THE LARVAL LIPIDS OF THE CHIRONOMID MIDGE

GLYPTOTENDIPES BARBIPES (STAEGER)

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This problem was concerned with determining the total lipid content and individual lipid composition of the larvae of a local chironomid, Glyptotendipes barbipes (Staeger). Lipid was obtained by homogenizing the larvae and then extracting with 20 volumes (w/v) of chloroform: methanol (2:1, v/v). After filtering to remove the residue, the filtrate was washed with 0.02% $CaCl_2$ (w/v), the water removed by freeze-filtering and use of Na_2SO_4, and the sample concentrated. The sample was then analyzed by chromatography, weighing, and spectrophotometry.

The results from column chromatography, thin layer chromatography, and gas chromatography indicate that these larvae are unique in their lipid properties. These can be summarized as follows:

(1) The total extractable lipid is two per cent of the wet weight, less than that reported for any other dipteran.

(2) The lipid recovered from column chromatography on silica gel consisted of about 72 per cent neutral lipid and 28 per cent phospholipid.

(3) The fatty acid composition of the neutral lipids differed from that of the phospholipids. The neutral lipids had 6.9 times more myristic acid, 3.8 times more palmitoleic acid, and 85 per cent less stearic acid.

(4) The total percentage of saturated fatty acids versus unsaturated fatty acids was about the same for both neutral lipids and phospholipids, with about 61 to 65 per cent saturation in each.

(5) Cephalin appeared to be a major component of the phospholipid fraction.

Several postulates are presented as an explanation of the above facts. The effect of an anaerobic environment may be a key factor. Possible compartmentation of biosynthetic sites and specific acyl-recognizing phosphates are also postulated.

THE LARVAL LIPIDS OF THE CHRONOMID MIDGE *GLYPTOTENDIPES BARBIPES* (STAEGER)

THESIS

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CHAPTER I

INTRODUCTION

The Midges

The true midges belong to the family Chironomidae of the order Diptera and are closely related to the flies and mosquitoes, but usually do not bite. Thienemann (1954) has published the most comprehensive review of the Chironomidae to date. Oliver (1971) has surveyed the more important work since Thienemann's publication. In the USA, Johannsen and Malloch have extensively studied and described the eastern and midwestern species (Johannsen, 1903, 1905, 1908, 1937, 1946; Malloch, 1915) and Townes (see Cole, 1969) has studied the western species. The family is world-wide in distribution, ranging from the Arctic to the Antarctic (Oliver, 1968; Wirth and Gressit, 1967). Most midge larvae are aquatic but some live in manure, in decaying vegetable matter, under bark, or in moist ground. At least one species lives in sap (see Cole, 1969, and Borror and Delong, 1955). The larvae of some of the Chironominae eat algae but those of the Tanypodinae are

carnivorous. The majority of the larvae are scavengers. Walshe (1951) has described in detail the feeding mechanisms of the Chironominae. Many of the aquatic larvae live in cases or tubes formed from bits of dead leaves and sand held together with silk-like threads secreted by the salivary glands (Walshe, 1951; Edgar and Meadows, 1969). Chironomid larvae form an important part of the diet of many fishes (Kugler and Chen, 1968). The adult midge tends to form huge swarms and Syrjamaki (1964) has grouped chironomids on the basis of their swarming times. The adults are frequently classified as a nuisance because of their population densities (Bay, 1964; Gerry, 1951; Jamnback, 1954) and research has been conducted on control measures (Edwards et al., 1964; Grodhaus, 1963; Hitchcock and Anderson, 1968; Hunter, 1967; Patterson and Wilson, 1966).

Chironomids and Pollution

With the rising concern over the problem of environmental pollution, chironomid larvae have become important as a possible index of pollution. Oliver (1971) has outlined the broad environmental requirements of the various

subfamilies of the Chironomidae and certain groups seem to be characteristic of particular environmental conditions. Thienemann (1954) even initiated a system of lake classification based on the type of chironomids found. This was further developed by Lenz and Lundberg (see Brundin, 1949). Concomitant with many types of pollution there is an alteration in the oxygen content of the water. Since chironomid larvae show specific differences in their resistance to low oxygen concentration (Harnisch, 1930; Pause, 1918), this factor frequently determines the presence or absence of certain species. Many of the species have larvae which are bright red in color due to the presence of a haemoglobin pigment (for which reason they are called "blood-worms"). Attempts have been made to correlate the presence of haemoglobin with the ability of Chironominae larvae to function at low oxygen concentrations (Braun, Formanek and Braunitzer, 1968; Harnisch, 1930, 1958; Lavrosky, 1966; Miall, 1900, 1903; Neuman, 1961; Platzer-Schultz, 1967, 1968, 1970; Scheer, 1934, Walshe, 1947a, b, 1951. See also Buck, 1953; Weber, 1965; and Wigglesworth, 1965). Since other larvae without haemoglobin can function at low oxygen concentrations, this problem needs also to

be investigated from many other aspects of physiology and intermediary metabolism.

Chironomid Metabolism

Although such areas as morphology, environmental habitat, life cycle, chromosomal puffing, and respiration physiology have been extensively studied in the Chironomidae, the intermediary metabolism has been neglected. A few studies have been done on carbohydrate metabolism in the Chironomidae. Harnisch (1938) studied changes in the metabolic constituents of larvae of Chironomus thummi under aerobic and anaerobic conditions. He found a tenfold increase in the total use of glycogen under anoxic conditions. Some of this response was due to a twofold increase in the rate at which glycogen was used. Augenfeld (1967) reported nearly twice as great a loss of glycogen under anoxic conditions as under aerobic conditions. He also reported that three anoxia-resistant species of Chironomus had nearly ten times more glycogen, percentage-wise, than did corresponding larvae of the anoxia-intolerant Tanytarsus species. Zebe and McShan (1957) and Augenfeld and Ness (1969) demonstrated the presence of lactic

dehydrogenase and of a glycolytic enzyme system in the tissues of Chironomus plumosus.

Lipids of Diptera

There are several good reviews on the subject of lipid content and metabolism in insects (Buck, 1953; Chefurka, 1965; Clayton, 1964; Fast, 1964; Gilbert, 1967; Gilbert and Schneiderman, 1961; Gilby, 1965; Gilmour, 1961; Hilditch, 1956; Jacobson and Beroza, 1963; Karlson, 1963; Karlson and Butenandt, 1959; Kilby, 1963; Niermierko, 1958; Scoggin and Tauber, 1950; Tietz-Devir, 1963; Wigglesworth, 1965; Wyatt, 1961). Diptera appear to differ from other animals both in fatty acid and phospholipid composition. As a group they are distinguished by a high percentage of palmitoleic acid (C 16:1) in both the glyceridic and phospholipid fractions (see Fast, 1964, 1966). Barlow (1964) found 19 to 60 per cent of palmitoleic acid in five species from four dipteran families, while most other species yielded no more than about 2.2 per cent. Gas chromatographic analyses have shown 34 per cent of palmitoleic acid in newly synthesized fatty acids of Aedes sollicitans adults (Van Handel and Lum, 1961) and more than 30 per cent

in larvae of Aedes aegypti (Fast and Brown, 1962). Hayashiya and Harwood (1968) found that palmitic, palmitoleic, and oleic acids collectively comprised at least 70 per cent of the total fatty acid in two strains of Anopheles freeborni. On the basis of previous reports (Takata and Harwood, 1964; Van Handel and Lum, 1961; Fast and Brown, 1962), they decided that the pattern of accumulation was characteristic for the Culicidae. Unlike mammals and most orders of insects, the major phospholipid fraction of Diptera is phosphatidyl ethanolamine (Bieber et al., 1961; Fast, 1966). In Aedes aegypti this phospholipid comprises 86 per cent of the total extractable phospholipid (Fast and Brown, 1962). Fast (1966) studied the phospholipids and fatty acids of 27 species of insects representing 6 orders and 20 families. He reported that the phosphorus in the ethanolamine phosphoglycerides accounted for approximately 50 per cent of the total lipid phosphorus in all but one family of Diptera examined, while the phosphorus of the choline phosphoglycerides accounted for only about 25 per cent. Fast and Brown (1962) found that the cephalin fraction from A. aegypti differed from the neutral lipids only in having slightly more 18-carbon acids and fewer

