduct tissue of the unstarved control worms. Following starvation of the worms under anaerobic conditions the quantity of volatile fatty acids in the ovary-oviduct tissue on day 2 was slightly less than the control value while on day 4 the amount of volatile fatty acids was 41% higher than the control value. When the worms were starved under aerobic conditions the amount of volatile fatty acids in the uterus plus eggs (Figure 8c) was higher than in the control value. Under anaerobic conditions on day 2 the quantity of volatile fatty acids in the uterus plus eggs was lower than the control value and on day 4 it was higher than the control value. When the results from the individual tissues were combined (Figure 8d), there was little difference between the results of the control group and the results of the groups starved under aerobic conditions. Under anaerobic conditions the quantity of volatile fatty acids in the combined tissues on day 2 was 25% less than in the combined tissue of the control worms but on day 4 was 12% higher than the control value.

Chromatography of the Non-volatile Fatty Acids: Methyl esters of the non-volatile fatty acids were analyzed by gas-liquid chromatography. The chromatograms obtained by using an ethylene glycol succinate column were analyzed for qualitative and quantitative results and a typical chromatogram is presented in Appendix G. The retention times of the methyl esters of the fatty acids in typical samples from each tissue and of the fatty acids in authentic standards relative to the retention time of methyl stearate are listed in Table III. The methyl esters are referred to by the numbering system developed by Ahrens, *et al*. (1).

TABLE III

GAS CHROMATOGRAPHIC IDENTIFICATION OF METHYL ESTERS OF NON-VOLATILE FATTY ACIDS OF THE NEUTRAL LIPIDS OF *ASCARIS* USING AN ETHYLENE GLYCOL SUCCINATE COLUMN

	RETENTION TIME RELATIVE TO 18:0				
	AUTHENTIC STANDARDS		*ASCARIS* TISSUE		
Fatty Acid	NIH	Applied Science	Body Wall	Ovary-Oviduct	Uterus/Eggs
6:0		0.035			
8:0	0.055	0.059			
9:0br			0.070	0.066	0.064
9:0		0.078			
10:0	0.099	0.104			
11:0		0.138			
12:0	0.177	0.184	0.179	0.175	0.173
13:0		0.242		0.232	0.227
14:0	0.316	0.324	0.316	0.315	0.311
15:0br			0.369	0.361	0.361
15:0		0.429	0.441	0.415	0.415
16:0	0.564	0.572	0.562	0.560	0.564
16:1	0.696		0.678	0.678	0.683
17:0		0.754	0.746	0.747	0.742
18:0	1.000	1.000	1.000	1.000	1.000
18:1	1.199	1.191	1.19	1.20	1.20
19:0		1.292	1.35	1.35	1.35
18:2		1.547	1.57	1.57	1.57
20:0	1.776	1.751			
18:3		2.129	2.16	2.16	2.16
21:1			2.73	2.74	2.74
22:0	3.158	3.069			
22:1		3.563			
23:0			3.89	3.89	3.89
24:0		5.329			
24:1		6.168			

Figure 9 is a plot of the log of the relative retention time of each acid versus the number of carbon atoms in its carbon skeleton. The identity of each acid was verified by gas-liquid chromatography of a few samples on an Apiezon L column and a typical chromatogram is presented in Appendix H. The retention times of the methyl esters of the fatty acids in the authentic standards and in selected experimental samples relative to the retention time of methyl stearate are listed opposite their identity in Table IV. The graph in Figure 10 is a plot of the data in Table IV. The identity of the methyl esters of the fatty acids was further checked by plotting the log of their relative retention times on the ethylene glycol succinate column against the log of their relative retention times on the Apiezon L column (Figure 11). Points representing acids having similar structures fall on a straight line on all of these graphs.

Two series of branched chain acids were tentatively identified as well as saturated straight chain, monounsaturated, diunsaturated and triunsaturated acids. All of the non-volatile fatty acids identified by Beames (6) were found in all experimental samples and in addition, the following non-volatile fatty acids were identified tentatively: 7:0br, 8:0, 9:0, 9:0br, 10:0, 10:0br, 11:0, 11:0br, 12:0br, 13:1, 12:0br, 11:0br, 13:0br, 13:0br, 15:1, 14:0br, 17:0br, 18:0br, 18:3, 19:2, 19:1, 19:0, 19:0br, 20:2, 20:0br, 21:1, 20:0br, 23:0. Most of these acids were found on the chromatogram from the Apiezon L column only, and were present in only trace amounts. In order to detect them, so much of the sample had to be added to the column that the more abundant acids were not quantitatively recorded. Table V contains the quantitative results

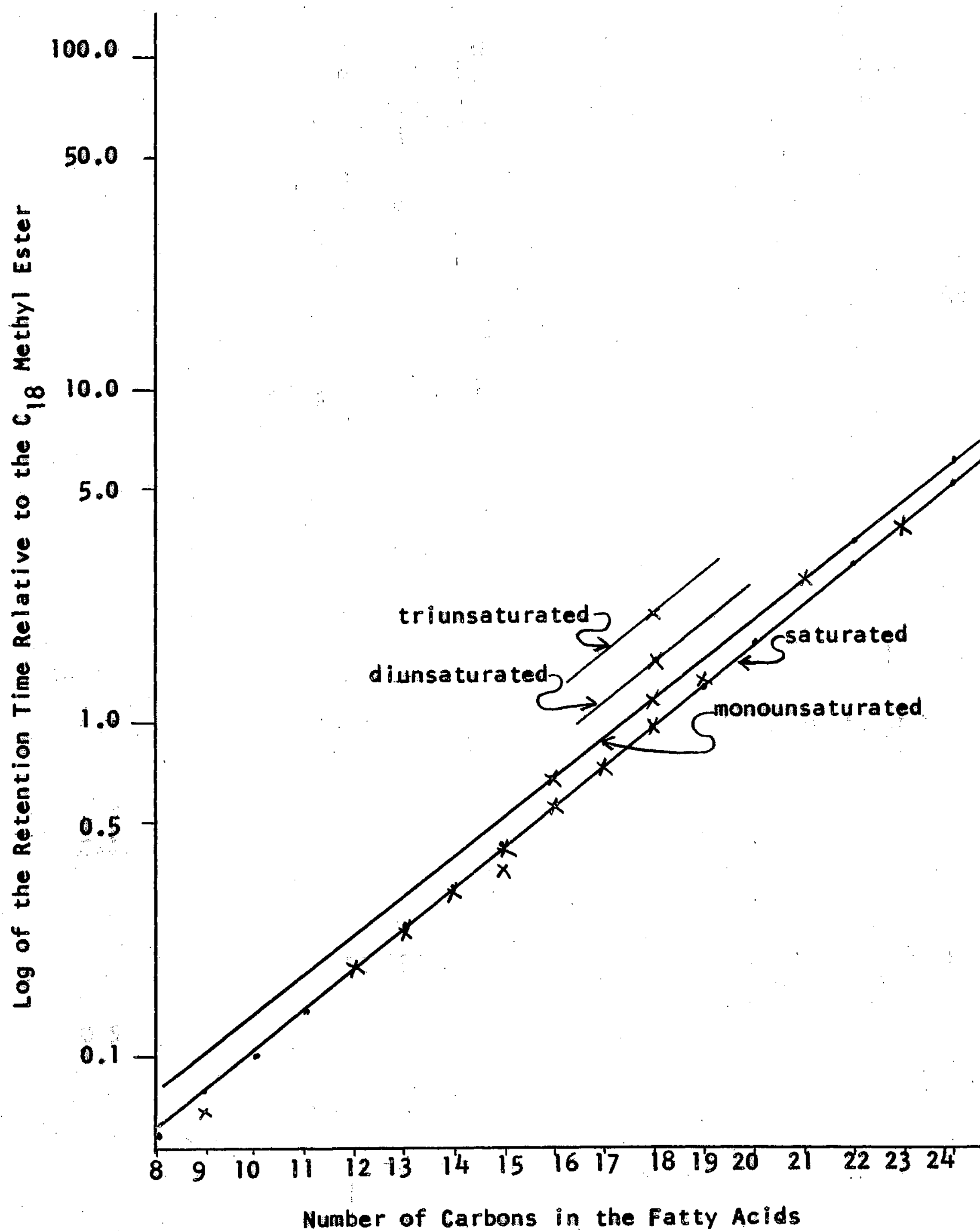


Figure 9. Plot of the Log of the Relative Retention Time Versus the Number of Carbons for the Non-volatile Fatty Acid Methyl Esters Chromatographed on an Ethylene Glycol Succinate Column.

TABLE IV

GAS CHROMATOGRAPHIC IDENTIFICATION OF METHYL ESTERS OF NON-VOLATILE FATTY ACIDS OF THE NEUTRAL LIPIDS OF ASCARIS USING AN APIEZON L COLUMN

Fatty Acid	RETENTION TIME RELATIVE TO 18:0				
	AUTHENTIC STANDARDS		ASCARIS TISSUE		
	NIH	Applied Science	Body Wall	Ovary-Oviduct	Uterus/Eggs
6:0		0.006			
7:0br				0.011	
8:0		0.014	0.017	0.015	
9:0		0.021	0.025	0.023	
9:0br			0.029	0.028	
10:0	0.035	0.034	0.035	0.032	
10:0br			0.043	0.041	
11:0		0.052	0.053	0.052	
11:0br			0.059	0.058	0.060
11:0br			0.074	0.072	
12:0	0.081	0.080	0.080	0.079	0.081
12:0br			0.090	0.090	0.091
13:1			0.102	0.109	
12:0br					0.116
13:0		0.123		0.124	
13:0br			0.134		
14:1			0.155		
13:0br			0.172		
14:0	0.189	0.189	0.186	0.186	0.189
15:1			0.243	0.241	0.244
14:0br			0.260		
15:0		0.288	0.286	0.283	0.287
15:0br			0.337		
16:1	0.385		0.382	0.381	0.383
16:0	0.436	0.437	0.435	0.433	0.438
17:1			0.574	0.570	0.572
17:0		0.661	0.660	0.659	0.660
17:0br				0.733	0.735
18:2&3		0.811	0.817	0.813	0.811
18:1	0.862	0.861	0.864	0.869	0.859
18:0	1.000	1.000	1.000	1.000	1.000
18:0br				1.090	
19:2			1.183	1.181	1.184
19:1			1.302	1.303	1.302
19:0		1.535	1.479	1.476	1.474
19:0br			1.621	1.635	1.625
20:2			1.888	1.845	1.839
20:0	2.279	2.305	2.290	2.294	2.280
20:0br			2.456		
21:1			2.994	2.981	2.960
20:0br			3.249		3.247
22:1		4.487			
22:0		5.200			

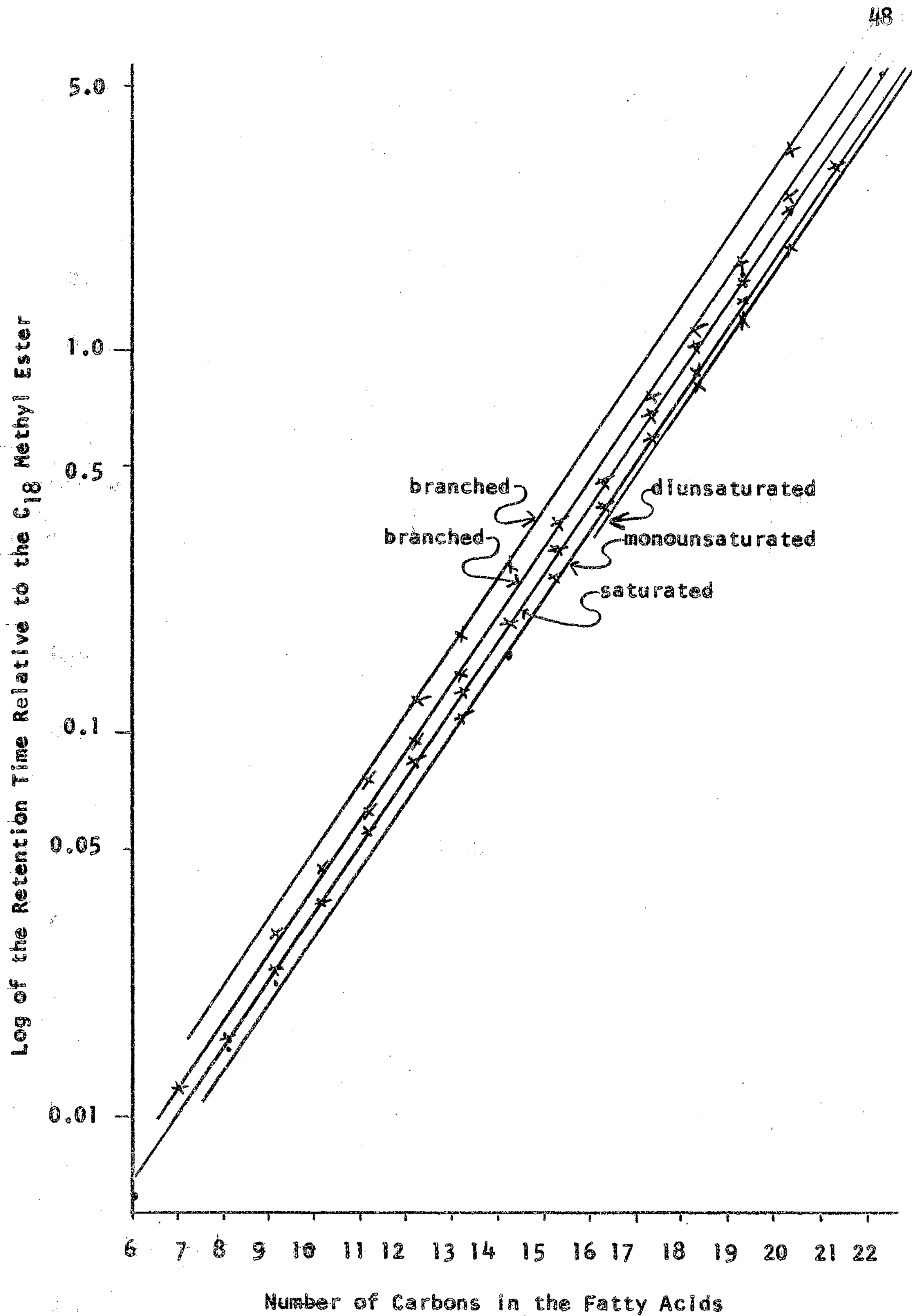


Figure 10. Plot of the Log of the Relative Retention Time Versus the Number of Carbons for the Non-volatile Fatty Acid Methyl Esters Chromatographed on an Apiezon L Column.

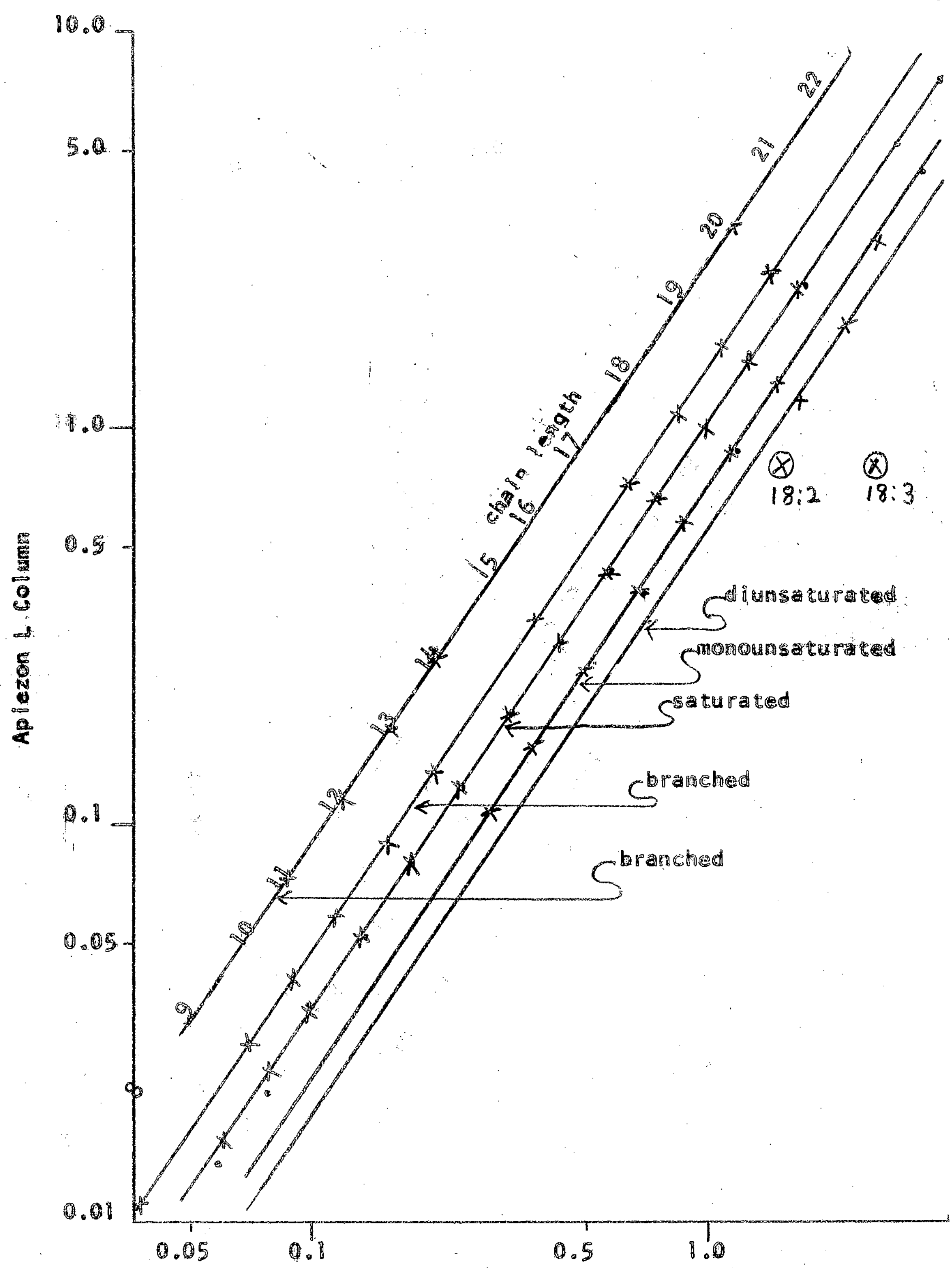


Figure 11. Plot of the Log of the Relative Retention Time of Methyl Esters on an Apiezon L Column Versus the Log of the Relative Retention Time of the Methyl Esters on an Ethylene Glycol Succinate Column.