12- and 14-carbon acids. Fast (1966) reported that Diptera in which the major phosphatides were ethanolamine phosphoglycerides were also characterized by high proportions of fatty acids less than 18 carbons long, particularly palmitoleic acid, in the neutral lipids. The fatty acid compositions of the phosphatides in Diptera differed from those found in other insects and vertebrates, particularly with respect to the distribution of 18-carbon acids. Stearic acid (18:0) levels were consistently higher in the phospholipids than in the neutral lipids, and usually higher in the ethanolamine phosphoglycerides than in the choline phosphoglycerides. Unlike vertebrates and other insects, the oleic acid (18:1) in Diptera tended to be higher in the ethanolamine phosphoglyceride fraction; linoleic acid (18:2) tended to be higher in the ethanolamine phosphoglycerides than in the choline phosphoglycerides. Generally, palmitic acid (C16:0) levels were higher in the neutral lipids than in any phospholipid fraction, and linoleic acid was always higher in the phospholipids than in the neutral lipid fraction. Fast (1966) concluded that Diptera, because of their ethanolamine phosphoglyceride predominance and unique phospholipid fatty

acid composition, may provide the means by which phospholipid function and the role of fatty acids in phospholipids can profitably be studied.

Chironomid Lipids

The literature on lipid content and metabolism in the Chironomidae is very sparse. Norman (see Timon-David, 1930, and Fast, 1964) reported that lipid constituted 8.3 per cent of the wet weight of adult Chironomus sp., and that 10.2 per cent of this was unsaponifiable material. Grindley (1952) reported that lipid composed 11.7 per cent of the dry weight of adult Tanytarsus lewisi (Freem.) and that it had an iodine number of 137.2. He also reported the presence of the fatty acids palmitic (C 16:0), palmitoleic (C 16:1), oleic (C 18:1), and linoleic (C 18:2), and stated that tetra- and pentaenoic fatty acids formed 18 per cent of the total. Fast (1966) reported on the phospholipid and fatty acid composition of Chironomus sp. adults. He found a choline phosphoglyceride/ethanolamine phosphoglyceride ratio of 0.56. Data for the choline phosphoglycerides was not presented, but the fatty acid patterns for the other lipid fractions was similar to

those of other Diptera except for the neutral lipid having more oleic acid than the ethanolamine phosphoglyceride fraction. The most noticeable feature was the nearly sixfold greater percentage of stearic acid in the ethanolamine phosphoglyceride fraction than in the neutral lipids. A small amount of myristoleic acid (C 14:1) was detected in the ethanolamine phosphoglyceride fraction but not in the others. These are the only species for which compositions have been reported; no one as yet has reported anything on the biosynthesis and degradation of lipid in the Chironomidae.

Purpose of the Experiment

Since so little is known about the metabolism (especially, lipid metabolism) of the Chironomidae despite their increasing importance in pollution studies and role as a minor pest in some areas of the country, this research was undertaken to try to describe some of the lipids normally found in a local species of chironomid. Because larvae are the easiest to obtain and to use, they were the stage selected for initial study.

CHAPTER II

METHODS AND MATERIALS

Materials

General Chemicals

Silica Gel powder, sodium nitrite ($NaNO_2$), concentrated sulfuric acid (H_2SO_4), glacial acetic acid, 60% perchloric acid ($HClO_4$), anhydrous sodium sulfate (Na_2SO_4), anhydrous calcium chloride ($CaCl_2$), potassium hydroxide (KOH), potassium dichromate (K_2CrO_4), and ammonium molybdate [$(NH_4)_2MoO_4$] were purchased from J. T. Baker Company, Phillipsburg, N.J. Chloroform, methanol, acetone, diethyl ether, hexane, and anhydrous monobasic potassium phosphate (KH_2PO_4) were obtained from Fisher Scientific Company, Fair Lawn, N.J. Ascorbic acid was bought from Eastman Organic Chemicals, Rochester, N.Y. Kieselgel G was purchased from E. Merk A.G., Darmstadt, Germany. Methylated derivatives of fatty acids for use as standards or reference were provided by Buford L. Brian of the Department of Biological Sciences, North Texas State University, Denton,

Texas. Cholesterol, cephalin, lecithin, monopalmitin, dipalmitin, tristearin, and fatty acids to be used as standards were purchased from Sigma Company, St. Louis, Mo. Methylamine hydrochloride was purchased from Aldrich Chemical Company, Milwaukee, Wis. Urea was obtained from Mallinkrodt Company, St. Louis, Mo. All chemicals were of reagent grade. The organic solvents were redistilled before use.

Special Reagents

Phosphate standard.--Some KH_2PO_4 was oven-dried overnight at 130 degrees centigrade (^oC) and then cooled over calcium chloride in a vacuum dessicator at room temperature. After temperature equilibration, 0.4397 grams (g) were weighed and dissolved in double-distilled water (H_2O) in an acid-washed 100 milliliter (ml) volumetric flask. From this vessel 10.0 ml were removed and diluted with double-distilled H_2O to the mark in an acid-washed 1-liter volumetric flask. This produced an inorganic phosphate standard of 10 μg P/ml or 30.645 μg PO_4/ml. A better standard can be made with the Na_2HPO_4 salt, however, since it is non-hygroscopic.

Phosphate determining reagent (PDR).--This was made by the method of McClare (1971). All glassware was acid-washed and only double-distilled H_2O was used. The final solution contained 40 ± 1 ml of 60% $HClO_4$, 5.0 g of ammonium molybdate, and 1.00 g of ascorbic acid per 500 ml of solution. The ascorbic acid was first mixed separately and then added to the main solution just before final dilution to the mark. If prepared properly the final solution was a clear, pale yellow. In the presence of phosphate or upon standing for extended periods of time, the solution turned deep blue. It had to be made fresh for each testing period.

Nitrosomethylurea and diazomethane.--A solution of 100 g of methylamine hydrochloride and 300 g of urea in 400 ml of water was boiled gently under reflux for 3 hours and then vigorously for 15 minutes. It was then cooled to room temperature and 100 g of sodium nitrite dissolved in it. This solution was further cooled to about 10°C and then added with vigorous stirring to a mixture of 600 g of ice and 110 g of concentrated sulfuric acid cooled in an ice-salt bath. Nitrosomethylurea immediately separated

as a fluffy, pinkish-white crystalline precipitate. It was collected on a Büchner funnel, washed with cold water, and dried in a vacuum desiccator for about one hour. The dried material was weighed and then added rapidly in very small quantities to a flask containing 70 ml of 45% aqueous KOH solution and 500 ml of ether for each 35 g of nitrosomethylurea. The flask was pre-cooled to 5°C in an ice bath and the mixture stirred by means of a magnetic stirrer throughout the addition. The deep yellow ethereal layer containing the resultant diazomethane was decanted and stored in the cold over pellets of KOH.

Biological Material

Larvae of a local chironomid, Glyptotendipes barbipes (Staeger), were collected during the months of January to July, 1971, from the municipal sewage pond of Aubrey, Texas. The larvae and accompanying sediment were transported to the laboratory and stored in 51 x 25 x 30 cm glass aquaria at 5 to 10°C until time of use. The aquaria were then allowed to warm to room temperature, whereupon the larvae would emerge from the mud and attach themselves to the sides of the aquaria. They could then be easily scraped off in large clusters and used.

Methods

Extraction of Lipids

The larvae were washed repeatedly on a Büchner funnel with distilled water to remove mucus and detritus. Between washings the larvae were dried by evaporation and transferred to fresh filter paper. This resulted in extremely clean samples. The larvae were then weighed. Small samples were homogenized for two or three minutes with two volumes (w/v) of chloroform:methanol (2:1, v/v) in a Potter-Elvejhem tissue grinder fitted with a teflon pestle. The samples were then transferred to an Erlenmeyer flask, the tissue grinder was washed twice with small amounts of chloroform:methanol. The washings were added to the flask, and the sample was then diluted to 20 volumes with more chloroform:methanol. The solution was flushed with nitrogen gas (N_2) and the flask sealed. If teflon-lined screw caps were not available, aluminum foil-wrapped rubber stoppers were used. The flask was then shaken over-night at room temperature on a rotary shaker (Eberbach Corporation, Ann Arbor, Mich.).

After extraction, the sample was filtered on a Büchner funnel through lipid-free filter paper (obtained by soaking regular filter paper in chloroform). The flask and residue were rinsed with more chloroform:methanol and the rinse solution was added to the first filtrate. The solution was transferred to a separatory funnel equipped with a teflon stopcock and was then washed with equal or lesser volumes of 0.02% $CaCl_2$ (w/v). This salt was chosen because Folch et al. (1957) reported that its use resulted in less loss of lipid than with the other salts or water. The bottom chloroform layer was drawn off and washed two or three more times until all the green pigment had been removed, leaving a clear yellow solution. The upper phases were discarded. The chloroform phase was transferred to a beaker, covered with aluminum foil, and stored in the freezer at -20°C for at least five hours. The resulting ice was removed by filtering the solution in the cold. The filtrate was then flushed with N_2 and stored at 5 to 10°C over a few grams of $CaCl_2$ in a sealed Erlenmeyer flask for several hours.

Determination of Total Lipid

The sample was removed from the cold and filtered. The filtrate was transferred to a round-bottom flask and the solvent removed under vacuum at 40 to 45°C on a flash-evaporator (Buchler Instruments, Fort Lee, N.J.). The residue was resuspended in chloroform, quantitatively transferred to a 10 ml volumetric flask, and brought to volume. One-milliliter portions were taken for weighing. These were spotted on small aluminum foil planchets and dried to a constant weight. The remainder of the sample was transferred to a screw cap test tube, flushed with N_2, and stored in the freezer at -20°C until needed.

Preparation of Separatory Columns

For separation of samples into neutral lipid and polar lipid fractions, ordinary separatory funnels were used. If stopcocks were not of teflon, they were left ungreased. If lubrication had to be applied to prevent sticking of the ground-glass joints, a silicone-based lubricant was applied lightly to the outside edges of the stopcocks.

For differential separation of the total mixed lipid samples a special column was prepared. A 10-ml Luer-lok

syringe was fused to the bottom of a 2.0 x 82 cm glass tube to give an overall length of 92.0 cm. A number 18-gauge needle, strip of polyethylene tubing, and pinch clamp were attached to the bottom to regulate flow. About 50 to 200 g of silica gel were heated overnight in an oven at 130°C, and 50 g portions then rapidly weighed and slurried in either hexane or chloroform. A pad of glass wool was placed in the bottom of the column and the slurry poured into the column. The gel was allowed to settle. It was then washed with 100-ml portions of hexane, chloroform, methanol, and chloroform (in that sequence). The column for differential separation was then washed with a final addition of 100 ml of hexane.

Separation of Neutral and Polar Lipids

The sample of total mixed lipids was concentrated under a stream of N_2 in a water bath at 45°C. It was then carefully pipetted onto the columns. The test tubes were rinsed with small volumes of chloroform which were then added to the columns. The neutral lipids were eluted with 200 ml of chloroform and collected in a round-bottom flask. The polar lipids were eluted next with 200 ml of

methanol and collected in a separate round-bottom flask. The migration of the methanol was traced by the dark bands of lipid moving down the columns and by the change in color of the gel to a snowy white. The two flasks were flushed with N_2, and the solvents were then flash-evaporated. The neutral residues were diluted to 10 ml with chloroform. The polar residues were diluted to 10 ml with methanol. Portions were taken from both for weighing. The remainder of the samples were flushed with N_2, sealed, and stored at -20°C.

Phosphate Determination

The amount of phosphate or phosphorus present in the polar lipid fraction was determined by the method of McClare (1971). All glassware was pre-cleaned with sulfuric-dichromate solution and only double-distilled water was used for rinsing and preparation of reagents. Approximately 0.1 ml portions of the lipid sample were placed in 20-ml test tubes, and the solvent was evaporated in an oven at 95°C. The tubes were cooled, 0.5 ml of 60% $HClO_4$ added, and the tubes then placed in a preheated tube heater (Warner-Chilcott Laboratories, Morris Plain, N.J.). The tubes were capped with marbles and heated at

200 to 218°C for 30 minutes. They were then cooled and 9.5 ml of PDR was added with a 10-ml Cornwall syringe pipette (Becton, Dickinson and Company, Rutherford, N.J.). The tubes were heated in a water bath for one hour and their optical density was determined on Spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, N.Y.) at 825 nanometers (nm).

Differential Elution of Lipid Classes

The solvent was evaporated from a total mixed lipid sample under a stream of N_2 at 45°C in a water bath. The residue was redissolved in a small volume of hexane and pipetted onto the separatory column. The tube was rinsed with hexane and this washing added to the column. The column was then sequentially eluted with appropriate volumes of hexane, decreasing hexane:ether ratios, ether, chloroform, decreasing chloroform:methanol ratios, and methanol. Samples were collected as 10-ml fractions on an SMI automatic fraction collector equipped with a 10-ml siphon (Scientific Manufacturing Industries, St. Emeryville, Cal.). The solvents were evaporated under a stream of N_2 in a water bath and the residues redissolved in

10.0 ml of chloroform. One-milliliter portions were taken for weighing.

Thin Layer Chromatography (TLC)

The major peaks resulting from differential elution were studied by TLC. Kiselgel G was slurried in water and layered with a Kensco spreader (Kensington Scientific Corporation, Oakland, Cal.) to a depth of 275 microns (μ) on 20 x 20 cm glass plates. The gel was activated by heating at 130°C for an hour. The plates were then washed in chloroform to remove impurities. They were then reactivated. Samples and standards were applied with 10-microliter (μl) capillary pipettes. Neutral lipids were usually developed in hexane:ether:acetic acid (60:40:1). Since the phospholipids did not migrate under these conditions they were developed first in one of various mixtures of chloroform:methanol:water, dried ten minutes, and redeveloped in hexane:ether:acetic acid. The second solution was allowed to migrate higher than the first so that the neutral lipid components would be separated from faster-moving phospholipid species. Spots were located with iodine vapor. For permanent retention of spots,

a spray consisting of 55% H_2SO_4 (w/w) and 0.6% K_2CrO_4 (w/w) was applied, followed by heating at 130°C.

Gas Chromatography

Saponification.--The neutral lipid and polar lipid (hereafter referred to as phospholipid) samples were each pooled in groups of five tubes. The solvents were evaporated under a stream of N_2 in the water bath. To each pooled sample were added 5 ml of 0.5 N methanolic KOH. The tubes were flushed with N_2, sealed, and heated overnight at 65°C. To further insure saponification, 5 ml of 10% methanolic KOH (w/v) were added and the tubes boiled under N_2 for about 30 minutes. On adding 3 ml of water and 10 ml of hexane, the soap which had formed gathered at the interface. The samples were then acidified with 12 N H_2SO_4 until all the soap disappeared. The hexane layer was transferred to a separate tube with a Pasteur pipette and the aqueous layer extracted twice more with 10-ml volumes of hexane. The hexane was evaporated under a stream of N_2 in the water bath and the lipid residue redissolved in ether.

Preparation of methylated fatty acids.--The diazomethane used was freshly distilled from the stock solution by gently warming a small amount of the ether solution in a long-necked distilling flask in a water bath and collecting the distillate in an identical flask packed in ice. The sample tubes were cooled in an ice bath and the fresh distillate added dropwise until the yellow color persisted for 30 minutes. The ether was then removed under a stream of N_2 after portions had been taken for weighing. On the basis of the sample weights the residues were redissolved in enough chloroform to give suitable concentrations for analysis.