TABLE V

QUANTITATIVE ANALYSIS OF NON-VOLATILE FATTY ACIDS IN FEMALE *ASCARIS* TISSUES FOLLOWING STARVATION UNDER 95% Air/5% CO_2 AND 95% N_2/5% CO_2

Fatty Acid	METHYL ESTERS AS PERCENTAGE OF TOTAL METHYL ESTERS				
	Control	Air/5% CO_2		N_2/5% CO_2	
		Day 2	Day 4	Day 2	Day 4
BODY WALL					
9:0br	3.0	2.4	1.8	4.1	6.0
12:0	0.3	Tr	0.3	0.7	Tr
13:0		Tr	0.3	Tr	
14:0	0.8	1.4	1.6	1.6	Tr
15:0br	1.8	2.3	2.8	2.3	Tr
15:0		0.6	0.4		Tr
16:0	9.8	13.7	15.9	8.5	4.0
16:1	Tr	0.9	0.7	Tr	0.7
17:0	Tr	0.9	0.5	Tr	0.6
18:0	11.1	10.0	8.8	10.1	11.7
18:1	24.3	22.8	22.5	21.6	22.5
19:0	2.1	1.0	1.5	4.2	6.9
18:2	27.1	35.6	35.0	32.4	27.2
18:3	1.4	1.3	1.2	1.8	1.5
21:1	2.3	2.8	2.3	4.0	3.9
23:0	16.2	3.6	4.4	8.6	14.3
OVARY-OVIDUCT					
9:0br	0.3	0.4	0.5	1.0	0.9
12:0	Tr	Tr	Tr	Tr	Tr
13:0	Tr	Tr	Tr	Tr	Tr
14:0	0.8	0.4	0.8	0.9	0.6
15:0br	1.4	1.0	1.5	1.5	1.2
15:0	0.7	0.3	0.3	0.3	0.2
16:0	15.0	13.0	15.6	15.4	15.1
16:1	1.0	0.6	0.6	1.0	0.7
17:0	1.4	0.7	0.6	1.2	0.6
18:0	9.0	9.8	9.3	8.1	8.7
18:1	28.4	26.1	25.0	27.2	29.6
19:0					0.7
18:2	29.1	37.0	35.9	29.3	29.3
18:3	1.0	1.4	1.8	1.8	1.1
21:1	2.6	3.1	3.3	2.8	2.8
23:0	9.3	5.7	4.8	8.9	7.8

TABLE V Continued

Fatty Acid	METHYL ESTERS AS PERCENTAGE OF TOTAL METHYL ESTERS				
	Control	Air/5% CO_2		N_2/5% CO_2	
		Day 2	Day 4	Day 2	Day 4
UTERUS AND EGGS					
9:0br	1.0	0.3	0.9	0.8	1.5
12:0	0.3	Tr	Tr	0.2	Tr
13:0		Tr	Tr	Tr	Tr
14:0	0.7	0.7	1.0	0.9	1.0
15:0br	1.7	1.6	1.9	1.6	1.8
15:0	0.5	0.6	0.7	0.4	0.5
16:0	15.8	14.0	15.3	14.0	13.0
16:1	0.6	0.7	0.6	0.7	0.9
17:0	1.2	0.7	0.7	0.8	0.6
18:0	9.5	10.1	9.6	8.5	8.2
18:1	29.8	26.9	25.7	27.2	28.1
19:0				Tr	1.4
18:2	29.1	37.4	36.8	30.7	32.0
18:3	0.9	2.0	1.6	1.6	1.2
21:1	2.3	2.8	2.8	3.1	2.7
23:0	6.8	2.4	2.5	8.4	6.5

of chromatography on the ethylene glycol succinate column. The most abundant acids were 16:0, 18:0, 18:1, 18:2, and 23:0 accounting for 88.5 to 91.0 per cent of the total volatile fatty acids.

Examination of Table V reveals that several fatty acids vary quantitatively from the control following starvation. In the body wall under aerobic conditions, palmitic acid increased to 6% of the total non-volatile fatty acids above the control and linoleic acid increased to 8% of the total non-volatile fatty acids above the control. Tricosanoic acid decreased from 16% of the total non-volatile fatty acid in the control sample to 4% following starvation. Several other fatty acids in the body wall show small changes following starvation under aerobic conditions. Under anaerobic conditions several of the non-volatile fatty acids in the body wall increased slightly by day 2 and tricosanoic acid decreased to about half of the control value. On day 4 under anaerobic conditions the tricosanoic acid in the body wall was near the control value and nonanoic acid and nonadecanoic acid were above the control value. The value for palmitic acid was about half the control value.

The amounts of specific acids following starvation of the worm varied less in the ovary-oviduct tissue than in the body wall. Under aerobic conditions in the ovary-oviduct, linoleic acid increased to 7% of the total non-volatile fatty acids above the control value and tricosanoic acid decreased to 4% below the control value. Several other acids varied from the control values by small amounts. Under anaerobic conditions the changes in amounts of the various fatty acids in the ovary-oviduct tissue were all less than 2% of the total non-volatile fatty acid.

Differences from the control following starvation were less pronounced in the uterus plus eggs than in the body wall. Following starvation under aerobic conditions linoleic acid was about 8% of the total non-volatile fatty acids higher than in the control while oleic acid and tricosanoic acid were each about 4% of the total non-volatile fatty acid lower than in the control. Under anaerobic conditions, linoleic acid was 3% of the total non-volatile fatty acids above the control on day 4 but none of the other acids varied much from the control value.

<u>Chromatography of the Volatile Fatty Acids</u>: The free volatile fatty acids isolated from the tissues were analyzed by chromatography on a DC 550/stearic acid column and a typical chromatogram is presented in Appendix I. Formic acid was not quantitatively recorded because some contaminating substance caused a negative peak on the chromatogram at the time formic acid should have been eluted. The fatty acids were identified by comparison of their relative retention times with those of known acids. Quantitative results of gas-liquid chromatography of the free volatile fatty acids are listed in Table VI.

The amount of the volatile fatty acids recovered from the body wall following starvation was so small that not enough of the sample could be placed on the chromatograph to be able to obtain quantitative results. The results that were obtained indicate that 2-methylbutyric acid and 2-methylvaleric acid were probably the most abundant volatile fatty acids in the body wall.

Larger amounts of volatile fatty acids were recovered from the ovary-oviduct tissue than from the body wall and it was possible to quantitatively analyze the chromatograms. Acetic acid was abundant in the ovary-oviduct

TABLE VI

QUANTITATIVE ANALYSIS OF VOLATILE FATTY ACIDS IN FEMALE
ASCARIS TISSUES FOLLOWING STARVATION UNDER
95% AIR/5% CO_2 AND 95% N_2/5% CO_2

Fatty Acid	Retention Time*	FATTY ACIDS AS PERCENTAGE OF TOTAL VOLATILE FATTY ACIDS				
		Control	Air/5% CO_2		N_2/5% CO_2	
			Day 2	Day 4	Day 2	Day 4
BODY WALL						
Formic	0.092	1.5				
Acetic	0.175	0.7				
Propionic	0.350	Tr	Tr	Tr	Tr	Tr
Isobutyric	0.419	1.0	2.4	Tr	1.4	Tr
2-methylbutyric	1.00	24.3	27.0	Tr	17.5	Tr
2-methylvaleric	2.03	72.6	70.5		81.1	
OVARY AND OVIDUCTS						
Formic	0.092		0.6	1.3	3.0	
Acetic	0.175	15.5	30.3	29.3	16.3	21.8
Propionic	0.350	6.2	1.1	0.7	7.3	7.7
Isobutyric	0.419	Tr		Tr	3.4	4.3
2-methylbutyric	1.00	23.0	23.3	23.1	23.3	23.2
2-methylvaleric	2.03	55.4	44.7	45.7	46.8	43.0

TABLE VI Continued

Fatty Acid	Retention Time	FATTY ACIDS AS PERCENTAGE OF TOTAL VOLATILE FATTY ACIDS				
		Control	Air/5% CO_2		N_2/5% CO_2	
			Day 2	Day 4	Day 2	Day 4
UTERUS PLUS EGGS						
Formic	0.092		0.4	1.7		
Acetic	0.197		4.7	0.3	Tr	Tr
Propionic	0.350	2.2	1.2	0.3	Tr	1.7
Isobutyric	0.419	0.7	0.2	0.8		
Butyric	0.671			0.6		3.2
2-methylbutyric	1.00	31.9	31.8	24.6	25.6	28.5
Valeric	1.39		1.8	Tr		
2-methylvaleric	2.03	65.2	60.0	71.6	74.0	66.6

*Relative to 2-methylbutyric

tissue and it varied more under the experimental conditions than any other volatile fatty acid. Following starvation under aerobic conditions 30% of the volatile fatty acids was acetic acid while in the control only 15% of the volatile fatty acids was acetic acid. Propionic acid and 2-methylvaleric acid were both present in smaller quantities following starvation under aerobic conditions than in the control. Following starvation under anaerobic conditions 2-methylvaleric acid was present in lower concentration than in the control but isobutyric acid increased from a trace in the control to about 4% of the volatile fatty acids. Acetic acid and propionic acid both increased above the control during starvation of the worms under anaerobic conditions but the increase in acetic acid under anaerobic conditions was only about 1/3 the increase under aerobic conditions.

In the uterus plus eggs over 90% of the volatile fatty acid was 2-methylbutyric acid and 2-methylvaleric acid in all of the samples. Small amounts of butyric and valeric acids were detected in some of the samples. The difference in the amount of specific volatile fatty acids in the uterus plus eggs under the various experimental conditions was of doubtful significance.

<u>Discussion</u>: Following starvation under aerobic conditions the total lipid, neutral lipid, phospholipid and volatile fatty acids in the body wall decreased while the non-volatile fatty acids increased slightly. The concentration of fatty acids in mg% (volatile and non-volatile combined) in the body wall was about the same following starvation as in the control. It appears that the decrease in total lipid was the result of a decrease in phospholipids and neutral lipids other than the fatty acids.

In the ovary-oviduct tissue under aerobic conditions the phospholipids appeared to be mobilized, while the other lipid fractions were all above the control value by day 2 but less than the control value by day 4. This indicates that in the ovary-oviduct tissue neutral lipids were synthesized during the first 2 days of starvation under aerobic conditions but were later mobilized more rapidly than they were synthesized. In the uterus plus eggs under aerobic conditions all lipid fractions increased to about the same extent. Since the concentration of all the lipid fractions under aerobic conditions was lower on day 4 than on day 2, it appeared that there was a high rate of incorporation of lipids into the uterus plus eggs the first 2 days of starvation followed by some mobilization of lipids from this tissue.

Under anaerobic conditions the lipid content of the tissues was slightly below the control on day 2 but near the control or slightly above it on day 4. The total fatty acids in the body wall was below the control value on both day 2 and day 4 but the changes in the other lipid fractions were irregular. It appeared that in the body wall the fatty acids were not synthesized and may have been mobilized to a limited extent under anaerobic conditions. In the ovary-oviduct tissue under anerobic conditions the phospholipids content decreased with time which indicated that they were mobilized in this tissue. The total fatty acids in the ovary-oviduct tissue under anaerobic conditions on day 2 was near the control level but on day 4 was much higher than in the control. This indicated that fatty acids were synthesized in the ovary-oviduct tissue or transferred to it from some other tissue. In the uterus plus eggs the phospholipids increased during starvation under anaerobic conditions. It is

possible that the phospholipids were transferred into the uterus from the ovary-oviduct as components of eggs that were produced by the ovary-oviduct tissue. All other lipid fractions in the uterus plus eggs were below the control level following starvation under anaerobic conditions. It appeared that the fatty acids in the uterus plus eggs could have been mobilized during starvation. The results indicated that lipid synthesis in *Ascaris* was lower under anaerobic conditions than under aerobic conditions.

Fairbairn reported that the weight of the volatile fatty acids in *Ascaris* tissue was less than the weight of the non-volatile fatty acids; however, on a molar basis they formed about half of the fatty acids of the saponifiable neutral lipids (54). Table VII contains a list of the ratio of moles of volatile fatty acids per mole of non-volatile fatty acids found in this study.

TABLE VII

MOLAR RATIO OF VOLATILE AND NON-VOLATILE FATTY ACIDS IN THE NEUTRAL LIPIDS

TREATMENT	BODY WALL	OVARY-OVIDUCT	UTERUS PLUS EGGS
Control	1.744	1.268	0.805
Air/5% CO_2			
Day 2	0.542	1.068	0.929
Day 4	0.637	1.296	0.882
N_2/5% CO_2			
Day 2	0.776	1.044	0.832
Day 4	1.500	1.092	1.133

The average molecular weight of the volatile fatty acids was assumed to be 100 for purposes of calculation. The average molecular weight of the

volatile fatty acids based on gas-liquid chromatography of the control sample for each tissue was: body wall, 101.7; ovary-oviduct, 93.7; uterus plus eggs, 99.1. The average molecular weight of the non-volatile fatty acids based on gas-liquid chromatography of the control sample from the various tissues was: body wall, 282; ovary-oviduct, 281; uterus plus eggs, 279. Fairbairn (54) reported the mean molecular weight of the volatile fatty acids was 90 and the mean molecular weight of the non-volatile acids was 290.

There were some large changes in ratio of the volatile fatty acids to non-volatile fatty acids in the body wall under aerobic conditions. The ratio decreased under aerobic conditions although the total fatty acid changed little from the control value. The actual amount of fatty acid isolated from the body wall was small so the ratio would be affected considerably by the completeness of separation of the volatile fatty acids from the non-volatile fatty acids by steam distillation. Since there was no increase in the total fatty acids it is likely that varying degrees of separation of the volatile fatty acids from the non-volatile fatty acids was the most probable explanation of the change in ratio.

In the ovary-oviduct tissue and the uterus plus eggs, there was little change in the ratio of the volatile fatty acids to non-volatile fatty acids although the concentration of the total fatty acids changed considerably under some experimental conditions. This indicated that the mobilization of volatile and non-volatile fatty acids was similar under the experimental conditions employed.

The amounts of specific fatty acids were more variable in the body wall than in the other tissues. There was no particular pattern associated

with the changes in specific non-volatile fatty acids of the body wall or the reproductive tissues. The changes involved saturated as well as unsaturated acids, although the quantity of three non-volatile acids changed more under experimental conditions than did the quantity of the other non-volatile fatty acids. Linoleic acid increased in amount in all tissues under all experimental conditions, although the increase was greatest under aerobic conditions. Palmitic acid increased in quantity in the body wall under aerobic conditions but decreased in quantity under anaerobic conditions. The amount of palmitic acid did not change to any extent in the reproductive tissues under any of the experimental conditions. The amount of tricosanoic acid in all three tissues decreased to a greater extent under aerobic conditions than under anaerobic conditions. It is possible that there was conversion of one acid to another although the pattern is not apparent. The worms used in this series of experiments were collected from many different hogs. It may be that the difference in amount of specific non-volatile fatty acids was a reflection of the difference in concentration of specific fatty acids in the diet of the hosts. The worms may have retained the fatty acid pattern of dietary triglycerides as was reported in mammals (22).

The most abundant volatile fatty acids in all three tissues examined were 2-methylbutyric acid and 2-methylvaleric acid. Saz and co-workers (132, 133) have demonstrated the formation of these acids from propionic acid and acetic acid. The pathway of formation of propionic acid by *Ascaris* has been elaborated (130) but the pathway of formation of acetate has not been determined in *Ascaris*. The accumulation of acetate in high concentration in the ovary-oviduct tissue, particularly under aerobic

conditions indicates that acetate was either not oxidized readily in this tissue, or it was not incorporated into long chain fatty acids or it had some important functional role in the ovary-oviduct tissue. It is known that most of the ascarosides, which are in high concentration in the reproductive tissue of Ascaris, occur largely as esters of acetic and propionic acids (116) but it is not known if this is a mechanism for removal of these acids or if these acids are an essential part of the structure of the ascarosides. If acetic acid and propionic acid are the only acids that can be incorporated into the ascarosides, this would partially explain why these two acids are in high concentration in the ovary-oviduct tissue. This series of experiments has indicated that the fatty acids in the tissues are in a dynamic state but has not shown whether any of the fatty acids are oxidized or not.

Third Experiment: Oxidation of Fatty Acids

The results of the previous experiments indicate that Ascaris has the ability to synthesis lipids at a high rate and possibly mobilize lipids. The purpose of this series of experiments was to determine whether or not mitochondria isolated from muscle tissue of the body wall were able to oxidize various fatty acids and to identify any fatty acid end products formed as a result of the oxidation of the fatty acids.

Oxidation of the sodium salts of several fatty acids by the homogenate, supernatant after removal of mitochondria, cellular debri sedimented by centrifuging at 500 g. and the mitochondria was measured. The rates of oxidation under the various experimental conditions are tabulated in Appendix D. All values were adjusted to the zero value by subtracting the rate of the control in each set of incubations.

Rate of Dye Reduction: Table VIII contains a summary of the rate at which 2,6-dichlorophenolindophenol was reduced by the cell fractions when the salts of several fatty acids were used as the substrates. A higher rate of dye reduction was stimulated by the volatile fatty acids than by the non-volatile fatty acids. Non-volatile fatty acids were used at lower concentrations than the volatile fatty acids because high concentrations of the non-volatile fatty acids inhibited dye reduction. Dye reduction by the homogenate was stimulated by all the fatty acids tested, although the rate of dye reduction when oleate was the substrate was so low as to be of doubtful meaning. Butyrate, 2-methylbutyrate and 2-methylvalerate stimulated dye reduction by the homogenate at a more rapid rate than other acids. The dye was reduced at a very low rate by the supernatant when 2-methylvalerate was the substrate and oleate inhibited endogenous activity by the supernatant. Dye reduction by the cell debri was stimulated by 2-methylvalerate but not by acetate, propionate, heptanoate, palmitate or oleate. Acetate, butyrate, 2-methylbutyrate, heptanoate and palmitate all stimulated dye reduction by the mitochondria but 2-methylvalerate stimulated a much higher rate of dye reduction than the other acids.