Analysis.--The methylated samples were analyzed with a Varian Aerograph 204-1C gas chromatograph equipped with a hydrogen flame detector (Varian Associates, Palo Alto, Cal.). The column consisted of 15% diethylene glycol succinate (DEGS) on Chromosorb W (60/80 mesh) packed in a 5 ft x 1/8 inch (o.d.) copper tube and conditioned overnight at 210°C with gas flow. The carrier gas was helium at 60 psi pressure and 25 ml/min flow. For the analysis, the column temperature was programmed from 150 to 200°C

at a rise of 6°C/min. The injection port was at 210°C and the detector at 215°C. For identification purposes methylated derivatives of decanoic acid (C 10:0), lauric acid (C 12:0), myristic acid (C 14:0), palmitic acid (C 16:0), palmitoleic acid (C 16:1), stearic acid (C 18:0), oleic acid (C 18:1), linoleic acid (C 18:2), linolenic acid (C 18:3), and arachidic acid (C 20:0) were run as standards. The nonsaponifiable components of the samples did not pass through the column so that the peaks observed represented only the fatty acid composition of the samples.

Hydrogenation of samples. --Representative samples of fatty acids from phospholipids and neutral lipids were hydrogenated by adding small amounts of platinum oxide catalyst to chloroform solutions of the fatty acids and then agitating the solutions with a magnetic stirrer at room temperature under a stream of hydrogen gas for 15 to 30 minutes.

CHAPTER III

RESULTS

Table 1 presents the results for total lipid, neutral lipid, phospholipid, and phosphorus content from 30 samples of larvae. Because use of wet weight measurements can lead to large errors in data of this nature, a large number of samples were used and the resulting data examined by statistical analysis.

In Table 1 the results for neutral lipid and phospholipid are expressed as percentages of the total recovered lipid rather than as percentages of the total extracted lipid. This is because the data show that there was usually a loss of lipid at the separation stage, so the two percentages may not be equivalent. The literature fails to make any distinctions between the two, so what is reported as "total lipid" in the literature might be synomymous with either of the two terms used here. If there is no difference in the percentage of loss of the neutral lipids and phospholipids, then all three are synonymous. Table 1 reveals that the larvae have an

TABLE I

PERCENTAGE CONTENT OF LIPIDS AND PHOSPHATE IN LARVAE OF GLYPTOTENDIPES BARBIPES

No.	WWt (g)	%TL / WWt	%NL / RL	%PL / RL	%RL / TL	%P / PL	TP (µg)	TP / WWt
1	3.87	2.6	76.5	23.5	72.5	1.3	128	33.1
2	4.72	2.9	82.9	17.1	53.4	2.2	192	40.7
3	3.49	2.4	74.5	25.5	73.9	2.3	168	49.4
4	3.02	2.0	75.1	24.9	82.1	2.0	149	49.3
5	3.01	2.2	71.5	28.5	79.7	1.6	141	46.8
6	2.94	2.4	85.7	14.3	45.5	4.1	115	39.1
7	2.49	2.5	87.4	12.6	66.1	3.6	112	45.0
8	2.47	2.1	86.7	13.3	66.9	4.0	99	40.1
9	3.68	3.3	40.7	59.3	20.6	0.8	69	18.8
10	2.66	2.6	62.7	37.3	59.2	1.6	141	53.0
11	1.55	2.0	64.0	36.0	126.3	1.0	85	54.8
12	3.95	1.6	68.5	31.5	48.7	3.0	173	43.8
13	2.91	3.0	56.9	43.1	99.6	1.2	275	94.5
14	2.76	2.0	22.6	77.4	43.6	4.3	472	171.0
15	3.03	2.2	64.1	35.9	61.0	1.8	160	52.8

TABLE I --Continued

No.	WWt (g)	TL / %WWt	NL / %RL	PL / %RL	RL / %TL	P / %PL	TP (µg)	TP / WWt
16	3.00	1.0	87.6	12.4	49.9	5.8	64	21.3
17	3.01	1.0	78.4	21.6	65.3	3.1	75	24.9
18	3.01	1.1	74.2	25.8	80.1	2.8	112	37.2
19	3.01	1.1	86.9	13.1	74.1	3.2	64	21.3
20	3.01	2.2	73.7	26.3	64.7	2.4	168	55.8
21	3.02	1.2	77.4	22.6	67.8	2.6	85	28.1
22	3.02	1.8	78.4	21.6	69.3	3.0	144	47.7
23	3.02	1.4	73.9	26.1	62.4	2.8	112	37.1
24	3.04	0.8	68.5	31.5	58.3	3.1	88	28.9
25	3.09	1.9	72.0	28.0	60.3	2.3	131	42.4
26	3.03	2.3	62.4	37.6	56.7	1.8	160	52.8
27	3.05	2.0	73.0	27.0	85.8	2.1	179	58.7
28	3.75	1.9	77.0	23.0	51.2	3.5	176	46.9
29	4.42	2.1	79.2	20.8	59.2	3.2	216	48.9
30	4.83	2.5	64.3	35.7	63.2	2.3	368	76.2
$\bar{X}$		2.0	71.6	28.4	65.6	2.6		48.7
UR		3.3	87.6	77.4	126.3	5.8		171.0

TABLE I --Continued

No.	%WWt (g)	%TL/WWt	%NL/RL	%PL/RL	%RL/TL	%P/PL	TP (μg)	TP/WWt
LR		0.8	22.6	12.4	20.6	1.0		18.8
SD		0.6	13.8	13.8	18.8	1.1		28.0
SE		0.1	2.5	2.5	3.4	0.2		5.1
L_1		2.2	76.7	33.6	72.6	3.0		59.1
L_2		1.8	66.4	23.3	58.6	2.2		38.3

Legend: No., sample number or statistic; WWt, wet weight; TL, total mixed lipid; NL, neutral lipid; PL, phospholipid; RL, recovered lipid = NL + PL; P, phosphorus; TP, total phosphorus in PL fraction; $\bar{X}$, mean; UR, upper limit of range; LR, lower limit of range; SD, standard deviation; SE, standard error; L_1 and L_2, upper and lower limits of 95% confidence interval by Student's t test.

exceedingly small concentration of lipid and that most of this is neutral lipid.

The results of gas chromatographic analysis of the fatty acids found in the saponifiable neutral lipids and phospholipids are presented in Table 2. In contrast to the data of Fast and Brown (1962) for A. aegypti but in agreement with the data of Fast (1966) for Chironomus sp. and other Diptera, the two fractions from G. barbipes have distinct differences in their fatty acid composition. The most noticeable differences occur at C 14:0, C 16:1, C 18:0, and C 20:0. The neutral lipids contain 6.9 times more myristic acid, 3.8 times more palmitoleic acid, and 85 per cent less stearic acid than the phospholipids. Although the C 18:3 and C 20:0 acids did not separate in the phospholipid analyses, it is apparent that the neutral lipids contain two to four times more arachidic acid than the phospholipids do. While there might be error in the ratios of myristic acid and arachidic acid, the amounts of palmitoleic acid and stearic acid are large enough to reflect a significant difference between the two lipid fractions. To prevent misinterpretation of the data as a result of oxidized or degraded samples, six pooled

TABLE II

FATTY ACID COMPOSITION OF LIPIDS IN LARVAE OF GLYPTOTENDIPES BARBIPES

Fatty Acid	Fraction	Relative percent fatty acid			
		X	SD	SE	n
<14	NL	nd			6
	PL	nd			3
14:0	NL	5.5	1.3	0.6	6
	PL	0.8	0.4	0.2	3
A	NL	1.5	0.5	0.3	6
	PL	0.2	0.1	0.1	3
B	NL	1.1	0.2	0.1	6
	PL	0.2	0.2	0.1	3
16:0	NL	37.1	5.8	2.4	6
	PL	30.9	2.2	1.3	3
16:1	NL	13.6	1.3	0.5	6
	PL	3.6	0.6	0.4	3
C	NL	ur			6
	PL	0.4	0.3	0.2	3
D	NL	ur			6
	PL	0.1	0.1	0.1	3
CD	NL	2.3	1.3	0.5	6
	PL	r			3

TABLE II --Continued

Fatty acid	Fraction	Relative percent fatty acid			
		X	SD	SE	n
18:0	NL	3.5	1.1	0.4	6
	PL	26.5	2.8	1.6	3
18:1	NL	17.0	2.8	1.1	6
	PL	22.0	1.1	0.6	3
18:2	NL	6.1	1.7	0.7	6
	PL	10.0	2.4	1.4	3
E*	NL	0.3	0.3	0.1	6
	PL	nd			3
18:3	NL	nd			6
	PL	ur			3
18:3, 20:0	NL	r			6
	PL	5.4	0.8	0.5	3
20:0	NL	12.0	2.5	1.0	6
	PL	ur			3
>20:0	NL	nd			6
	PL	nd			3

TABLE II --Continued

Fatty Acid	Fraction	Relative percent fatty acid			
		$\bar{X}$	SD	SE	n
Total saturates	NL	ca. 61 to 63			6
	PL	ca. 61 to 65			3
Total unsaturates	NL	ca. 37 to 39			6
	PL	ca. 35 to 39			3

Legend: $\bar{X}$, mean; SD, standard deviation; SE, standard error; n, number of observations; 16:1, etc., 16 carbon acid with one double bond; A, etc., unknown fatty acid appearing at that position in the sequence; nd, not detected or not looked for; ur, not resolved into distinguishable peaks; r, resolved into distinguishable peaks; NL, neutral lipid; PL, phsopholipid.

*These may be the same peaks. They appear at nearly the same position and overlap.

samples of each fraction were analyzed. Those showing obvious oxidation or degradation were discarded.

Some of the fatty acid peaks were not identified due to a lack of standards with matching retention times. These, however, comprised less than five per cent of the total. The nature of peak E was not decided, but hydrogenation of a few samples (Fig. 1 and 2) confirmed the identity of the known peaks and indicated that the unknown peaks A, B, C, and D represent saturated fatty acids. The neutral lipids and phospholipids have about the same total percentage of saturated fatty acids, and this value is about twice that of the unsaturated acids.

The results of differential elution are shown in Figure 3. The lipid was extracted from 148 g of larvae and aliquots taken for weighing. Calculations indicated that there was about 1.95 g of total mixed lipid in the sample. It was transferred to hexane and loaded on the column. The lipid components were then sequentially eluted by the solvents shown. Analysis of the peaks by TLC (Fig. 4) indicates that the column was overloaded, since the first peak is unquestionably phospholipid. The loading capacity of silica gel is 100 mg of total lipid

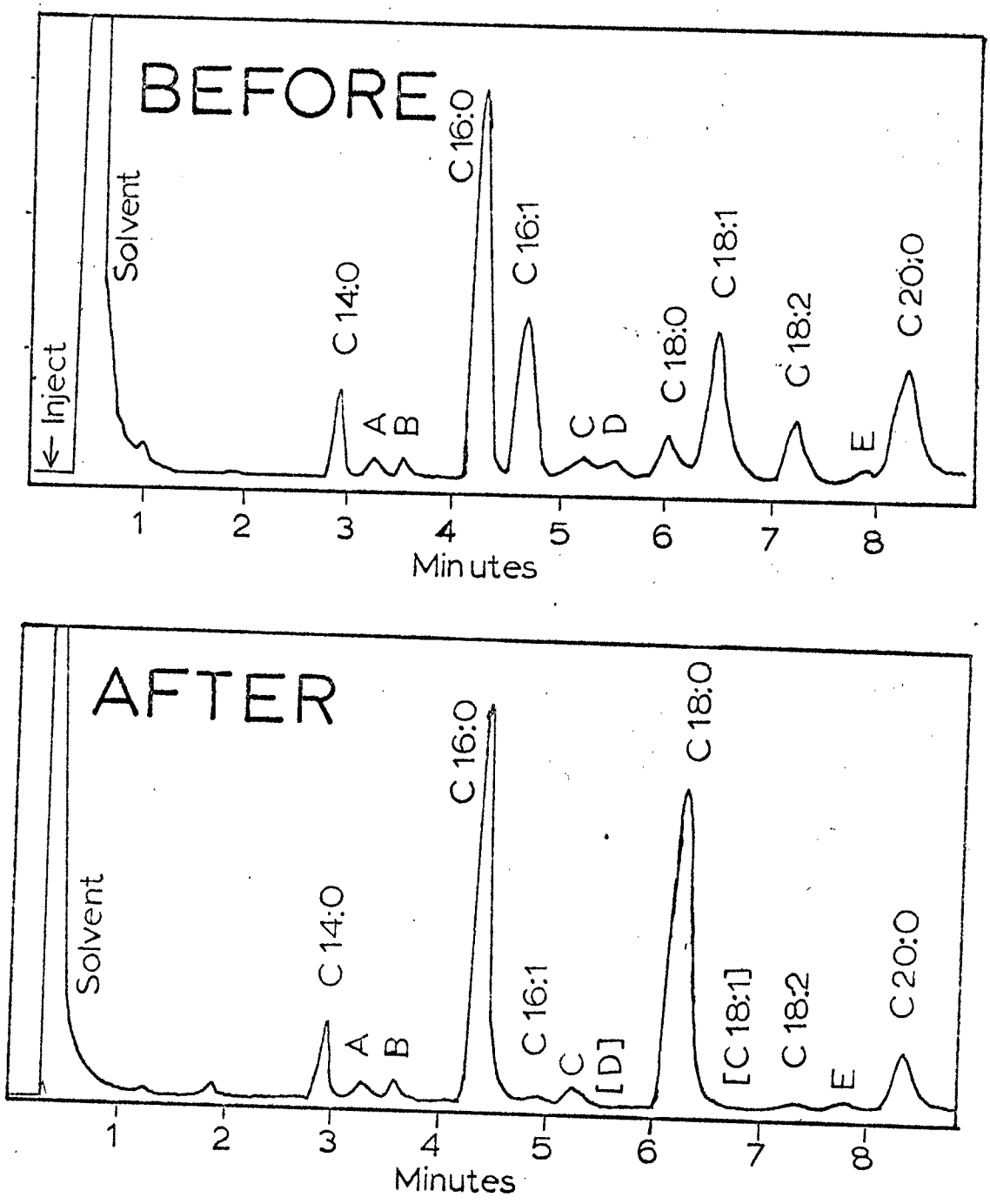

Fig. 1--Gas chromatography of fatty acid methyl esters from saponifiable neutral lipids. Before and after hydrogenation.