Figure 12 indicates that increasing the amount of 2-methylvalerate in each incubation tube to more than 4 μMoles caused only a slight increase in the rate of dye reduction, so 4 μMoles of the volatile fatty acids per tube was used in most incubations in this series of experiments. Figure 13 indicates the optimal amount of mitochondria to add to each incubation tube was the mitochondria isolated from 0.04 or 0.05 gm. of muscle tissue. The effect of the pH of the phosphate buffer (Figure 14)

TABLE VIII

FATTY ACID OXIDATION BY SUBCELLULAR FRACTIONS OF
ASCARIS MUSCLE

Acid	Amount of Acid per Tube	µMoles Dye Reduced/gm. Muscle/hr.*			
		Homogenate	Supernatant	Debri	Mitochondria
Acetic	4.0 µMoles	2.40 (H1)		0.00 (D4)	0.16 (M3)
Propionic	4.0 µMoles	3.60 (H1)		0.51 (D4)	0.00 (M3)
Butyric	4.0 µMoles	5.62 (H1)			2.10 (M6)
2-methylbutyric	4.0 µMoles	4.01 (H1)			2.50 (M6)
2-methylvaleric	4.0 µMoles	9.22 (H1)	1.68 (S1)	6.78 (D4)	10.85 (M6)
Heptanoic	0.1 µMoles			0.28 (D1)	0.17 (M5)
Palmitic	0.4 µMoles	1.20 (H1)		-0.79 (D2)	0.83 (M6)
Oleic	0.4 µMoles	0.41 (H1)	-1.68 (S1)	0.51 (D4)	-0.43 (M6)

* Micromoles of dye reduced per gram of muscle tissue from which the fraction was taken per hour of incubation.

( ) Numbers in parenthesis indicate the set of incubations listed in Appendix D from which the data was taken.

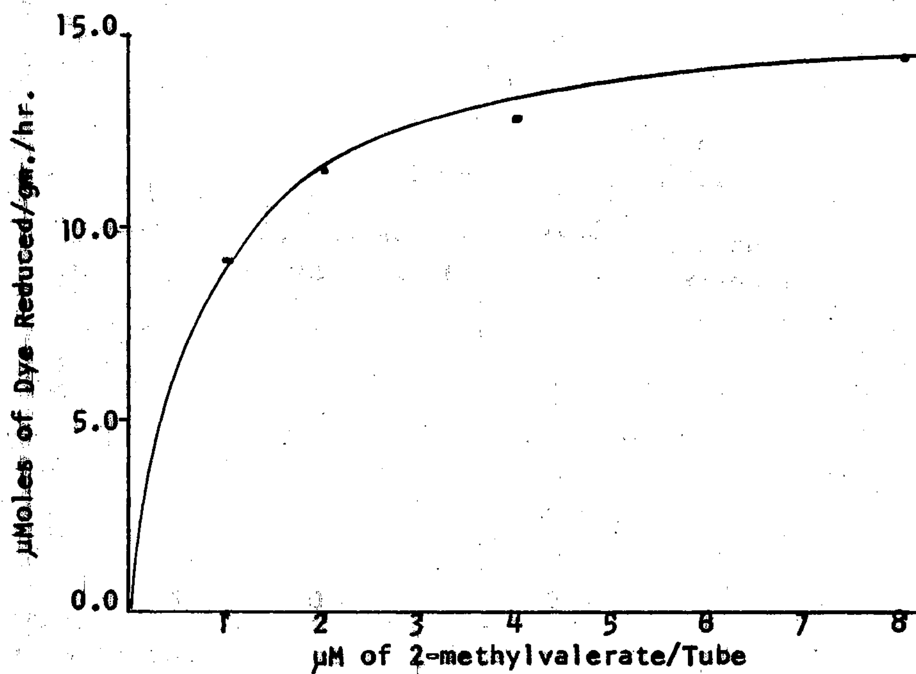


Figure 12. The Effect of Increasing Concentrations of Substrate on the Rate of Oxidation of 2-methylvalerate.

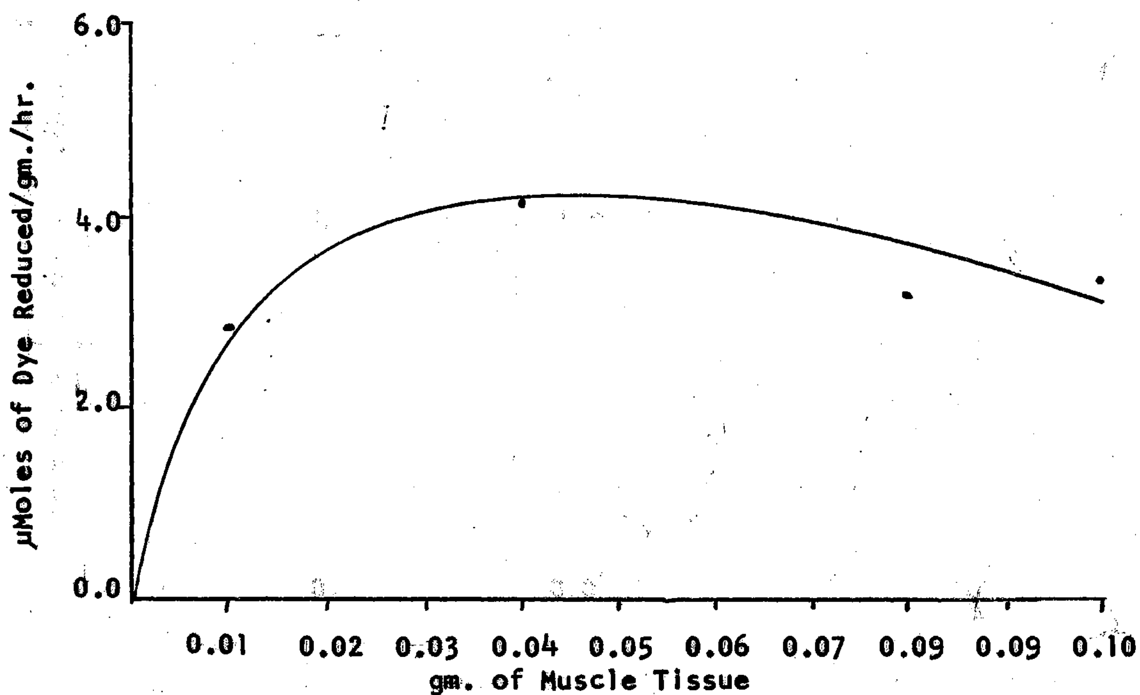


Figure 13. The Effect of Increasing Concentrations of Enzyme on the Rate of Oxidation of 2-methylbutyrate Plus ATP.

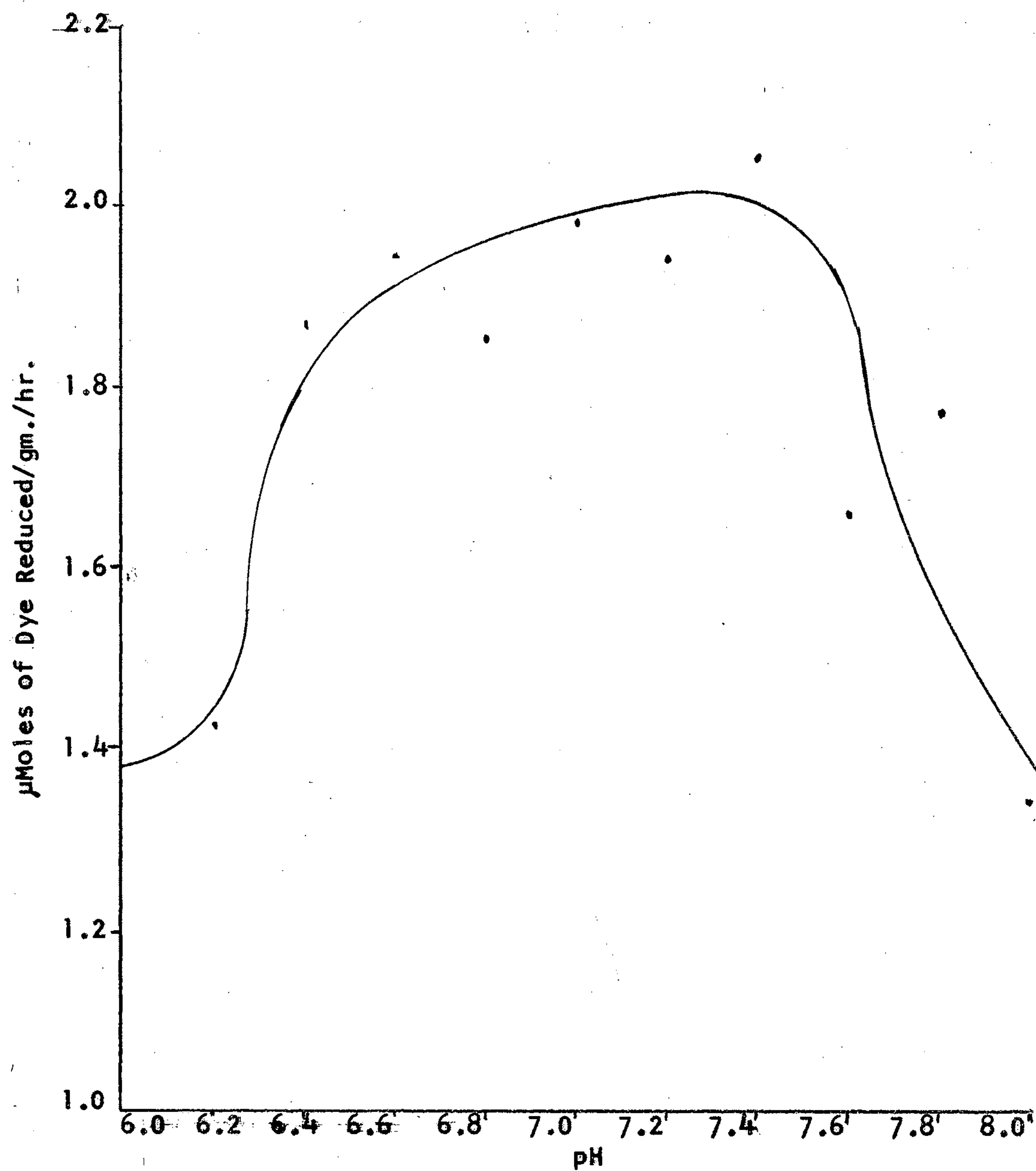


Figure 14. The Effect of pH on the Rate of Oxidation of 2-methylvalerate.

indicates that the highest rate of activity occurred when the buffer pH was 7.4. While pH values as low as 6.4 caused only a small decrease in the rate of dye reduction, when the pH was below 6.4 the rate of dye reduction decreased sharply. When the pH was above 7.4 the rate of dye reduction dropped sharply. The homogenate possessed only very low activity following freezing for 24 hours at -20°C, but refrigeration of the mitochondria for as long as 48 hours had little effect on enzyme activity. The activity of mitochondria isolated from worms starved for 3 days was similar to that of mitochondria from fresh worms but mitochondria from worms starved for 5 days had low activity. The rate of dye reduction was greater under 95% N_2/5% CO_2 than under 95% air/5% CO_2.

The effects of several substances on the rate of dye reduction when 2-methylbutyrate and 2-methylvalerate were used as the substrate are tabulated in Table IX. Succinate and malonate stimulated a substantial increase in the rate of dye reduction, while fumarate, cytochome c, and cytochrome c plus fumarate stimulated an increase in the rate of dye reduction of less than 10%. ATP plus cytochrome c plus fumarate and ATP plus coenzyme A slowed the rate of dye reduction by 14.5% and 17.5% respectively. ATP and ATP plus cytochrome c caused a slowing of the rate of dye reduction of less than 10%. Bicarbonate had no effect on the rate of dye reduction. Changes in the rate of dye reduction of less than 10% were small enough to be of doubtful importance. The rate of dye reduction was strongly stimulated when succinate was added to this system. Oleate, on the other hand, inhibited the reduction of 2,6-dichlorophenolindophenol in the presence of added succinate (Figure 15). Malonate is a specific inhibitor of succinic dehydrogenase (67) and was expected to have little

TABLE IX

THE EFFECT OF VARIOUS SUBSTANCES ON THE RATE OF FATTY ACID OXIDATION BY *ASCARIS* MUSCLE MITOCHONDRIA

Substances Added	2-methylbutyrate		2-methylvalerate	
	Change in µMoles Dye Reduced/gm./hr.*	% Change	Change in µMoles Dye Reduced /gm./hr.	% Change
Succinate (1.0 µMole)	2.25 (M 9)	28		
ATP (9.0 µMoles)	-0.01 (M13)	- 0.5		
Cytochrome c (0.06 µMole)	0.15 (M13)	8.9		
Fumarate (1.0 µMole)	0.15 (M13)	8.9		
ATP (9.0 µMoles) + Cytochrome c (0.06 µMole)	-0.15 (M13)	- 8.9		
ATP (9.0 µMoles + Fumarate (1.0 µMole)	0.15 (M13)	8.9		
Cytochrome c (0.06 µMole) + Fumarate (1.0 µMole)	0.45 (M13)	26.8		
ATP (9.0 µMoles) + Cytochrome c (0.06 µMole) + Fumarate (1.0 µMole)	-0.31 (M13)	-14.5		
ATP (1.5 µMole) + Coenzyme A (0.025 µMole)			-2.33 (M14)	-17.5
Malonate (1.0 µMole)			0.34 (M17)	20

* Micromoles of dye reduced per gram of muscle tissue from which the fraction was taken per hour of incubation.

( ) Numbers in parenthesis indicate the set of incubations listed in Appendix D from which the data was taken.

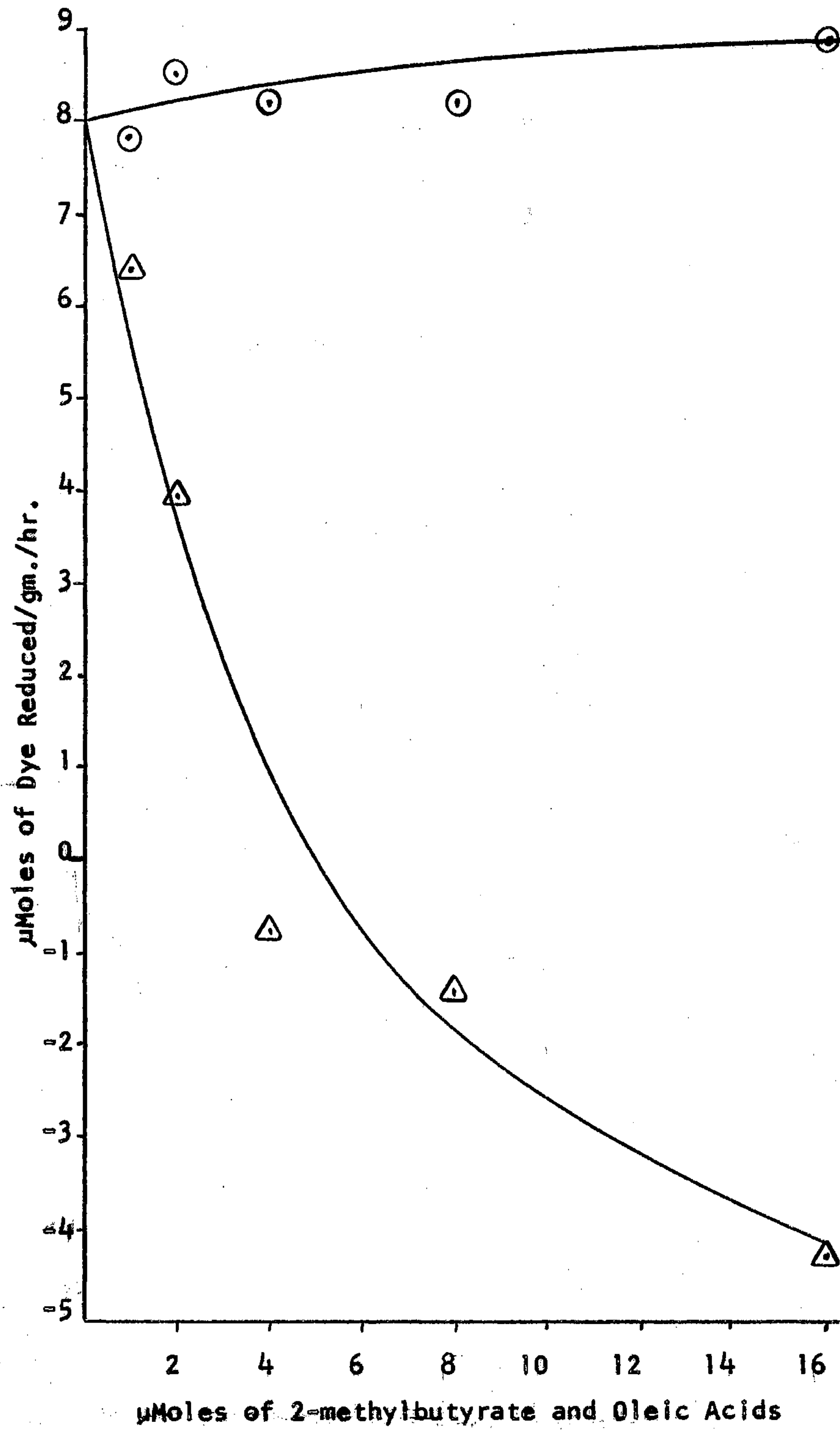


Figure 15. The Effect of Increasing Concentrations of 2-methylbutyric Acid (○) and Oleic acid (△) on the Rate of Oxidation os Succinate Acid.

effect on the rate of dye reduction in this system, however, it consistently stimulated the rate of dye reduction when 2-methyl acids were used as substrates.

End Products: A second series of experiments on the oxidation of volatile fatty acids by mitochondria from muscle tissue of the body wall was performed in an attempt to accumulate sufficient end products for identification by column and paper chromatography. The rate of dye reduction was higher under anaerobic conditions than under aerobic conditions (Table X). This may have been due to oxidation of the dye by oxygen or to competition of oxygen with 2,6-dichlorophenolindophenol as a hydrogen acceptor under aerobic conditions. Propionate, 2-methylbutyrate, tiglate, 2-methylvalerate, pyruvate and succinate all caused dye reduction when mitochondria were present. No dye reduction occurred in the absence of mitochondria or in the absence of a suitable substrate. Dye reduction proceeded at a rapid rate in the absence of KCl, $MgCl_2$ and phenazine methosulfate.

The end products positively identified as the result of oxidation of propionate, 2-methylbutyrate and 2-methylvalerate are acetate and propionate (Table XI). A substance, present in low concentration in several incubations, migrated on the chromatograms in a manner similar to formate but was not positively identified. Paper chromatography of the oxidation products of succinate revealed only 1 spot. This spot absorbed ultraviolet light and was possibly fumarate. Chromatography of incubation mixtures containing propionate revealed a small quantity of material that might have been a 5 or 6 carbon acid.