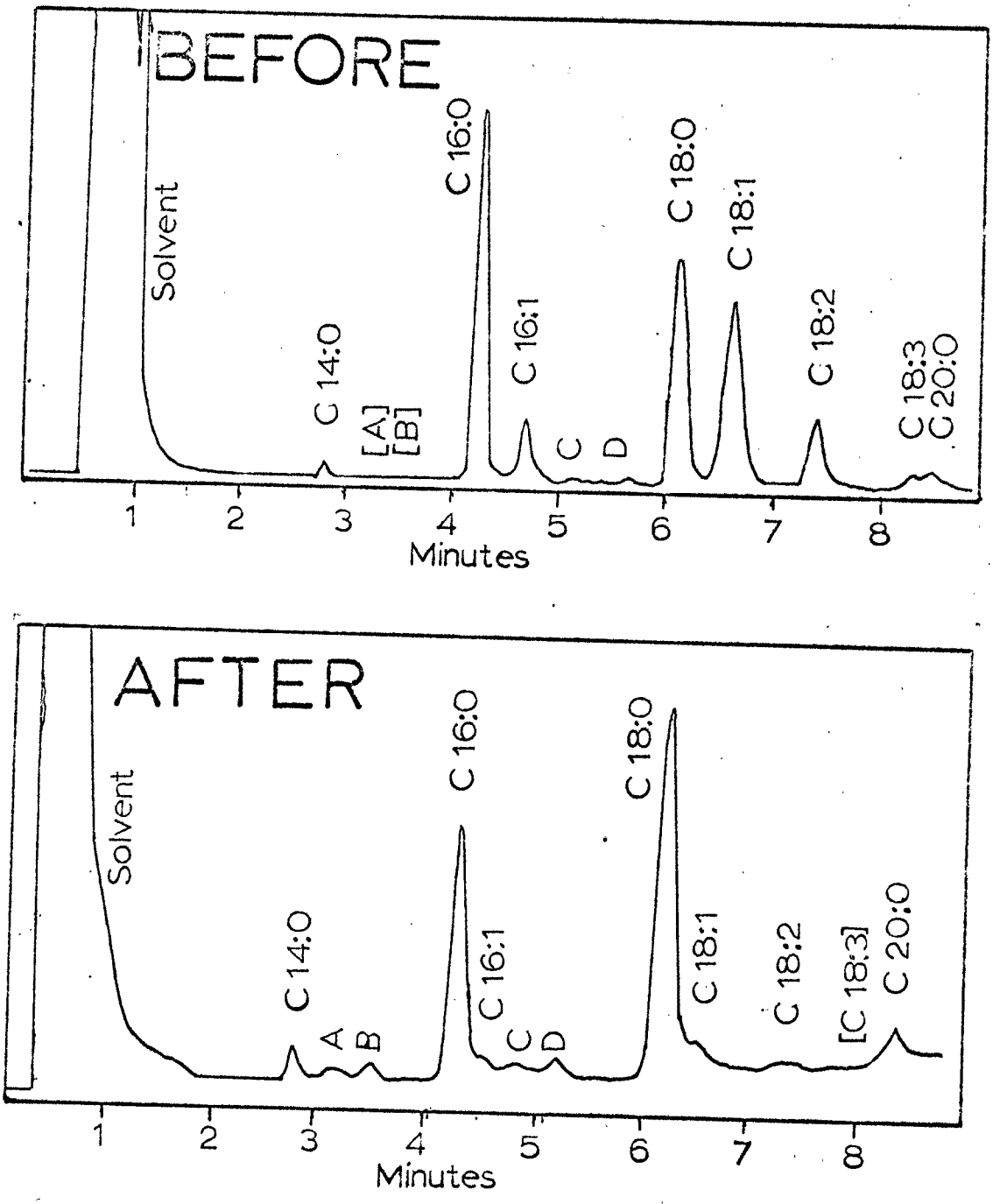

Fig. 2--Gas chromatography of fatty acid methyl esters from saponifiable phospholipids. Before and after hydrogenation.

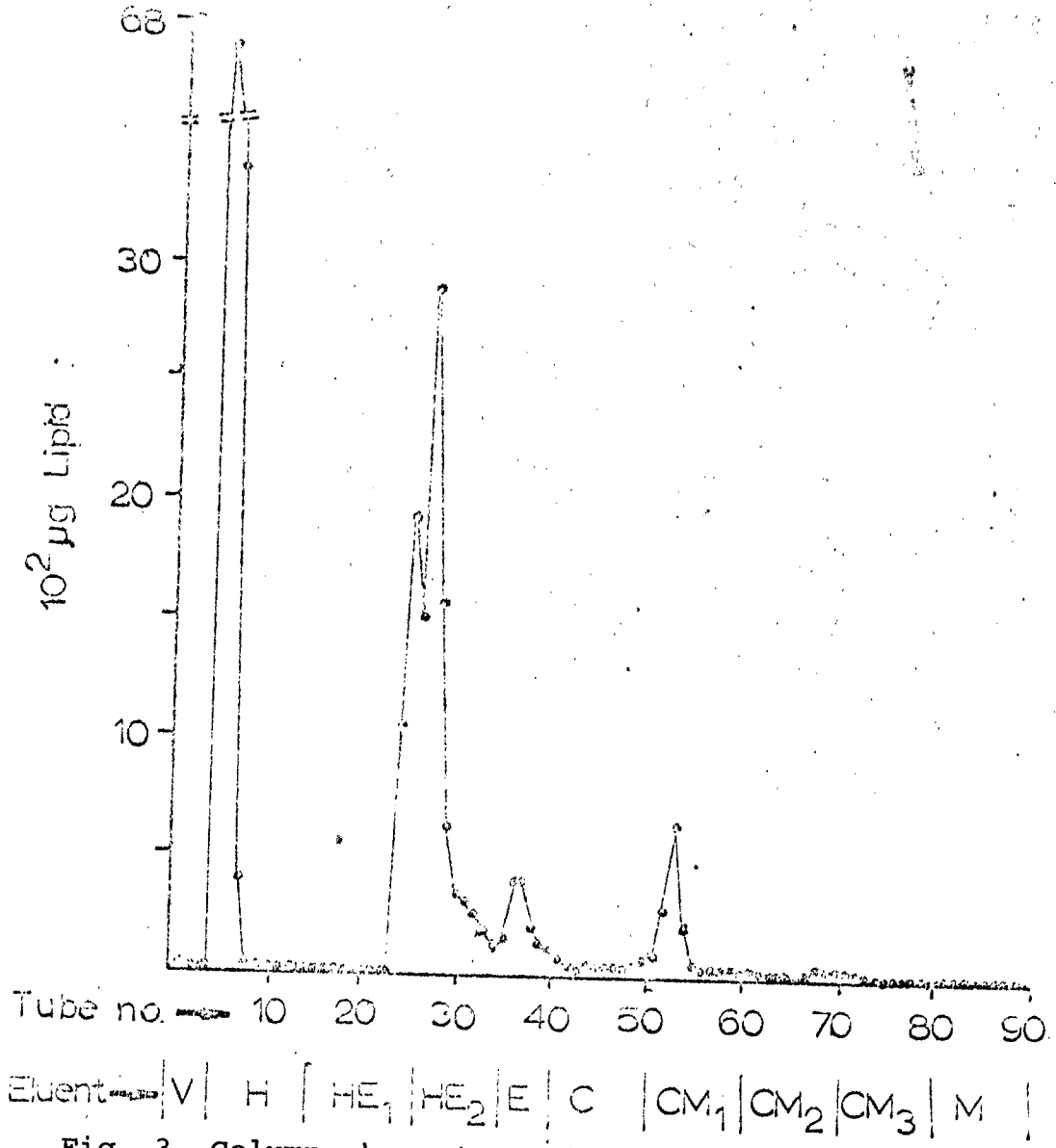

Fig. 3--Column chromatography of 1.95 g total mixed lipid sample on 50 g of silica gel. Legend: V, hexane void volume; H, 100 ml of hexane; HE_1, 100 ml of hexane:ether (9:1); HE_2, 100 ml of hexane:ether (3:1); E, 50 ml of ether; C, 100 ml of chloroform; CM_1, 100 ml of chloroform:methanol (8:2); CM_2, 100 ml of chloroform:methanol (6:4); CM_3, 100 ml of chloroform:methanol (2:8), M, 100 ml of methanol.

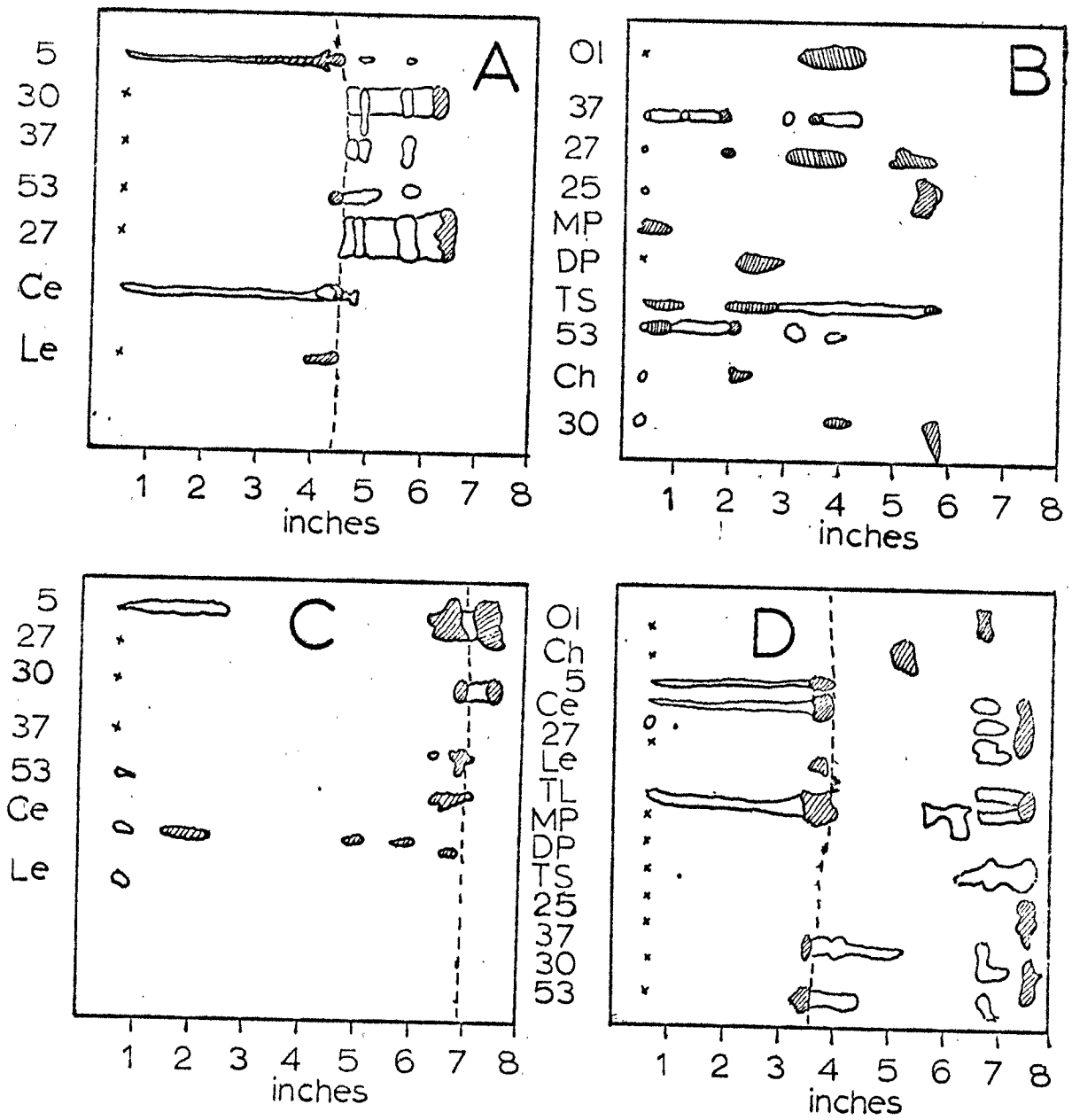

Fig. 4--Thin-layer chromatography of peaks obtained by column chromatography of a total mixed lipid sample on silica gel. Legend: A, development in chloroform:methanol:water (70:30:5) followed by hexane:ether:acetic acid (60:40:1); B, development in hexane:ether:acetic acid (60:40:1); C, development in chloroform:methanol (8:2) followed by hexane:ether:acetic acid (60:40:1); D, development in chloroform:methanol:water (60:30:5) followed by hexane:ether:acetic acid (60:40:1); Ol, oleic acid; Ch, cholesterol; Ce, cephalin; Le, lecithin; TL, total mixed lipid; MP, monopalmitin; DP, dipalmitin; TS, tristearin; hatching, dominant spots; dashed line, solvent front; numbers, peaks.

and 10 mg of phospholipid for each gram of silica gel. This is about half as much phospholipid as calculations indicate was in the applied sample. Except for the anomalous phospholipid peak around tube 5, nothing significant was eluted by the pure hexane. The neutral lipids came off the column between tubes 25 and 45, coincident with the increasing ether gradient. The resolution of the peaks was poor. The results from the TLC determinations imply that peak 25 consists of cholesteryl esters, while peak 27 is a mixture of fatty acids, cholesteryl esters, and possibly aclglycerols and cholesterol. Due to the ambiguity of the spots, cholesterol may have been anywhere between tubes 25 to 40. Peak 37 consists of acylglycerols. Pure chloroform failed to elute anything from the column (probably because all the neutral lipid had been removed by the ether). Application of increasing methanol solutions to the column eluted only a single peak, and that one early during the first wash. Failure to elute more phospholipid peaks with methanol can be ascribed to the fact that most of the phospholipid had failed to stick to the column during loading. Although resolution

on TLC was poor, peak 5 gave a vigorous ninhydrin reaction and can be identified as cephalin, while peak 53 failed to give a reaction and may be lecithin.

CHAPTER IV

DISCUSSION

The value of 2 per cent of wet weight for the total lipid of G. barbipes larvae is lower than that reported for adults of other chironomid species: Chironomus sp., 8.3 per cent of wet weight (by Norman, see Timon-David, 1930); and Tanytarsus lewisi (Freem.), 11.7 per cent of dry weight (calculatable as about 3 per cent of wet weight) (Grindley, 1952). It is even low when compared to larvae of other families of the Diptera for which percentage of wet weight data have been reported (cf. Table 3).

In the Diptera, only E. tenax is close to the per cent of wet weight value reported here for lipid of G. barbipes. Such data are not available for other chironomid larvae, so no comparisons can be made.

Without comparable data for other chironomid larvae, the meaning of such a low total extractable lipid value for the larvae of G. barbipes is difficult to ascertain. To further confuse matters, other work in this laboratory

TABLE III

PERCENT TOTAL LIPID OF DIPTERAN LARVAE

Insect Larvae	$\%\frac{TL}{WWT}$	Reference
Pegomyia ulmaria (Rond.)	5.6	Timon-David, 1930
Blowflies (unspecified)	6.8	Frew, 1929
Phormia terraenovae (R.-D.)	12.2	Cherry, 1959
Phormia regina (Meig.)	7.1	Patton et al., 1941
" "	8.9	Haub and Hitchcock, 1941
Aedes aegypti (Linn.)	3.7	Fast and Brown, 1962
Musca domestica (Linn.)	7.1	Pearincott, 1960
Musca domestica vincina (Macq.)	4.9	Levinson and Silverman, 1954
Gastrophilus intestinalis (DeGreer)	5.0	Timon-David, 1930
Eristalis tenax (Linn.)	1.8	Timon-David, 1930

Legend: TL, total lipid; WWt, wet weight.

indicates that the adults may have about the same percentage of total lipid. Why should this species have such a low lipid content? The higher values that have been reported for adults of other chironomid species are understandable on the basis that the adults do not feed and therefore have to rely on stored lipid for energy. Also, the production of eggs by the females involves a great deal of lipid synthesis. But these factors hold true for G. barbipes also; so no satisfactory explanation is now available for the adults. A tentative explanation can be offered for the larvae: that they have no large lipid reserve simply because they can not utilize one. Carbohydrates can be utilized either aerobically or anaerobically to provide energy, but the utilization of fat requires oxygen. The larvae of G. barbipes live in the sediment at the bottom of sewage lagoons and presumably have very little oxygen available. It would be pointless for an organism to build up a supply of lipid if it could not be used. Against this explanation is the fact that larvae have 2.6 times more neutral lipid than phospholipid. Most storage lipid is usually in the form of triglycerides, while the phospholipids contribute heavily to structural

lipid. Therefore, the explanation offered here leads one to expect a much lower neutral lipid/phospholipid ratio than the 5:2 found here.

The values of about 72 per cent and 28 per cent of the total lipid for neutral lipid and phospholipid, respectively, fall well into the range of values reported for other dipterans (cf. Table 4). To determine whether the neutral lipid/phospholipid ratio is affected by aerobic versus anaerobic conditions, it will be necessary to raise the larvae under both conditions. This will have to be done in the laboratory where such conditions can be explicitly defined and controlled.