Discussion: The dye, 2,6-dichlorophenolindophenol, is readily

TABLE X

THE EFFECT OF OXYGEN ON THE RATE OF FATTY ACID OXIDATION BY *ASCARIS* MUSCLE MITOCHONDRIA

Substrate	μMoles Dye Reduced/gm.*	
	in Air	in Nitrogen
Acetate	0.00 (8)	0.00 (8)
Propionate	0.00 (8)	3.387 (7)
2-methylbutyrate	0.510 (8)	3.948 (7)
Tiglate	0.572 (1)	
2-methylvalerate	0.510 (8)	2.040 (8)
Pyruvate	0.00 (9)	1.527 (9)
Succinate	3.567 (9)	5.607 (9)

* Micromoles of dye reduced per gram of muscle tissue that the mitochondria were isolated from.

( ) indicates the set of incubations listed in Appendix E from which the data was taken.
(1) LS 1
(7) LS 7
(8) LS 8
(9) LS 9

TABLE XI

IDENTIFICATION OF THE PRODUCTS OF FATTY ACID OXIDATION BY *ASCARIS* MUSCLE MITOCHONDRIA

Substrate	End Products of Oxidation				
	Formate	Acetate	Propionate	Fumarate	C5 or C6
Propionate	?	x	x		?
2-methylbutyrate	?	x	x		
2-methylvalerate		x	x		
Pyruvate	?	x			
Succinate				?	

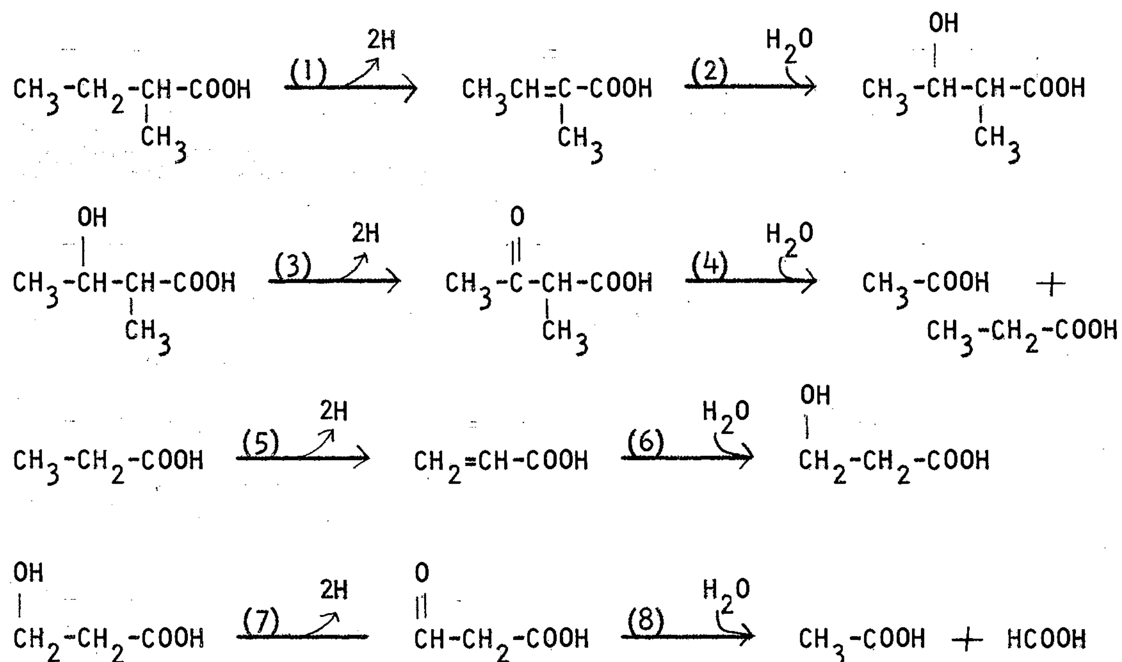
Based on data in Appendix F

reduced in a system in which dehydrogenation reactions are proceeding. In the enzyme system used in this study it was assumed that the substrates were being dehyrogenated with the transfer of hydrogen to the dye by a pathway terminating with a flavoprotein. The reduction of the dye in the absence of phenazine methosulfate supports the postulate that the electron transfer system terminated with a flavoprotein rather than a cytochrome. The high rate of dye reduction by the mitochrondria when the volatile fatty acids were used as the substrate indicates that the enzyme activity measured in this study was associated with the mitochondria.

The stimulating effect of fumarate and succinate on fatty acid oxidation in mammals is thought to be associated with the oxidation of acetate by way of the tricarboxylic acid cycle (88, 94) but in Ascaris this cycle is incomplete and there is little evidence that acetate is oxidized. So the stimulating effect of succinate and fumarate was due to some extra-tricarboxylic acid cycle activity not apparent at present. ATP was expected to stimulate oxidation but did not, and seemed to inhibit the slight positive effect of cytochrome c and fumarate. Coenzyme A with ATP had a negative effect on the rate of dye reduction. Coenzyme A has previously been found to have little effect in mitochondrial preparations of mammalian tissues (72).

The identification of acetate among the end products indicates that the mitochondria were not able to oxidize it under the conditions employed in this experiment. Propionate was present among the end products in most cases which indicated that it also was not readily oxidized under the conditions of this experiment. Perhaps the oxidation of

propionate was inhibited by the accumulation of acetate. The scheme below is the probable route of oxidation of 2-methylbutyrate and propionate by Ascaris mitochondria:



Steps 1 through 4 were proposed as the method of oxidation of 2-methylbutyrate in mammals (40, 41). The oxidation of 2-methylvalerate could proceed along a similar pathway resulting in the formation of 2 moles of propionate at step 4 instead of a mole of acetate and a mole of propionate. Saz and Weil (132) reported that 2-methylbutyrate is formed in Ascaris muscle by a pathway which is essentially a reversal of steps 4 through 1 in the above scheme.

Formate has been identified in the medium in which Ascaris was cultured (111, 141) and also as a constituent of the lipids extracted from Ascaris (6, 62). There was some indication in this experiment that formate may have been one of the end products of the oxidation of

volatile fatty acids. Formate could be formed as a result of the oxidation of propionate as illustrated in steps 5 through 8 in the above scheme. The oxidation of propionate by peanut cotyledons has been found to proceed as shown in steps 5 and 6 above although the final step is ATP dependent and results in the production of acetate and CO_2 (68). In Ascaris steps 7 and 8 as shown above would be similar to steps 3 and 4 in the oxidation of the longer chain acids and could possibly have been mediated by the same enzyme system.

The oxidation of propionate by a pathway in which succinate is an intermediate was not supported by the results of this experiment. Succinate was readily oxidized by this system, however, the only end product that was detected was an unsaturated acid. The major end product detected following propionate oxidation was acetate and no unsaturated compounds were detected.

The lack of oxidation of palmitate and oleate in this experiment may indicate that the mitochondria either did not have the enzymes needed for oxidation of the long chain fatty acids or had only small amounts of them. It is also possible that a cofactor that was not added in this experiment would be required for oxidation of the long chain fatty acids. Since Ascaris possesses an active system of carbohydrate metabolism and carbohydrates are plentiful in the intestine of the hog it is possible that oxidation of fatty acids has little importance in the metabolism of the worm in vivo.

CHAPTER V

SUMMARY AND CONCLUSIONS

Previous studies have shown that Ascaris lumbricoides females contain large amounts of lipids and that most of the lipid in the worm is in the reproductive tissues; however, the metabolism of lipids in this worm has not been thoroughly investigated. It is known that *in vivo* the worm incorporates about 1.6% of its body weight into eggs each day and that developing larvae are capable of mobilizing lipid. It is not known if the adult can synthesize lipid or if it can mobilize lipid in its own tissues during starvation. This study was designed to measure lipid mobilization during starvation in Ascaris females and oxidation of fatty acids by mitochondria isolated from muscle tissue of the body wall of Ascaris females.

This study was divided into three experiments. In the first experiment the total lipid of the body wall and reproductive system plus eggs of Ascaris lumbricoides was determined following starvation of the worms under atmospheres of either air, 95% air/5% CO_2, N_2 or 95% N_2/5% CO_2. In the second experiment the content of total lipid, neutral lipid, phospholipid and fatty acids of the saponifiable neutral lipids of the body wall, ovary-oviduct tissue and uterus plus eggs was determined following starvation of the worms under atmospheres of either 95% air/5% CO_2 or 95% N_2/5% CO_2. The third experiment was an investigation of the oxidation of fatty acids by mitochondria isolated from the muscle tissue

of the body wall of Ascaris lumbricoides.

In the first experiment the lipid content of the tissues following starvation was significantly greater than that of the control. Most of the increase in lipid was in the reproductive system plus eggs, and the lipid content of the reproductive system plus eggs was similar regardless of the atmosphere under which the worms were starved. This indicated that lipid synthesis continued during starvation probably at the expense of the glycogen reserves in the animal.

In the second experiment the lipids of the ovary-oviduct tissue and of the uterus plus eggs were analyzed separately. The total lipid of the uterus plus eggs of the worms starved under aerobic conditions was above the control value while the total lipid content of the uterus plus eggs of the worms starved under anaerobic conditions was below the control value. The total lipid of the body wall and ovary-oviduct tissue following starvation showed only small changes from the control values. Examination of the various fractions of the total lipid revealed a higher concentration of phospholipid in the body wall than in the reproductive tissues and a higher concentration of neutral lipid in the reproductive tissues than in the body wall.

The phospholipid content of the body wall and ovary-oviduct tissue decreased during starvation while in the uterus plus eggs the phospholipid content increased during starvation. The increase in phospholipid content of the uterus plus eggs was greater under aerobic conditions than under anaerobic conditions. It is likely that the increase in lipid in the uterus plus eggs was due to the incorporation of lipid into the eggs that were maturing in the uterus or to movement of developing eggs

from the ovary-oviduct tissue to the uterus. It appeared that the transfer of lipid into the uterus plus eggs was inhibited by a lack of oxygen.

The fatty acid content of the ovary-oviduct tissue after 2 days of starvation under aerobic conditions was above the control value but after 4 days it was below the control value. Under anaerobic conditions there was a continued increase in the fatty acid content of the ovary-oviduct tissue. This indicated that the synthesis of fatty acids in the ovary-oviduct continued during starvation but following more than two days starvation in air or starvation in the absence of air the fatty acids were not transferred to the uterus plus eggs. The change in ratio of moles of volatile fatty acid per mole of non-volatile fatty acid in the body wall was great, but the validity of the results was questionable since the fatty acid content of the body wall was low and changed little during starvation. The ratio of the moles of volatile fatty acid to non-volatile fatty acid in both portions of the reproductive tissue was near 1 under all experimental conditions even though some of the differences in content of fatty acid were great. This indicated that in the reproductive system the volatile fatty acids were mobilized in a manner similar to non-volatile fatty acid mobilization.

Analysis of the gas-liquid chromatograms of the methyl esters of the non-volatile fatty acids of the saponifiable neutral lipids allowed tentative identification of the fatty acids extracted from the tissues. The following non-volatile acids had not been identified previously in <u>Ascaris</u> tissues: 7:0br, 8:0, 9:0, 9:0br, 10:0, 10:0br, 11:0, 11:0br, 12:0br, 13:1, 12:0br, 13:0br, 13:0br, 15:1, 14:0br, 17:0br, 18:0br, 18:3, 19:2, 19:1, 19:0, 19:0br, 20:2, 20:0br, 21:1, 20:0br, 23:0.

The most abundant fatty acids were the 16:0, 18:0, 18:1, 18:2 and 23:0 acids, accounting for about 90% of the total non-volatile fatty acids in the tissues examined under all experimental conditions. The amount of several of the non-volatile fatty acids of the saponifiable neutral lipids changed more during starvation than did other acids. The differences were most pronounced in the body wall. Linoleic acid increased in concentration in relation to the other fatty acids in all the tissues under all conditions, however, the increase was greatest under aerobic conditions. Tricosanoic acid decreased in concentration in relation to the other fatty acids in all the tissues under all conditions but more under aerobic conditions than under anaerobic conditions. Palmitic acid increased in concentration in relation to the other fatty acids in the body wall under aerobic conditions and decreased under anaerobic conditions. In the reproductive system palmitic acid changed little in concentration in relation to the other acids under the experimental conditions. The significance of these changes in the concentration of certain fatty acids was not clear.

The most abundant volatile fatty acid in all tissues was 2-methylvalerate. In the body wall and uterus plus eggs it accounted for about 70% of the total non-volatile fatty acid in the saponifiable neutral lipid. In the ovary-oviduct tissue it accounted for about one-half of the volatile fatty acid. 2-methylbutyric acid accounted for one-fifth to one-fourth of the volatile fatty acid in all the tissues under all experimental conditions. In the body wall and uterus plus eggs, formic acid, acetic acid, propionic acid and isobutyric acid were present in small amounts. Traces of butyric acid and valeric acid were

identified in some samples from the uterus plus eggs. In the ovary-oviduct tissue, acetic acid amounted to 15% of the volatile acid in the control sample while it amounted to less than 1% in the control samples of the body wall and uterus plus eggs. Following starvation under aerobic conditions the amount of acetic acid in the ovary-oviduct tissue relative to the other volatile fatty acids in this tissue doubled while 2-methylvaleric acid and propionic acid decreased relative to the other volatile fatty acids. Under anaerobic conditions acetic acid increased a small amount and isobutyric acid increased from a trace to 3-4% of the total volatile fatty acid while 2-methylvaleric acid decreased relative to the other volatile fatty acids. The significance of this effect of oxygen was not determined.

In the third experiment it was found that enzymes for oxidation of volatile fatty acids were present in the mitochondria of Ascaris muscle tissue. The rate of oxidation was determined by measuring the rate of reduction of 2,6-dichlorophenolindophenol. The highest rate of dye reduction was stimulated by 2-methylvalerate, but 2-methylbutyrate, butyrate, propionate and tiglate also stimulated dye reduction. Acetate, heptanoate, palmitate and oleate stimulated dye reduction at a very low rate or not at all. Dye reduction proceeded most rapidly when the phosphate buffer pH was 7.4 although activity was high in the pH range from 6.4 to 7.4. The reaction was not stimulated by ATP, Coenzyme A, cytochrome c or fumarate. Succinate and malonate stimulated oxidation of the 2-methyl acids.

Analysis of the fatty acid end products following the oxidation of 2-methylbutyric acid and 2-methylvaleric acid indicated that propionate

is an intermediate product of the oxidation of the 2-methyl acids. Propionate was oxidized to acetate and either formate or CO_2. The oxidation of the volatile fatty acids in the presence of a suitable hydrogen acceptor indicated that the volatile fatty acids may serve as a source of metabolic energy for Ascaris. The worm may be able to utilize the low oxygen content of the small intestine for the oxidation of the volatile fatty acids as well as other products. The lack of oxidation of acetate was a further indication that the tricarboxylic acid cycle is incomplete in Ascaris.

The following conclusions were drawn on the basis of the results of this study: 1. Lipid synthesis continued for at least two days during starvation of Ascaris. 2. The lipid content of Ascaris following starvation was not affected by the presence or absence of oxygen and carbon dioxide in the atmosphere under which the worms were starved. 3. The greatest increase in lipid during starvation occurred in the reproductive tract and was probably associated with continued egg production in the ovary-oviduct tissue. 4. When oxygen was not included in the atmosphere or if starvation continued for more than two days in the presence of oxygen the transfer of lipid from the ovary-oviduct tissue to the uterus plus eggs appeared to have been inhibited. 5. Enzymes associated with volatile fatty acid oxidation were present in mitochondria isolated from the muscle tissue of the body wall. 6. The pH optimum for volatile fatty acid oxidation was 7.4. 7. Volatile fatty acids were oxidized to acetate and either formate or CO_2 and propionate was an intermediate in the oxidation of the 2-methyl acids. 8. There was a very large number of non-volatile fatty acids present in Ascaris

tissues, however, the 16:0, 18:0, 18:1, 18:2 and 23:0 acids accounted for about 90% of the non-volatile fatty acids of the saponifiable neutral lipids. 9. The most abundant volatile fatty acids in the saponifiable neutral lipids were 2-methylbutyric acid and 2-methylvaleric acid. 10. In the ovary-oviduct tissue during starvation acetate increased to a much greater extent under aerobic conditions then under anaerobic conditions, while propionate decreased under aerobic conditions and increased to only a slight extent under anaerobic conditions. The 4-carbon acids increased in the ovary-oviduct tissue during starvation under anaerobic conditions but were present in only trace amounts in the control and in worms starved under aerobic conditions. Similar changes were not noted in the body wall or uterus plus eggs. 11. The lipids in *Ascaris* were in a dynamic state but it was not determined to what extent they are used for metabolic energy in this worm.

It has recently been shown that *Ascaris lumbricoides* will grow to maturity in the rabbit (10, 11). Worms grown in the rabbit under laboratory conditions would tend to be less variable at the beginning of an experiment than worms collected at random in packing plants. Using such worms could result in data which would be less variable and easier to interpret. Studies with whole worms on the oxidation of radioactively labelled non-volatile and volatile fatty acids should be performed to determine whether the intact worm is capable of oxidizing fatty acids. Use of radioactively labelled fatty acids would also aid in identification of the end products of fatty acid oxidation. More can be learned about fatty acid oxidation in *Ascaris* tissues by isolating the enzymes from the mitochondria.

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APPENDICES

APPENDIX A

TOTAL LIPID CONTENT OF BODY WALL AND REPRODUCTIVE TRACT OF *ASCARIS LUMBRICOIDES* FEMALES

Worm No.	Body Wt. (gm.)	Total Lipid					
		Body Wall		Reprod. Tract		Combined	
		(mg.)	(mg%)*	(mg.)	(mg%)	(mg.)	(mg%)
Group I CONTROL							
C- 1	2.92	9.04	309.58	45.75	1566.78	54.79	1876.30
C- 2	2.08	8.30	399.03	17.92	861.53	26.22	1260.57
C- 3	2.59	8.68	335.13	33.23	1283.01	41.91	1618.14
C- 4	3.47	10.49	302.30	40.26	1160.23	50.75	1462.53
C- 5	2.42	8.52	352.06	27.21	1124.38	35.73	1476.44
C- 6	3.80	10.89	282.57	33.03	869.21	43.92	1155.78
C- 7	2.91	7.67	263.57	42.47	1459.45	50.14	1723.02
C- 8	2.71	7.39	272.69	39.62	1461.99	47.01	1734.68
C- 9	2.19	6.50	296.80	15.93	727.39	22.43	1024.20
C-10	1.21	3.14	259.50	12.00	991.73	15.14	1251.23
C-11	6.48	27.02	416.97	74.62	1151.54	101.64	1568.51
C-12	4.98	17.97	360.84	48.05	964.85	66.02	1325.70
C-13	6.21	21.98	353.94	90.90	1463.76	112.88	1817.71
C-14	5.45	16.71	306.60	70.30	1289.90	87.01	1596.51
C-15	7.79	32.36	415.40	115.09	1477.40	147.45	1892.81
C-16	6.06	23.25	383.66	45.31	747.68	68.56	1131.35
C-17	4.10	15.71	383.17	42.03	1025.12	57.74	1408.29
C-18	5.00	19.35	387.00	52.77	1055.40	72.12	1442.40
C-19	5.60	17.51	312.67	57.22	1021.78	74.73	1334.46
C-20	3.13	9.87	315.33	47.10	1504.73	56.97	1820.12
Mean			335.44		1160.40		1498.54
S.E.**			11.27		59.32		58.56

APPENDIX A Continued

Worm No.	Body Wt. (gm.)	Total Lipid: Body Wall (mg.)	Body Wall (mg%)	Reprod. Tract (mg.)	Reprod. Tract (mg%)	Combined (mg.)	Combined (mg%)
Group II INCUBATED UNDER NITROGEN							
A.SACRIFICED AFTER 2 DAYS							
N-21	4.08	19.93	488.48	64.95	1591.91	84.52	2071.56
N-22	5.03	23.46	466.40	71.55	1422.46	95.01	1888.86
N-23	5.16	35.39	685.85	81.01	1569.96	116.40	2255.81
N-24	4.37	33.65	770.02	63.36	1449.88	97.01	2219.90
N-25	6.21	26.26	422.86	88.27	1421.41	114.53	1844.28
N-26	4.49	22.97	511.58	81.34	1811.58	104.31	2323.16
N-27	3.16	20.76	656.96	35.58	1125.94	56.34	1782.91
N-28	4.48	24.30	542.41	65.64	1465.17	89.94	2007.58
N-29	3.17	18.56	585.48	61.59	1942.90	80.15	2528.39
Mean			570.03		1533.47		2102.49
S.E.			38.08		79.26		82.65
B.SACRIFICED AFTER 4 DAYS							
N-41	3.68	18.59	505.17	68.40	1858.69	90.19	2363.85
N-42	5.71	29.35	514.01	81.44	1426.26	110.79	1940.28
N-43	5.72	24.48	427.97	71.73	1254.02	96.21	1681.99
N-44	6.19	22.66	366.07	82.52	1333.11	105.18	1699.19
N-45	5.21	21.42	411.13	70.81	1359.11	92.23	1770.24
N-46	5.51	22.46	407.62	70.91	1286.93	93.37	1694.55
N-47	4.50	20.82	462.66	90.58	2012.88	111.40	2475.55
N-48	4.02	19.13	475.87	56.63	1408.70	75.76	1884.57
N-49	5.48	21.26	387.95	78.01	1423.54	99.27	1811.49
N-50	4.89	23.01	470.55	89.15	1823.10	112.16	2293.66
Mean			442.90		1588.63		1961.54
S.E.			15.83		85.99		93.88

APPENDIX A Continued

Worm No.	Body Wt. (gm.)	Total Lipid					
		Body Wall		Reprod. Tract		Combined	
		(mg.)	(mg%)	(mg.)	(mg%)	(mg.)	(mg%)
C. SACRIFICED AFTER 6 DAYS							
N-61	5.41	18.51	342.14	84.59	1563.58	103.10	1905.73
N-62	4.05	17.91	442.22	45.92	1133.82	63.83	1576.05
N-63	2.89	13.54	468.51	45.21	1564.35	58.75	2032.87
Mean			417.62		1420.58		1838.22
S.E.			38.52		143.47		136.21
Group III INCUBATED UNDER 95% NITROGEN: 5% CO_2							
A. SACRIFICED AFTER 1 DAY							
NC-11	6.04	21.18	350.66	74.74	1237.41	95.92	1588.07
NC-12	4.38	13.20	301.36	73.00	1666.66	86.20	1968.03
NC-13	5.43	16.27	299.63	69.34	1276.97	85.61	1576.60
NC-14	4.89	18.27	373.61	74.99	1533.53	93.26	1907.15
NC-15	4.01	17.16	427.93	52.72	1314.71	69.88	1742.64
NC-16	3.66	11.13	304.09	64.39	1759.28	75.52	2063.38
NC-17	4.64	14.28	307.75	71.45	1539.87	85.73	1847.62
NC-18	5.48	19.09	348.35	84.37	1539.59	103.46	1887.95
NC-19	5.60	31.45	561.60	84.16	1502.85	115.61	2064.46
NC-20	5.43	17.03	313.62	86.61	1595.02	103.64	1908.65
Mean			358.86		1496.59		1855.46
S.E.			25.97		53.86		54.48

APPENDIX A Continued

Worm No.	Body Wt. (gm.)	Total Lipid: Body Wall (mg.)	Body Wall (mg%)	Reprod. Tract (mg.)	Reprod. Tract (mg%)	Combined (mg.)	Combined (mg%)
B. SACRIFICED AFTER 2 DAYS							
NC-21	6.99	19.03	272.24	117.84	1685.83	136.87	1958.08
NC-22	5.03	17.84	354.67	78.85	1567.59	96.69	1922.26
NC-23	3.82	10.90	285.34	49.75	1302.35	60.65	1587.69
NC-24	3.31	11.94	360.72	59.62	1801.20	71.56	2161.93
NC-25	4.09	14.45	353.30	71.84	1756.47	86.29	2109.77
NC-26	4.31	16.64	386.07	72.13	1673.54	88.77	2059.62
NC-27	3.88	13.33	343.55	67.85	1748.71	81.18	2092.26
NC-28	6.49	17.60	271.18	98.32	1514.94	115.92	1786.13
NC-29	2.91	10.45	359.10	61.08	2098.96	71.53	2458.07
Mean			331.80		1683.29		2015.09
S.E.			14.44		72.95		81.79
C. SACRIFICED AFTER 3 DAYS							
NC-31	3.98	13.11	329.39	58.48	1469.34	71.59	1798.74
NC-32	4.76	16.07	337.60	41.98	881.93	58.05	1219.53
NC-33	5.49	14.95	272.31	82.86	1509.28	97.81	1781.60
NC-34	4.24	14.43	340.33	57.84	1364.15	72.27	1704.48
NC-35	5.36	15.43	287.87	67.49	1259.14	82.92	1547.01
NC-36	5.46	16.12	295.23	73.50	1346.15	89.62	1641.39
NC-37	5.32	16.61	312.21	67.00	1259.39	83.61	1571.61
NC-38	4.34	14.52	334.56	59.79	1377.64	74.31	1712.21
NC-39	6.08	16.91	278.12	90.72	1492.10	107.63	1770.23
NC-40	5.33	17.67	332.51	45.06	845.40	62.73	1167.92
Mean			312.01		1280.45		1592.38
S.E.			8.03		75.71		70.96

APPENDIX A Continued

Worm No.	Body Wt. (gm.)	Total Lipid: Body Wall (mg.)	Body Wall (mg%)	Reprod. Tract (mg.)	Reprod. Tract (mg%)	Combined (mg.)	Combined (mg%)
D. SACRIFICED AFTER 4 DAYS							
NC-41	2.82	11.07	392.55	46.51	1649.29	57.58	2041.84
NC-42	4.25	14.60	343.52	52.63	1238.35	67.23	1581.88
NC-43	4.25	13.01	306.11	64.46	1516.70	77.47	1822.82
NC-44	3.73	17.18	460.58	34.69	930.02	51.87	1390.61
NC-45	3.62	13.95	385.35	50.42	1392.81	64.37	1778.17
NC-46	3.41	10.21	299.41	51.86	1520.82	62.07	1820.23
NC-47	3.18	11.42	359.11	47.48	1493.08	58.90	1852.20
NC-48	3.22	12.91	400.93	45.75	1420.80	58.66	1821.73
NC-49	4.08	11.74	287.74	72.66	1780.88	84.40	2068.62
NC-50	2.89	9.46	327.33	46.82	1620.06	56.28	1947.40
Mean			356.26		1456.28		1812.55
S.E.			17.15		74.87		62.62
E. SACRIFICED AFTER 6 DAYS							
NC-61	4.24	15.56	366.98	65.93	1554.95	81.49	1921.93
NC-62	3.03	8.79	290.09	61.12	2017.16	70.01	2310.56
NC-63	4.33	12.82	296.07	68.02	1570.90	80.84	1866.97
NC-64	4.52	20.32	449.55	44.86	992.47	65.18	1442.03
NC-65	4.93	15.33	310.95	77.04	1562.67	92.37	1873.63
NC-66	3.39	12.07	356.04	53.31	1572.56	65.38	1928.61
NC-67	5.12	17.51	341.99	48.46	946.48	65.97	1288.47
NC-68	4.26	13.38	314.08	67.25	1578.63	80.63	1892.72
NC-69	3.00	10.38	346.00	48.34	1611.33	58.72	1957.33
NC-70	3.00	9.28	309.34	41.90	1396.66	51.18	1706.00
Mean			338.11		1480.38		1818.83
S.E.			14.85		98.53		90.05

Worm No.	Body Wt. (gm.)	Total Lipid: Body Wall (mg.)	Body Wall (mg%)	Reprod. Tract (mg.)	Reprod. Tract (mg%)	Combined (mg.)	Combined (mg%)
F. SACRIFICED AFTER 8 DAYS							
NC-81	3.12	11.27	361.21	49.37	1582.39	60.64	1943.58
NC-82	3.70	10.45	282.43	46.53	1257.56	56.98	1540.00
NC-83	3.03	11.08	365.67	46.87	1546.86	57.95	1912.54
NC-84	3.50	14.54	415.42	56.01	1600.28	70.55	2015.71
NC-85	3.38	15.62	462.13	31.72	938.46	47.34	1400.59
NC-86	3.18	7.95	250.00	41.61	1308.49	49.56	1558.40
Mean			356.14		1372.34		1728.47
S.E.			32.43		105.18		105.59
Group IV INCUBATED UNDER AIR							
A. SACRIFICED AFTER 2 DAYS							
A-21	3.30	10.50	318.18	78.23	2370.60	88.73	2688.78
A-22	2.70	7.51	278.14	42.56	1576.29	50.07	1854.44
A-23	4.36	11.94	273.85	60.92	1397.24	72.86	1671.10
A-24	5.27	17.48	331.68	70.41	1336.05	87.89	1667.74
A-25	3.83	13.45	351.17	47.47	1239.42	60.92	1590.60
A-26	4.68	15.08	322.22	73.89	1578.84	88.97	1901.06
A-27	4.70	15.05	320.21	64.51	1372.55	79.56	1692.76
A-28	2.69	8.78	326.39	37.56	1396.28	46.34	1722.67
A-29	4.78	14.40	301.25	67.25	1406.90	81.65	1708.15
A-30	4.12	11.04	267.96	61.42	1490.77	72.46	1758.73
Mean			309.11		1516.49		1825.60
S.E.			8.76		100.44		100.10

APPENDIX A Continued

Worm No.	Body Wt. (gm.)	Total Lipid Body Wall (mg.)	Body Wall (mg%)	Reprod. Tract (mg.)	Reprod. Tract (mg%)	Combined (mg.)	Combined (mg%)
B. SACRIFICED AFTER 4 DAYS							
A-41	3.68	14.11	383.42	62.24	1691.30	76.35	2074.72
A-42	2.91	9.81	337.11	49.82	1712.02	59.63	2049.14
A-43	4.55	10.43	229.23	87.63	1925.93	98.06	2155.16
A-44	3.43	12.49	364.13	50.99	1486.58	63.48	1850.70
A-45	4.01	9.72	242.39	52.47	1308.47	62.19	1550.87
A-46	5.59	15.51	277.45	58.50	1046.51	74.01	1323.97
A-47	4.20	14.62	348.09	52.23	1243.57	66.85	1591.66
A-48	3.74	12.37	330.74	57.51	1537.70	69.88	1868.44
A-49	4.70	17.53	372.97	76.53	1628.29	94.06	2001.27
A-50	5.87	18.95	322.82	51.98	885.51	70.93	1208.34
Mean			320.84		1446.59		1767.43
S.E.			17.01		102.08		104.48
C. SACRIFICED AFTER 6 DAYS							
A-61	4.39	16.25	370.15	45.46	1035.53	61.71	1405.69
A-62	5.00	19.04	380.80	44.46	889.20	63.50	1270.00
A-63	3.57	16.51	462.46	52.56	1472.26	69.07	1934.73
A-64	3.80	17.40	457.89	49.16	1293.68	66.56	1751.57
A-65	5.04	17.32	343.65	64.91	1287.89	82.23	1631.54
A-66	3.81	18.04	473.49	58.72	1541.20	76.36	2004.19
A-67	5.68	17.64	310.56	78.24	1377.46	95.88	1688.02
A-68	3.38	12.33	364.79	56.00	1656.80	68.33	2021.59
A-69	4.54	15.64	344.49	67.85	1494.49	83.49	1838.98
A-70	3.70	15.01	405.67	52.66	1423.24	67.67	1828.91
Mean			391.40		1347.18		1737.52
S.E.			17.85		73.83		78.29

APPENDIX A Continued

Worm No.	Body Wt. (gm.)	Total Lipid: Body Wall (mg.)	Body Wall (mg%)	Reprod. Tract (mg.)	Reprod. Tract (mg%)	Combined (mg.)	Combined (mg%)
Group V. INCUBATED UNDER AIR/5% CO_2							
A. SACRIFICED AFTER 1 DAY							
AC-11	4.68	14.77	315.59	78.40	1675.21	93.17	1990.81
AC-12	5.99	20.78	346.91	68.82	1148.91	89.60	1495.82
AC-13	4.06	17.34	427.09	49.14	1210.34	66.48	1637.43
AC-14	4.60	16.02	348.26	75.04	1631.30	91.06	1949.56
AC-15	4.65	12.12	260.64	63.58	1367.31	75.70	1627.95
AC-16	5.85	19.47	332.82	69.89	1194.70	89.36	1527.52
AC-17	3.21	12.65	394.08	55.53	1729.90	68.18	2123.98
AC-18	3.27	6.73	205.81	44.16	1350.45	50.89	1556.26
AC-19	4.08	12.51	306.61	64.21	1573.77	76.62	1880.39
AC-20	2.73	11.25	412.08	57.92	2121.61	69.17	2533.69
Mean			334.99		1500.35		1835.34
S.E.			21.50		96.18		105.75
B. SACRIFICED AFTER 2 DAYS							
AC-21	6.00	21.96	366.00	93.48	1558.00	115.44	1924.00
AC-22	4.41	19.12	433.56	60.90	1380.95	80.02	1814.51
AC-23	6.33	20.22	319.43	71.88	1135.54	92.10	1454.97
AC-24	4.30	15.24	354.41	75.51	1756.04	90.75	2110.46
AC-25	4.19	15.86	378.52	48.20	1150.35	64.06	1528.87
AC-26	3.82	13.80	361.25	58.55	1532.72	72.35	1893.97
AC-27	2.76	13.94	505.07	29.53	1069.92	43.47	1575.00
AC-28	5.62	18.25	324.73	74.56	1326.69	92.81	1651.42
AC-29	5.21	20.01	384.06	112.51	2159.50	132.52	2543.57
AC-30	4.19	13.61	324.82	61.81	1475.17	75.42	1800.00
Mean			375.18		1454.49		1829.68
S.E.			18.03		103.74		101.64

APPENDIX A Continued

Worm No.	Body Wt. (gm.)	Total Lipid: Body Wall (mg.)	Body Wall (mg%)	Reprod. Tract (mg.)	Reprod. Tract (mg%)	Combined (mg.)	Combined (mg%)
C. SACRIFICED AFTER 3 DAYS							
AC-31	5.90	17.15	290.67	85.59	1450.67	102.74	1741.35
AC-32	3.86	13.45	348.44	62.15	1610.10	75.60	1958.54
AC-33	4.61	17.56	380.91	52.94	1148.37	70.50	1529.28
AC-34	2.60	8.01	308.07	47.39	1822.69	55.40	2130.76
AC-35	4.51	15.35	340.35	72.86	1615.52	88.21	1955.87
AC-36	2.35	9.17	390.21	35.23	1499.14	44.40	1889.36
AC-37	4.43	16.03	361.85	72.48	1636.11	88.51	1997.96
AC-38	4.43	13.67	308.57	58.16	1312.86	71.83	1621.44
AC-39	2.61	8.63	330.65	45.13	1729.11	53.76	2059.77
AC-40	4.07	12.85	315.72	59.32	1457.49	72.17	1773.21
Mean			337.54		1528.21		1865.75
S.E.			10.43		62.84		61.42
D. SACRIFICED AFTER 4 DAYS							
AC-41	5.83	18.19	312.00	61.39	1053.00	79.58	1365.00
AC-42	7.66	30.65	400.14	106.65	1392.29	137.30	1792.42
AC-43	7.54	31.62	419.36	75.89	1006.49	107.51	1425.86
AC-44	8.24	30.01	364.19	82.56	1001.94	112.57	1366.14
AC-45	6.37	20.21	317.26	74.73	1173.15	94.94	1490.40
AC-46	4.11	12.59	306.32	68.11	1657.17	80.70	1963.50
AC-47	7.91	29.56	373.70	87.24	1102.90	116.80	1476.61
AC-48	2.60	7.24	278.40	49.10	1888.46	56.34	2166.92
AC-49	5.13	15.88	309.55	64.61	1259.45	80.49	1669.00
AC-50	5.41	15.76	291.31	96.72	1787.80	112.48	2079.10
Mean			337.22		1332.27		1679.50
S.E.			15.29		105.52		93.37

APPENDIX A Continued

Worm No.	Body Wt. (gm.)	Total Lipid					
		Body Wall		Reprod. Tract		Combined	
		(mg.)	(mg%)	(mg.)	(mg%)	(mg.)	(mg%)
	E. SACRIFICED AFTER 6 DAYS						
AC-61	4.70	13.56	288.51	49.47	1052.55	63.03	1341.06
AC-62	4.51	13.08	290.02	75.79	1680.48	88.87	1970.50
AC-63	2.78	11.01	396.04	33.27	1196.76	44.28	1592.80
AC-64	5.28	16.53	313.06	87.35	1654.35	103.88	1967.42
AC-65	7.56	24.74	327.24	113.64	1503.17	138.38	1830.42
AC-66	3.35	10.55	314.92	80.37	2399.10	90.92	2714.02
AC-67	7.41	25.81	348.31	108.19	1460.05	134.00	1808.36
AC-68	3.07	8.33	271.33	48.50	1579.80	56.83	1851.14
AC-69	3.34	11.09	332.03	48.91	1464.37	60.00	1796.40
AC-70	4.64	8.37	180.38	63.30	1364.22	71.67	1544.61
Mean			306.18		1535.49		1841.67
S.E.			17.85		114.09		115.33

*mg%-mg. lipid/100 gm. body wt. **S.E.-standard error of mean

APPENDIX B

lsd VALUES FOR COMPARING MEANS OF TOTAL LIPID CONTENT OF TISSUES OF *ASCARIS*

Number of observations in means compared	least significant difference .1*	.05*	.01*
COMBINED BODY WALL AND REPRODUCTIVE TRACT			
20-10	175.50	210.54	277.45
20- 9	182.37	217.42	286.52
20- 6	213.73	254.95	335.97
20- 3	280.29	335.09	441.58
10-10	203.79	243.09	320.35
10- 9	208.82	249.09	328.25
10- 6	236.79	282.45	372.22
10- 3	298.81	356.44	469.72
9- 6	241.12	287.62	379.03
9- 3	302.27	360.57	475.18
6- 3	322.22	384.36	506.51
REPRODUCTIVE TRACT AND EGGS			
20-10	173.67	207.17	273.00
20- 9	179.38	213.97	281.97
20- 6	210.32	250.89	330.62
20- 3	276.43	329.75	434.54
10-10	200.55	239.23	315.25
10- 9	205.49	245.13	323.02
10- 6	233.02	277.96	366.29
10- 3	294.05	350.75	462.23
9- 6	237.30	283.07	373.02
9- 3	297.46	354.82	467.58
6- 3	317.09	378.24	498.45
BODY WALL			
20-10	37.20	44.37	58.47
20- 9	38.54	45.97	60.58
20- 6	44.64	53.25	70.17
20- 3	59.44	70.91	93.44
10-10	42.95	51.24	67.52
10- 9	44.13	52.64	69.37
10- 6	49.54	59.09	77.87
10- 3	63.22	75.41	99.37
9- 6	50.56	60.31	79.48
9- 3	64.01	76.36	100.62
6- 3	67.93	81.03	106.78

*level of significance

APPENDIX C

COMPOSITION OF LIPIDS OF ADULT FEMALE *ASCARIS LUMBRICOIDES*

Group	Body Wt. gm.	Tissue Wt. gm.	Total Lipid (mg.)	Total Lipid (mg%)*	Neutral Lipid (mg.)	Neutral Lipid (mg%)
BODY WALL						
Control	43.06	19.49	164.0	380.9	57.67	133.9
Air/CO_2						
2nd day	42.58	16.62	134.2	315.2	48.67	114.3
4th day	42.05	15.29	126.4	300.6	35.70	84.9
N_2/CO_2						
2nd day	42.52	16.53	144.8	340.5	54.52	128.2
4th day	28.60	11.11	101.0	353.1	28.80	100.7
OVARY AND OVIDUCT						
Control	43.06	3.27	340.2	790.1	282.07	655.1
Air/CO_2						
2nd day	42.58	3.15	352.0	826.7	298.54	701.1
4th day	42.05	2.73	303.4	721.5	257.43	612.2
N_2CO_2						
2nd day	42.52	2.76	322.6	758.7	270.65	635.5
4th day	28.60	2.06	240.8	842.0	207.48	725.5
UTERUS AND EGGS						
Control	43.06	5.24	228.2	530.0	145.40	337.7
Air/CO_2						
2nd day	42.58	7.61	344.0	807.9	213.66	501.8
4th day	42.05	4.87	298.4	709.6	190.62	453.3
N_2CO_2						
2nd day	42.60	4.99	190.8	448.7	102.38	240.8
4th day	28.60	3.19	153.8	537.8	90.32	315.8

*(mg %) mg. lipid/100 gm. weight

APPENDIX C Continued

TISSUES AFTER STARVATION UNDER AIR/5% CO_2 AND NITROGEN/5% CO_2

Phospho-Lipid (mg.)	Phospho-Lipid (mg%)	Volatile Fatty Acids (mg.)	Volatile Fatty Acids (mg%)	Nonvolatile Fatty Acids (mg.)	Nonvolatile Fatty Acids (mg%)
106.43	247.6	13.60	31.58	22.03	51.16
85.52	200.9	5.80	13.62	30.13	70.76
90.75	215.8	7.15	17.00	31.74	75.48
90.87	213.7	5.92	13.92	21.43	50.40
72.18	252.4	6.87	24.02	13.08	45.73
58.34	135.5	53.50	124.24	118.54	275.29
53.49	125.6	56.70	133.16	149.12	350.21
47.21	112.3	46.80	111.30	101.37	241.07
52.67	123.9	47.33	111.31	127.31	299.41
33.50	117.1	49.80	174.13	128.06	447.76
82.76	192.2	26.00	60.38	90.00	209.01
130.11	305.6	40.60	95.35	122.02	286.57
107.99	256.8	38.30	91.08	121.03	287.82
88.34	207.8	15.80	37.16	52.91	124.44
63.58	222.3	18.70	65.38	46.00	161.05

APPENDIX D

RESULTS OF OXIDATION OF FATTY ACIDS BY MITOCHONDRIA ISOLATED FROM MUSCLE FROM ASCARIS BODY WALL

Each of the test tubes in the following incubation system contained 60 µMoles sodium phosphate buffer, pH 7.4, 10 µMoles $MgCl_2$, 10 µMoles KCl, 10 µMoles KCN, 1.53 x 10-7 M 2,6-dichlorophenolindophenol and 0.15 mg. phenazine methosulfate in a final volume of 6 ml. The amount and kind of substrate and enzyme preparation are indicated for each tube. All incubations were carried out at room temperature under air unless otherwise indicated. The results are expressed in micromoles of dye reduced per gram of muscle tissue per hour of incubation.

The eight sets of tubes (H1-H8) all contained a homogenate of the body wall. Set S1 contained supernatant. Sets D1-D5 contained unbroken cells and debri sedimented at 500 g. Sets M1-M24 contained mitochondria.

HOMOGENATE:

H1. Each tube contained the homogenate of .05 gm. of muscle.

	µMoles/gm./hr.
4.0 µMole acetate	2.40
4.0 µMole propionate	3.60
4.0 µMole butyrate	5.62
4.0 µMole 2-methylbutyrate	4.01
4.0 µMole 2-methylvalerate	9.22
0.4 µMole palmitate	1.20
0.4 µMole oleate	0.41

H2. Each tube contained the homogenate of 0.1 gm. of muscle

		µMoles/gm./hr.
1.0 µMole 2-methylvalerate		1.43
8.0 µMole 2-methylvalerate		3.83
1.0 µMole 2-methylvalerate	10.0 µMole $NaHCO_3$	2.40
8.0 µMole 2-methylvalerate	10.0 µMole $NaHCO_3$	4.06
0.1 µMole oleate		0.23
0.8 µMole oleate		2.63
0.1 µMole oleate	10.0 µMole $NaHCO_3$	0.23
0.8 µMole oleate	10.0 µMole $NaHCO_3$	1.66

H3. Each tube contained the homogenate of 0.01 gm. of muscle. These tubes were incubated under $N_2/5\%CO_2$.

	µMoles/gm./hr.
4.0 µMole 2-methylvalerate	1.88
4.0 µMole 2-methylvalerate + 10.0 µMole $NaHCO_3$	1.24
0.4 µMole oleate	1.24
0.4 µMole oleate + 10.0 µMole $NaHCO_3$	0.00

APPENDIX D Continued

H4. Each tube contained homogenate from 0.05 gm. of muscle tissue.

	μMole/gm./hr.
4.0 μMole 2-methylvalerate	14.04
4.0 μMole 2-methylvalerate + 80.0 μMole malonate	17.04
0.4 μMole palmitate	4.02
0.4 μMole palmitate + 80.0 μMole malonate	1.98
0.4 μMole oleate	3.00
0.4 μMole oleate + 80.0 μMole malonate	4.02

H5. Each tube contained homogenate (which had been frozen for 24 hours) from 0.01 gm. of muscle tissue.

	μMole/gm./hr.
4.0 μMole acetate	.99
4.0 μMole acetate + 10.0 μMole $NaHCO_3$	1.50
4.0 μMole propionate	.99
4.0 μMole propionate + 10.0 μMole $NaHCO_3$	1.50
4.0 μMole 2-methylvalerate	.75
4.0 μMole 2-methylvalerate 10.0 μMole $NaHCO_3$	.99
0.4 μMole oleate	.24
0.4 μMole oleate + 10.0 μMole $NaHCO_3$	.24

H6. Each tube contained homogenate from 0.1 gm. of muscle tissue from worms starved 48 hours.

	μMole/gm./hr.
4.0 μMole acetate	2.52
4.0 μMole acetate + 10.0 uMole $NaHCO_3$	4.02
4.0 μMole propionate	1.98
4.0 μMole propionate + 10.0 uMole $NaHCO_3$	2.52
4.0 μMole 2-methylvalerate	5.52
4.0 μMole 2-methylvalerate + 10.0 uMole $NaHCO_3$	5.52
0.4 μMole oleate	1.98
0.4 μMole oleate + 10.0 uMole $NaHCO_3$	3.48

H7. Each tube contained homogenate from 0.03 gm. of muscle tissue.

	μMole/gm./hr.
1.0 μMole 2-methylvalerate	−3.30
1.0 μMole 2-methylvalerate + 1.0 μMole malonate	11.58
2.0 μMole 2-methylvalerate	16.56
2.0 μMole 2-methylvalerate + 2.0 μMole malonate	26.46
0.1 μMole 2-methylvalerate	0.00
0.1 μMole 2-methylvalerate + 1.0 μMole malonate	4.98
0.2 μMole 2-methylvalerate	-3.30
0.2 μMole 2-methylvalerate + 2.0 μMole malonate	3.30

APPENDIX D Continued

H8. Each tube contained homogenate from 0.03 gm. of muscle tissue.

	µMole/gm./hr.
2.0 µMole 2-methylvalerate	-0.36
0.01 µMole palmitate + 1.0 µMole malonate	0.00
0.02 µMole palmitate + 2.0 µMole malonate	0.36
0.08 µMole palmitate + 8.0 µMole malonate	-2.15
0.01 µMole palmitate	0.72
0.08 µMole palmitate	0.36
0.01 µMole oleate + 1.0 µMole malonate	-0.72
0.02 µMole oleate + 2.0 µMole malonate	-1.43
0.08 µMole oleate + 8.0 µMole malonate	-2.51
0.01 µMole oleate	0.36
0.08 µMole oleate	1.08

SUPERNATANT:

S1. Each tube contained supernatant from 0.02 gm. of muscle tissue.

	µMole/gm./hr.
1.0 µMole 2-methylvalerate	0.00
8.0 µMole 2-methylvalerate	1.68
1.0 µMole 2-methylvalerate + 10.0 uMole $NaHCO_3$	0.00
8.0 µMole 2-methylvalerate + 10.0 uMole $NaHCO_3$	1.68
0.1 µMole oleate	-1.68
0.8 µMole oleate	0.00
0.1 µMole oleate + 10.0 uMole $NaHCO_3$	-1.68
0.8 µMole oleate + 10.0 uMole $NaHCO_3$	0.00

CELL DEBRI:

D1. Each tube contained cell debri from 0.1 gm. of muscle tissue.

	µMole/gm./hr.
1.0 µMole 2-methylvalerate	3.25
4.0 µMole 2-methylvalerate	4.41
0.1 µMole heptanoate	-0.28
1.0 µMole heptanoate	0.00
0.01 µMole palmitate	-0.28
0.1 µMole palmitate	0.28
0.01 µMole oleate	-0.28
0.1 µMole oleate	-0.28

APPENDIX D Continued

D2. Each tube contained cell debri from 0.077 gm. of muscle from worms starved 3 days.

	μMole/gm./hr.
1.0 μMole 2-methylvalerate	2.62
4.0 μMole 2-methylvalerate	4.44
1.0 μMole heptanoate	-0.79
4.0 μMole heptanoate	-1.03
0.1 μMole palmitate	-0.26
0.4 μMole palmitate	0.00
0.1 μMole oleate	-0.79
0.4 μMole oleate	0.00

D3. Each tube contained cell debri from 0.05 gm. of muscle from worms starved four days.

	μMole/gm./hr.
1.0 μMole succinate	8.04
1.0 μMole succinate + 0.01 μMole oleate	7.36
1.0 μMole succinate + 0.04 μMole oleate	8.68
1.0 μMole succinate + 1.0 μMole 2-methylvalerate	10.04
1.0 μMole succinate + 4.0 μMole 2-methylvalerate	10.04

D4. Each tube contained cell debri 0.01 gm. of muscle tissue.

	μMole/gm./hr.
4.0 μMole acetate	0.00
4.0 μMole acetate + 10.0 μMole $NaHCO_3$	0.24
4.0 μMole propionate	0.51
4.0 μMole propionate + 10.0 μMole $NaHCO_3$	0.51
4.0 μMole 2-methylvalerate	6.78
4.0 μMole 2-methylvalerate + 10.0 μMole $NaHCO_3$	7.02
0.4 μMole oleate	0.51
0.4 μMole oleate + 10.0 μMole $NaHCO_3$	0.24

D5. Each tube contained cell debri plus mitochondria from 0.05 gm of muscle tissue.

	μMole/gm./hr.
1.0 μMole 2-methylvalerate	4.86
8.0 μMole 2-methylvalerate	9.71
1.0 μMole 2-methylvalerate + 10.0 μMole $NaHCO_3$	5.15
8.0 μMole 2-methylvalerate + 10.0 μMole $NaHCO_3$	9.42
0.1 μMole oleate	0.56
0.8 μMole oleate	0.29
0.1 μMole oleate + 10.0 μMole $NaHCO_3$	-0.86
0.8 μMole oleate + 10.0 μMole $NaHCO_3$	-0.29

APPENDIX D Continued

MITOCHONDRIA:

M1. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	μMole/gm./hr.
1.0 μMole 2-methylvalerate	9.02
2.0 μMole 2-methylvalerate	11.36
4.0 μMole 2-methylvalerate	12.70
8.0 μMole 2-methylvalerate	14.36

M2. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	μMole/gm./hr.
0.1 μMole oleate	-0.51
0.2 μMole oleate	-0.73
0.4 μMole oleate	-0.73
0.8 μMole oleate	-0.92
1.0 μMole butyric	-1.09
2.0 μMole butyric	2.19
4.0 μMole butyric	3.28
8.0 μMole butyric	4.01

M3. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	μMole/gm./hr.
1.0 μMole butyrate	1.69
2.0 μMole butyrate	0.16
4.0 μMole butyrate	0.92
1.0 μMole propionate	0.92
2.0 μMole propionate	0.46
4.0 μMole propionate	0.00
1.0 μMole acetate	0.16
2.0 μMole acetate	0.00
4.0 μMole acetate	0.16
4.0 μMole 2-methylvalerate	4.92

M4. Each tube contained mitochondria from 0.05 gm. of muscle from worms starved for 5 days

	μMole/gm./hr.
1.0 μMole 2-methylvalerate	0.67
4.0 μMole 2-methylvalerate	0.84
0.1 μMole heptanoate	0.17
0.4 μMole heptanoate	0.17
0.01 μMole palmitate	0.17
0.04 μMole palmitate	0.00
0.01 μMole oleate	0.00
0.04 μMole oleate	0.33

APPENDIX D Continued

M5. Each tube contained mitochondria from 0.02 gm. of muscle tissue.

	µMole/gm./hr.
1.0 µMole 2-methylbutyrate + 2.0 µMole succinate	7.85
2.0 µMole 2-methylbutyrate + 2.0 µMole succinate	8.57
4.0 µMole 2-methylbutyrate + 2.0 µMole succinate	8.21
8.0 µMole 2-methylbutyrate + 2.0 µMole succinate	8.21
16.0 µMole 2-methylbutyrate + 2.0 µMole succinate	8.93
1.0 µMole oleate + 2.0 µMole succinate	6.43
2.0 µMole oleate + 2.0 µMole succinate	3.93
4.0 µMole oleate + 2.0 µMole succinate	-0.72
8.0 µMole oleate + 2.0 µMole succinate	-1.44
16.0 µMole oleate + 2.0 µMole succinate	-4.29

M6. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	µMole/gm./hr.
4.0 µMole acetate	-1.25
4.0 µMole propionate	-1.25
4.0 µMole butyrate	2.10
4.0 µMole 2-methybutyrate	2.50
4.0 µMole 2-methylvalerate	10.85
0.4 µMole palmitate	0.83
0.4 µMole oleate	-0.43

M7. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	µMole/gm./hr.
1.0 µMole 2-methylvalerate	27.06
4.0 µMole 2-methylvalerate	27.06
0.1 µMole palmitate	-1.02
0.4 µMole palmitate	0.00
0.01 µMole palmitate	0.00
0.04 µMole palmitate	-1.02
0.1 µMole oleate	-1.02
0.4 µMole oleate	-1.02
0.01 µMole oleate	-1.02
0.04 µMole oleate	0.00

M8. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	µMole/gm./hr.
10.0 µMole 2-methylbutyrate	2.07
*20.0 µMole 2-methylbutyrate	1.07
40.0 µMole 2-methylbutyrate	3.01
80.0 µMole 2-methylbutyrate	4.26

*diluted by mistake

APPENDIX D Continued

M9. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	µMole/gm./hr.
10.0 µMole 2-methylbutyrate	2.76
10.0 µMole 2-methylbutyrate + 1.0 µMole succinate	10.28
10.0 µMole 2-methylbutyrate + 2.0 µMole succinate	11.28
10.0 µMole 2-methylbutyrate + 4.0 µMole succinate	13.29
10.0 µMole 2-methylbutyrate + 8.0 µMole succinate	13.29
1.0 µMole succinate	5.27
2.0 µMole succinate	8.03
4.0 µMole succinate	9.27
8.0 µMole succinate	12.03

M10. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	µMole/gm./hr.
1.0 µMole succinate	0.66
1.0 µMole succinate + .01 µMole oleate	0.66
1.0 µMole succinate + .02 µMole oleate	0.66
1.0 µMole succinate + .04 µMole oleate	0.66
1.0 µMole succinate + .08 µMole oleate	0.66
1.0 µMole succinate + .01 µMole palmitate	0.66
1.0 µMole succinate + .02 µMole palmitate	0.34
1.0 µMole succinate + .04 µMole palmitate	0.66
1.0 µMole succinate + .08 µMole palmitate	0.34
1.0 µMole succinate + .1 µMole heptanoate	0.34
1.0 µMole succinate + .2 µMole heptanoate	0.34
1.0 µMole succinate + .4 µMole heptanoate	0.84
1.0 µMole succinate + .8 µMole heptanoate	0.84

M11. Each tube contained mitochondria from 0.05 gm. of muscle from worms starved for 3 days.

	µMole/gm./hr.
0.1 µMole oleate	0.48
0.2 µMole oleate	0.48
0.4 µMole oleate	0.25
0.8 µMole oleate	0.25
0.1 µMole oleate + 1.0 µMole fumarate	0.48
0.2 µMole oleate + 1.0 µMole fumarate	0.25
0.4 µMole oleate + 1.0 µMole fumarate	0.48
0.8 µMole oleate + 1.0 µMole fumarate	0.48
0.1 µMole oleate + 1.0 µMole succinate	2.44
0.2 µMole oleate + 1.0 µMole succinate	2.19
0.4 µMole oleate + 1.0 µMole succinate	1.46
0.8 µMole oleate + 1.0 µMole succinate	1.23
1.0 µMole fumarate	0.46
1.0 µMole succinate	2.93

APPENDIX D Continued

M12. Each tube contained mitochondria from 0.08 gm. of muscle tissue.

	µMole/gm./hr.
80.0 µMole 2-methylbutyrate	0.94
80.0 µMole 2-methylbutyrate + 4.5 µMole ATP	1.26
80.0 µMole 2-methylbutyrate + 20.0 µMole succinate	10.02
80.0 µMole 2-methylbutyrate + 20.0 µMole succinate + 4.5 µMole ATP	10.34
40.0 µMole oleate	0.22
40.0 µMole oleate + 4.5 µMole ATP	0.22
40.0 µMole oleate + 20.0 µMole succinate	7.52
40.0 µMole oleate + 20.0 µMole succinate + 4.5 µMole ATP	7.42

M13. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	µMole/gm./hr.
4.5 µMole ATP	.46
2.0 µMole 2-methylbutyrate	1.68
2.0 µMole 2-methylbutyrate + 9.0 µMole ATP	2.13
2.0 µMole 2-methylbutyrate + 9.0 µMole ATP + 0.06 µMole Cyt 6	1.53
2.0 µMole 2-methylbutyrate + 9.0 µMole ATP + 0.06 µMole Cyt c + 1.0 µMole fumarate	1.83
2.0 µMole 2-methylbutyrate + 0.06 µMole Cyt c	1.83
2.0 µMole 2-methylbutyrate + 0.06 µMole Cyt c + 1.0 µMole fumarate	2.13
2.0 µMole 2-methylbutyrate + 1.0 µMole fumarate	1.83
2.0 µMole 2-methylbutyrate + 9.0 µMole ATP + 1.0 µMole fumarate	1.83

M14. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	µMole/gm./hr.
4.0 µMole 2-methylvalerate	13.34
1.5 µMole ATP + 0.025 µMole CoA	2.01
1.5 µMole ATP + 0.025 uMole CoA + 4 µMole 2-methylvalerate	13.02
1.5 µMole ATP + 0.025 µMole CoA + .4 µMole oleate	0.00
1.5 µMole ATP + 0.025 µMole CoA + .4 µMole palmitate	-0.51
3.0 µMole ATP + 0.025 µMole CoA	4.02
3.0 µMole ATP + 0.025 µMole CoA + 4 µMole 2-methylvalerate	14.55
3.0 µMole ATP + 0.025 µMole CoA + .04 µMole oleate	4.02
3.0 µMole ATP + 0.025 µMole CoA + .04 µMole palmitate	4.02

APPENDIX D Continued

M15. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	μMole/gm./hr.
4.0 μMole 2-methylvalerate + 60.0 μMole each of KCl, $MgCl_2$, NaCN	12.54
4.0 μMole 2-methylvalerate + 60.0 μMole $MgCl_2$, NaCN, + 10.0 μMole KCl	14.04
4.0 μMole 2-methylvalerate + 60.0 μMole KCl, $MgCl_2$, + 10.0 μMole NaCN	13.02
4.0 μMole 2-methylvalerate + 60.0 μMole KCl, NaCN, + 10.0 μMole $MgCl_2$	12.54
0.4 μMole oleate + 60.0 μMole KCl, $MgCl_2$, NaCN	0.00
0.4 μMole oleate + 60.0 μMole $MgCl_2$, NaCN + 10 μMole KCl	0.00
0.4 μMole oleate + 60.0 μMole KCl, $MgCl_2$, + 10.0 μMole NaCN	0.00
0.4 μMole oleate + 60.0 μMole KCl, NaCN + 10.0 μMole $MgCl_2$	0.00
0.4 μMole oleate + 10.0 μMole KCl, NaCN, $MgCl_2$	-0.51

M16. Each tube contained mitochondria from 0.05 gm. of muscle tissue.

	μMole/gm./hr.
4.0 μMole 2-methylvalerate	20.04
4.0 μMole 2-methylvalerate + 80 μMole malonate	28.08
0.4 μMole palmitate	1.02
0.4 μMole palmitate + 80 μMole malonate	1.02
0.4 μMole oleate	1.98
0.4 μMole oleate + 80 μMole malonate	1.98

M17. Each tube contained mitochondria from 0.1 gm. of muscle tissue.

	μMole/gm./hr.
1.0 μMole 2-methylvalerate	1.70
1.0 μMole 2-methylvalerate + 1.0 μMole malonate	2.04
2.0 μMole 2-methylvalerate	2.38
2.0 μMole 2-methylvalerate + 2.0 μMole malonate	2.38
0.1 μMole 2-methylvalerate	0.00
0.1 μMole 2-methylvalerate + 1.0 μMole malonate	0.23
0.2 μMole 2-methylvalerate	0.57
0.2 μMole 2-methylvalerate + 2.0 μMole malonate	1.25

M18. Each tube contained mitochondria from 0.08 gm. of muscle tissue.

	μMole/gm./hr.
2.0 μMole 2-methylvalerate	1.95
0.01 μMole palmitate + 1.0 μMole malonate	-0.21
0.02 μMole palmitate + 2.0 μMole malonate	0.00
0.08 μMole palmitate + 8.0 μMole malonate	0.39
0.01 μMole palmitate	0.00
0.08 μMole palmitate	-0.21
0.01 μMole oleate + 1.0 μMole malonate	0.00
0.02 μMole oleate + 2.0 μMole malonate	0.21
0.08 μMole oleate + 8.0 μMole malonate	0.39
0.01 μMole oleate	0.00
0.08 μMole oleate	-0.21

APPENDIX D Continued

M19. Each tube contained mitochondria from 0.05 gm. of muscle tissue incubated under $N_2/5\% CO_2$.

	µMole/gm./hr.
2.0 µMole 2-methylvalerate	3.20
2.0 µMole 2-methylvalerate + 2.0 µMole malonate	4.01
0.02 µMole palmitate	-0.40
0.02 µMole palmitate + 2.0 µMole malonate	0.00
0.02 µMole oleate	-0.40
0.02 µMole oleate + 2.0 µMole malonate	-0.20

M20. Each tube contained mitochondria from 0.05 gm. of muscle tissue incubated under air/5% CO_2.

	µMole/gm./hr.
2.00 µMole 2-methylvalerate	2.81
2.00 µMole 2-methylvalerate + 2.0 µMole malonate	2.75
0.02 µMole palmitate	0.20
0.02 µMole palmitate + 2.0 µMole malonate	-0.40
0.02 µMole oleate	0.00
0.02 µMole oleate + 2.0 µMole malonate	-1.20

M21. Each tube contained mitochondria from 0.02 gm. of muscle tissue.

	µMole/gm./hr.
1.0 µMole 2-methylvalerate	4.51
8.0 µMole 2-methylvalerate	10.02
1.0 µMole 2-methylvalerate + 10 µMole $NaHCO_3$	5.51
8.0 µMole 2-methylvalerate + 10 µMole $NaHCO_3$	10.02
0.1 µMole oleate	-0.50
0.8 µMole oleate	0.50
0.1 µMole oleate + 10 µMole $NaHCO_3$	0.00
0.8 µMole oleate + 10 µMole $NaHCO_3$	0.50

M22. Each tube contained mitochondria from 0.01 gm. of muscle tissue.

	µMole/gm./hr.
1.0 µMole 2-methylvalerate	1.50
8.0 µMole 2-methylvalerate	2.84
1.0 µMole 2-methylvalerate + 10.0 µMole $NaHCO_3$	1.34
8.0 µMole 2-methylvalerate + 10.0 µMole $NaHCO_3$	3.00
0.1 µMole oleate	-0.16
0.8 µMole oleate	0.00
0.1 µMole oleate + 10.0 µMole $NaHCO_3$	-0.50
0.8 µMole oleate + 10.0 µMole $NaHCO_3$	0.00

APPENDIX D Continued

M23. Each tube contined 4.5 micromoles of ATP and 40.0 micromoles of 2-methylbutyrate. The amount of mitochondria varied.

	μMole/gm./hr.
mitochondria from 0.01 gm. of muscle	2.82
mitochondria from 0.02 gm. of muscle	1.79
mitochondria from 0.04 gm. of muscle	4.10
mitochondria from 0.08 gm. of muscle	3.14
mitochondria from 0.10 gm. of muscle	3.23

M24. Each tube contained mitochondria (which had been stored in the refrigerator for 48 hours) from 0.29 gm. muscle, and 80.0 micromoles of 2-methylvalerate. 60 micromoles of sodium phosphate buffer at each of the indicated pH values was used.

buffer pH	μMole/gm./hr.
6.0	1.38
6.2	1.43
6.4	1.87
6.6	1.94
6.8	1.85
7.0	1.98
7.2	1.94
7.4	2.05
7.6	1.66
7.8	1.77
8.0	1.34

APPENDIX E

OXIDATION OF FATTY ACIDS ON LARGE SCALE

Each of the following incubations was carried out in a 125 ml. flask immersed in a water bath at 37°C. Dye was added at intervals as the color disappeared. All incubations were allowed to continue until dye reduction stopped. In all cases dye reduction continued for 1½ to 2 hours. The results are listed in terms of total micromoles of dye reduced and micromoles of dye reduced per gram of muscle.

LS1. Each flask contained the mitochondria from 1.07 gm. of muscle, 300 micromoles of sodium phosphate buffer pH 7.4, 50 micromoles each of $MgCl_2$, KCl, and KCN. 200 micromoles of substrate was added to each flask.

	DYE REDUCED	
Substrate	µMoles	µMoles/gm.
tiglate	0.612	0.572
2-methylbutyrate	1.071	1.001
2-methylvalerate	1.836	1.716

LS2. The experiment was designed as above except that each flask contained mitochondria from 2.7 gm. of muscle tissue and the phosphate buffer was reduced to 150 micromoles.

	DYE REDUCED	
Substrate	µMoles	µMoles/gm.
tiglate	0.612	0.227
2-methylbutyrate	1.224	0.453
2-methylvalerate	1.836	0.680

LS3. Each flask contained mitochondria from 3.34 gm. of muscle, otherwise the contents were the same as LS2.

	DYE REDUCED	
Substrate	µMoles	µMoles/gm.
100 µMoles 2-methylvalerate	2.448	1.733
100 µMoles 2-methylvalerate plus 100 µMoles malonate	3.366	1.008

APPENDIX E Continued

LS4. Two identical flasks were set up each containing mitochondria from 15 gm. of muscle. The amount of sodium phosphate buffer was increased to 750 micromoles but the other ingredients were the same. The final volume of each flask was 90 ml.

Substrate	DYE REDUCED µMoles	µMoles/gm.
300 µMoles 2-methylbutyrate	91.8	6.120

LS5. Each flask contained mitochondria from 30 gm. of muscle, 3 millimoles of sodium phosphate buffer, and the same amount of the other salts as listed above. 300 micromoles of substrate was added to each tube. The final volume was brought to 60 ml. with distilled water.

Substrate	DYE REDUCED µMoles	µMoles/gm.
2-methylbutyrate in air	7.65	0.255
2-methylbutyrate in N_2	38.25	1.275
2-methylvalerate in air	22.95	0.765
2-methylvalerate in N_2	53.55	1.785

LS6. Each flask contained mitochondria from 21.5 gm. of muscle and 200 µMoles of substrate in addition to the buffer and salts as in LS5. Incubation was under nitrogen.

Substrate	DYE REDUCED µMoles	µMoles/gm.
2-methylvalerate	76.5	3.558

LS7. Each flask contained 3 millimoles of sodium phosphate buffer 300 micromoles of KCN, mitochondria from 15.5 gm. of muscle and 200 µMoles of substrate under nitrogen.

Substrate	DYE REDUCED µMoles	µMoles/gm.
2-methylbutyrate	61.2	3.948
propionate	52.5	3.387

LS8. Incubation was as in LS7. Each tube contained mitochondria from 15 gm. of muscle.

Substrate	DYE REDUCED µMoles	µMoles/gm.
2-methylvalerate in air	7.65	0.510
2-methylvalerate in N_2	30.6	2.040
2-methylbutyrate in air	6.65	0.510
propionate in air	0.00	0.000
acetate in air	0.00	0.000
acetate in N_2	0.00	0.000

APPENDIX E Continued

LS9. The contents of each flask were the same as in LS8 with the exception of the substrate.

Substrate	DYE REDUCED µMoles	DYE REDUCED µMoles/gm.
pyruvate in air	0.0	0.000
pyruvate in N_2	22.9	1.527
succinate in air	53.5	3.567
succinate in N_2	84.1	5.607

LS10. This series of flasks was incubated in the absence of dye. Mitochondria from 14 gm. of muscle was added to each flask. The other ingredients were as in LS8.

Substrate	DYE REDUCED µMoles	DYE REDUCED µMoles/gm.
2-methylbutyrate in air		
2-methylbutyrate in N_2		
2-methylvalerate in air	No dye was added to these flasks	
2-methylvalerate in N_2		
propionate in air		
propionate in N_2		

APPENDIX F

END PRODUCT ANALYSIS

Column chromatography r value = migration of band in cm/movement of the meniscus of the solvent in cm.
Paper chromatography rf value = distance of center of the spot from the origin/distance solvent front from the origin

LS1-3 Insufficient end product to identify by the methods used.

LS4-5 Methods used resulted in loss of volatile fatty acids.

	Substrate	r	rf	Fatty Acids
LS6	2-methylvalerate in N_2	0.035		?
		0.47		propionate
		1.73		2-methylvalerate
LS7	2-methylbutyrate in N_2	0.035		?
		0.20	0.085	acetate
		0.38 (faint)	0.179	propionate
		1.81	0.382	2-methylbutyrate
	propionate in N_2	0.031		?
		0.20	0.080	acetate
		0.38	0.176	propionate
		1.78	0.43	a C_5 acid?

APPENDIX F Continued

	Substrate	r	rf	Fatty Acids
LS8	2-methylvalerate in air	0.45		propionate
		1.66		2-methylvalerate
	2-methylvalerate in N_2	0.39 (faint)		propionate
		1.78	0.57	2-methylvalerate
	2-methylbutyrate in air	0.79		?
		1.25		?
		1.68	0.42	2-methylbutyrate
	propionate in air	0.44	0.18	propionate
		1.66		a C_6 acid?
	acetate in air	0.17	0.11	acetate
	acetate in N_2	0.15	0.11	acetate
		0.44 (very faint)		propionate?
LS9	pyruvate in air	0.005		?
		0.098	0.11	pyruvate
		0.174 (faint)		acetate
	pyruvate in N_2	0.016		?
		0.104	0.11	pyruvate
	succinate in air	0.0076	0.00	succinate
		0.086	0.08*	fumarate?
	succinate in N_2		0.00	succinate
		0.082	0.07*	fumarate

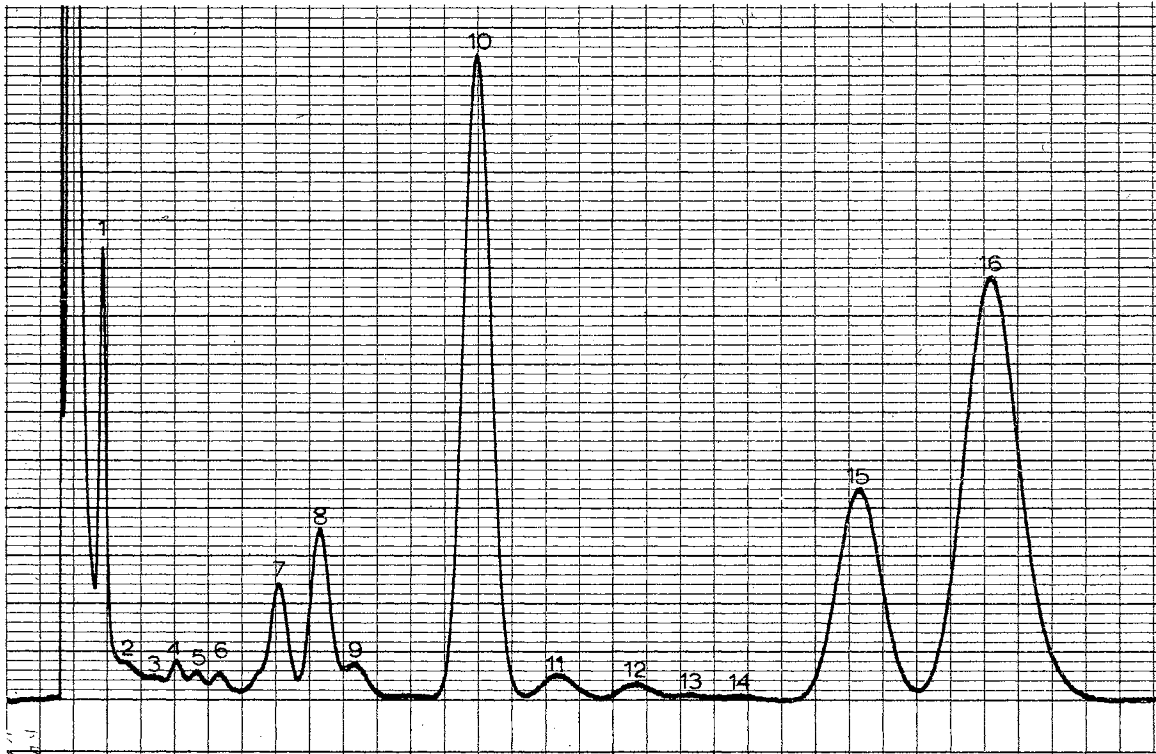
*absorbed ultraviolet light

APPENDIX F Continued

	Substrate	r	rf	Fatty Acids
LS10	Incubation in the absence of dye			
	2-methylbutyrate in air	0.20 (very faint)	0.10	acetate
		0.52		propionate
		1.65	0.43	2-methylbutyrate
	2-methylbutyrate in N_2		0.13	acetate
		1.7	0.56	2-methylvalerate
	2-methylvalerate in air		0.13	acetate
		1.7	0.56	2-methylvalerate
	2-methylvalerate in N_2		0.11	acetate
		1.7	0.56	2-methylvalerate
	propionate in air	0.42	0.20	propionate
		1.7		a C_6 acid?
	propionate in N_2	0.34	0.21	propionate

Appendix G. Gas-liquid chromatogram of the methyl-esters of the non-volatile acids of the saponifiable neutral lipids of the body wall of worms starved 4 days under 95% air/5% CO_2. The sample was chromatographed on a column packed with Gas Chrom CLH coated with 14.5% ethylene glycol succinate, at 168°C. and an argon pressure of 16 psi. The peaks were identified as follows:

1.	9:0br	12.	17:0
2.	10:0	13.	16:2
3.	11:0	14.	18:0br
4.	12:0	15.	18:0
5.	13:0br	16.	18:1
6.	13:0	17.	18:2
7.	14:0	18.	20:1
8.	15:0br	19.	18:3
9.	15:0	20.	21:0
10.	16:0	21.	21:1
11.	16:1	22.	23:0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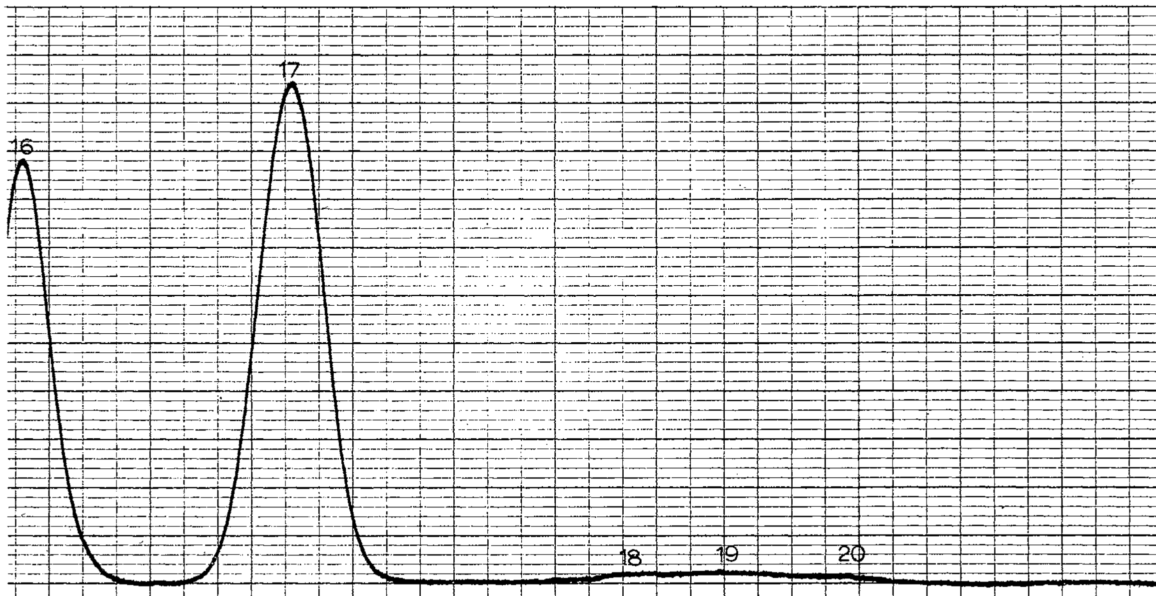
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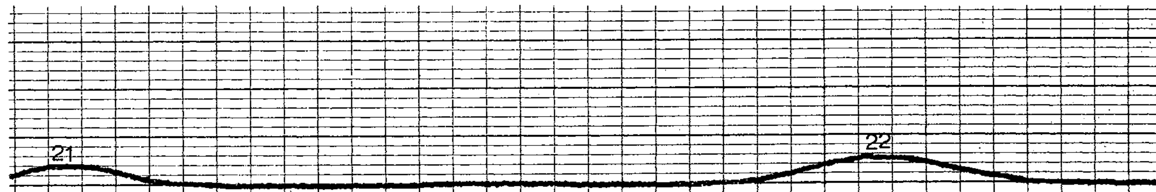
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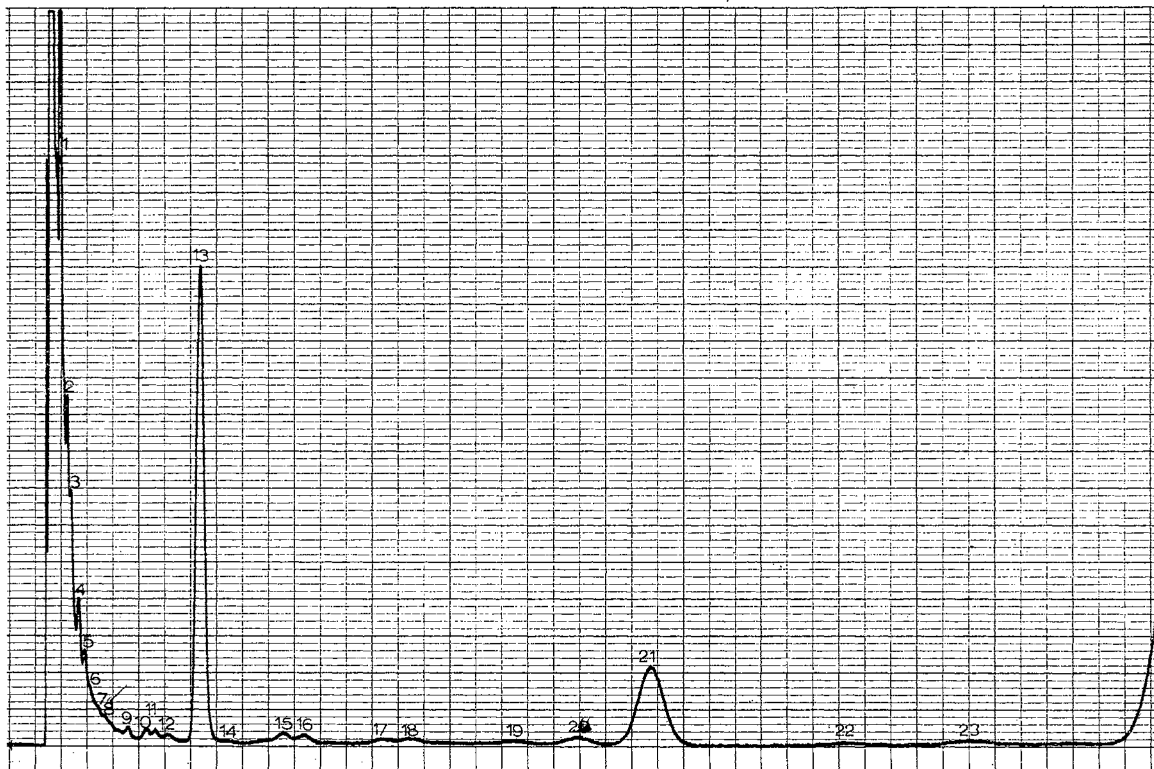
Appendix H. Gas-liquid chromatogram of the methyl esters of the fatty acids of the saponifiable neutral lipids of the body wall of worms starved 4 days under 95% N_2/5% CO_2. The sample was chromatographed on a column packed with Gas Chrom CLH coated with 20% Apiezon L at 200° C., and at an agron pressure of 20 psi. Tentative identification of the peaks was as follows:

1. 7:0br
2. 8:0
3. 8:0br
4. 9:1
5. 9:0
6. 9:0br
7. 10:0
8. 10:0br
9. 11:0br
10. 11:0br
11. 12:0
12. 12:0br
13. 13:1
14. 13:0br
15. 13:0br
16. 14:0
17. 15:1
18. 14:0br
19. 15:0br
20. 16:1
21. 16:0
22. 17:1
23. 17:0
24. 18:2 & 3
25. 18:1
26. 18:0
27. 19:2
28. 19:0
29. 19:0br
30. 20:2
31. 20:1
32. 20:0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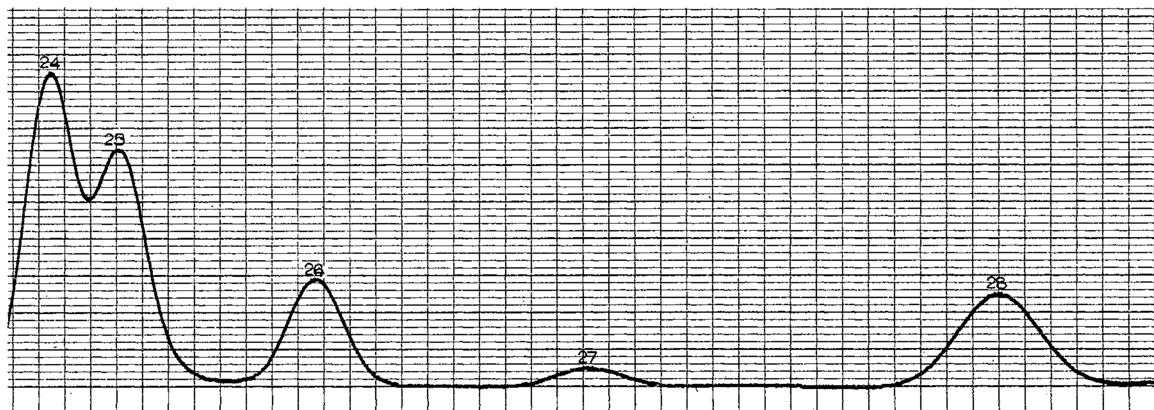
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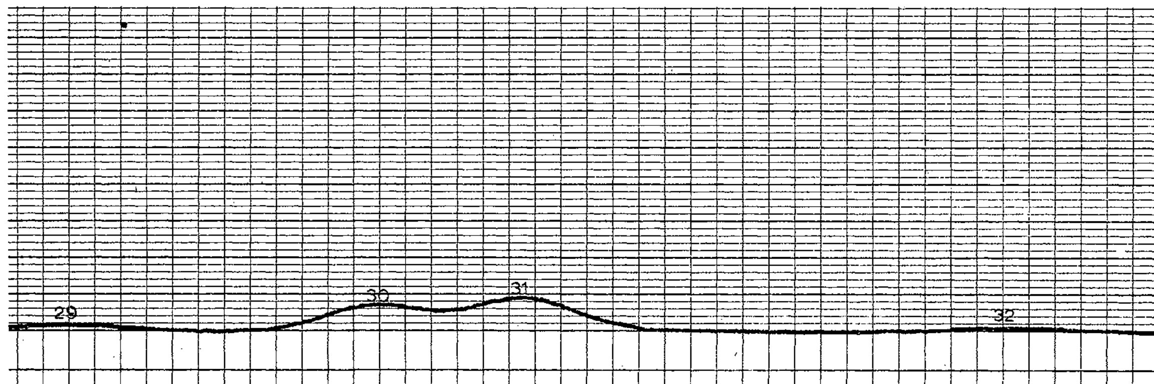
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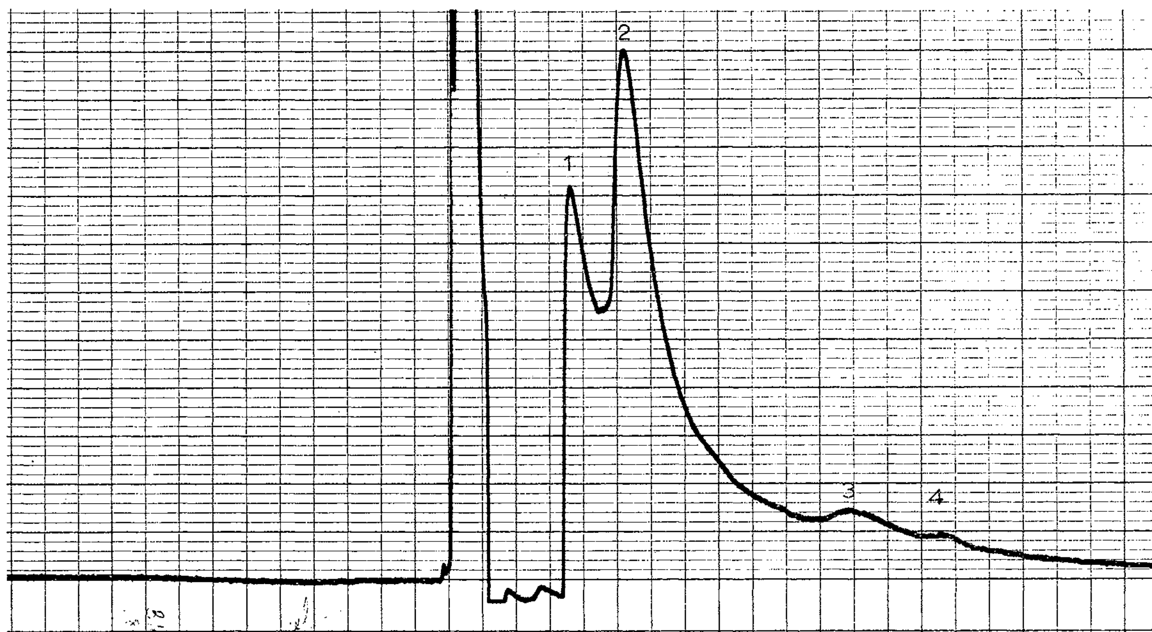
Appendix I. Gas-liquid chromatogram of the volatile fatty acids from the saponifiable lipids of the uterus plus eggs of *Ascaris lumbricoides*. The sample was chromatographed on a column packed with Gas Chrom CLP coated with DC550/15% Stearic acid at 100°C and an argon pressure of 16 psi. The peaks are identified as follows: 1. formic acid, 2. acetic acid, 3. propionic acid, 4. unknown, 5. isovaleric acid, 6. 2-methylbutyric acid, 7. n-valeric acid, 8. 2-methylvaleric acid.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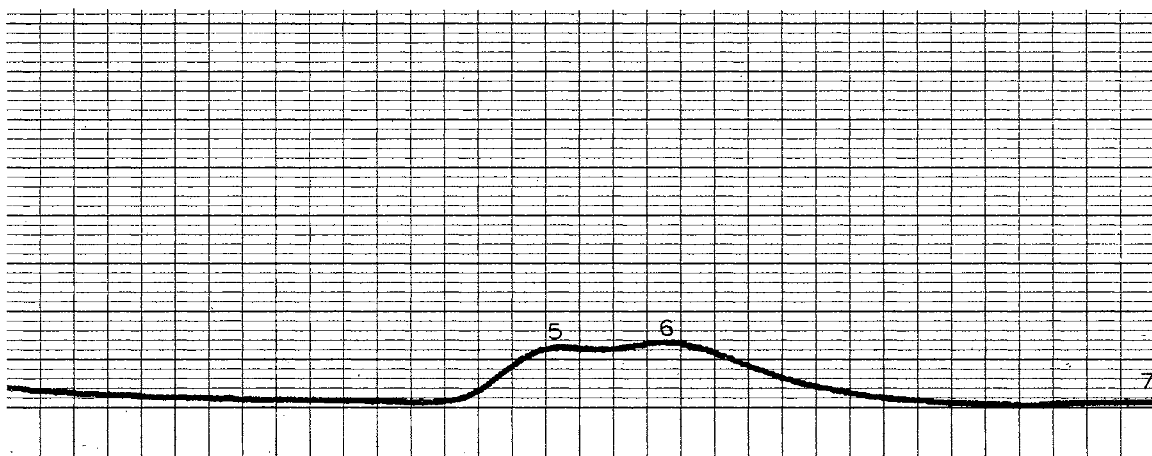
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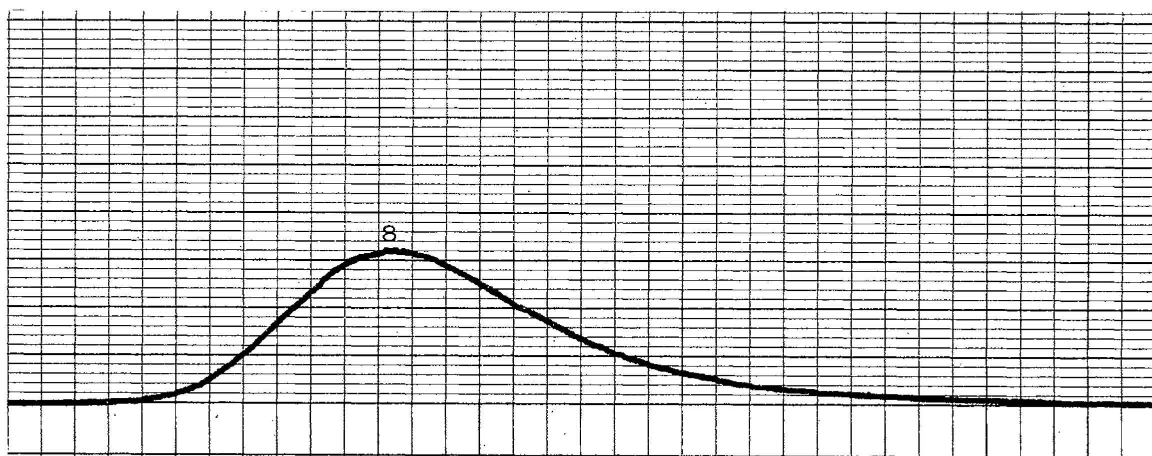
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VITA

Neil Soren Jacobsen

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