Except for the 2.83 per cent of total reported by Albrecht (1961) for adults of Calliphora erythrocephala, the percentages of palmitoleic acid found in the larvae of G. barbipes are below those reported for similar fractions in other Diptera (cf. Table 5). Although the value of 13.6 per cent of total for palmitoleic acid in G. barbipes larval neutral lipid is lower than in most of the other Diptera, it is still higher than the one to ten per cent of total found in other orders (cf. Fast, 1964, 1966). Except for the palmitoleic acid values and phospholipid

TABLE IV

DIPTERAN NEUTRAL AND PHOSPHOLIPID PERCENTAGES

Insect	Stage	Lipid type	% reported	Reference	Calculated % total
Calliphora erythrocephala	A	PL	9.5 T	Albrecht, 1961	
Phormia regina	A	PL	22.6 D	Bieber et al., 1961	ca. 80
Musca domestica	A	PL	1.71W	Pearincott, 1960	ca. 81
Drosophilia melanogaster	A	PL	39.5 T	Wren and Mitchell, 1959	
Aedes aegypti	L	PL	16 T	Fast and Brown, 1962	
Musca domestica	L	PL	1.33W	Pearincott, 1960	ca. 20
Phormia regina	L	PL	16.8 D	Bieber et al., 1961	ca. 60
Musca domestica	P	PL	0.45W	Pearincott, 1960	ca. 7

Legend: A, adult; L, larva; P, pupa; PL, phospholipid; T, total lipid; D, dry weight; W, wet weight.

TABLE V

PERCENT PALMITOLEIC ACID IN DIPTERA

Insect	Stage	Lipid type	% C 16:1 / Total	Reference
Aedes aegypti (Linn.)	L	Ceph	36.4	Fast and Brown, 1962
" "	L	NL	32.7	" " "
" "	P	TL	19	Barlow, 1964
Aedes sollicitans (Linn.)	A	TL	34	Van Handel and Lum, 1961
Anopheles freeborni Aitken	L	TL	20-30	Hayashiya and Harwood, 1968
" "	10d,F	TL	35-50	" " "
Culex pipiens pipiens (Linn.)	A,F	TL	34.4-50.2	Buffington and Zar, 1968
Chironomus sp.	A	NL	21.2	Fast, 1966
" "	A	Ceph	13.5	" "
" "	A	Resid	19.3	" "
Musca domestica Linn.	P	TL	22	Barlow, 1964
Hylema brassicae (Bouche)	P	TL	62	" "

Legend: L, larva; P, pupa; A, adult; d, days; F, female; TL, total extracted lipid; NL, neutral lipid fraction; Ceph, cephalin fraction; Resid, residue fraction.

stearic acid value, the percentages found here for the fatty acids of G. barbipes correspond well to those reported for the Culicidae (Barlow, 1964; Buffington and Zar, 1968; Fast and Brown, 1962; Hayashiya and Harwood, 1968; Van Handel and Lum, 1961. See also Fast, 1964). The pattern of fatty acid composition in the two fractions from G. barbipes is similar to data reported by Fast (1966) for fatty acids from Chironomus sp. (cf. Table 6). The major differences occur in the ratios of C 14:0, C 16:1, and C 18:3 plus C 20:0, and in the detection of C 14:1, C 16:2, and C 17:0. Fast also reported a measurable amount of acids less than 14-carbons and greater than 20-carbons in length, but this is not a discrepancy. With G. barbipes fractions, chains shorter than 10-carbon came off the column at the same time as the solvent and could not be detected. Those longer than 20-carbons failed to come off the column during the time alloted but could have been present.

The difference in the fatty acid compositions of phospholipid and neutral lipid is puzzling. It implies either a specific recognition of the different phosphatidic acids and diacylglycerols by the lipid phosphatase system,

TABLE VI

FATTY ACIDS OF ADULT CHIRONOMUS SP. (FAST, 1966)

Fatty Acid	Relative Percentage		
	Neutral lipid fraction	EPG fraction (cephalin)	Residue fraction
14		3.4	2.9
14:0	2.2	2.7	2.9
14:1		1.2	
16:0	26.6	20.2	15.2
16:1	21.2	13.5	19.3
16:2		1.7	3.0
17:0		4.8	
18:0	3.4	17.2	6.8
18:1	30.2	27.6	23.9
18:2	9.3	7.0	12.7
18:3, 20:0	3.1	3.0	6.5
20	1.8		4.2

or a difference in the synthetic mechanisms used to form the various lipid classes. Mechanisms operative after the phosphatidic acid intermediate might include transacylation. Variation in fatty acid composition prior to the phosphatase step could be brought about by differences in the biosynthetic de novo and elongation schemes or by differences in the utilization of their products. Differences between the classes of lipid could also be caused by selective utilization of ingested fatty acyl groups, or by formation of triacylglycerols directly from monoacylglycerols. The alternative to an employment of one or more of these schemes as an explanation for different fatty acid composition of neutral lipids and phospholipids is to postulate a hitherto unknown method of producing triacylglycerol and phosphoglyceride.

This variation in the fatty acid composition of lipids is not a new phenomenon. There is ample documentation of selective esterification of fatty acids in both the neutral lipids and phospholipids of mammals (e.g. Karmen, Goodman, and Whyte, 1963; and Whyte, Goodman, and Karmen, 1963) and plants (Kates, 1970). Karmen and his colleagues found that there was a high degree of positional specificity

in the lecithins but that the positional distribution tended to be random in triglycerides. The presence of a pathway for making triacylglycerol directly from monoacylglycerol has been confirmed for the intestinal mucosa of mammals (Clark and Hubscher, 1961), but most organisms seem to depend on the classical scheme of Kennedy (1961, 1963) where phosphatidic acid is the major intermediate in the formation of triacylglycerols and phospholipids. According to this scheme, differences in utilization or biosynthesis of fatty acyl groups would have little effect unless one also postulates a compartmentation of the sites for production of triacylglycerol and phosphoglyceride. There is currently no valid evidence for this, although the mitochondria and cell membranes tend to have larger concentrations of phospholipid than the rest of the cell. The questions of biosynthetic differences and compartmentation will have to be settled by isotopic tracer studies and centrifugal fractionation of cell components. The question of whether there is a difference in the utilization of ingested fatty acids can easily be answered by an analysis of the substances upon which the larvae feed.

The discrepancy between fatty acid composition of the neutral lipid and that of the phospholipid from G. barbipes can not be ascribed to oxidative degradation. That all the samples of one sample were oxidized and the effects therefore not noticeable, is not borne out by the data. It is implausible that oxidation of C 16:1 would occur without corresponding oxidation of C 18:2 and C 18:1. Except for palmitoleic acid content of the phospholipid, the pattern of fatty acids for G. barbipes closely resembles that for Chironomus sp. (Table 6). If oxidation had occurred, it is unlikely that there would be such a similarity; nor would there be such similarities between so many of the individual fatty acids in the lipids of G. barbipes itself.

The percentages of total saturated fatty acids for these larvae are considerably above most of those calculated from literature for other Diptera (Table 7). This high proportion of saturated fatty acids in G. barbipes larvae requires explanation. In most animals, increased saturation of the fatty acids corresponds to a prolonged exposure to elevated temperatures, and decreased saturation to a prolonged exposure to lowered temperatures. Since these

TABLE VII

PERCENTAGE OF TOTAL SATURATED FATTY ACIDS IN DIPTERA

Insect	Stage	Lipid type	%Satur* Total	Reference
Aedes aegypti	L	Ceph	28	Fast and Brown, 1962
" "	L	NL	52	" " "
" "	P	TL	29	Barlow, 1964
Aedes sollicitans	A	TL	42	Van Handel and Lum, 1961
Anopheles freeborni	A	TL	25-40	Hayashiya and Harwood, 1968
Culex pipiens pipiens	A	TL	30-50	Buffington and Zar, 1968
Musca domestica	P	TL	34	Barlow, 1964
Agria affinis (Fall.)	P	TL	25	" "
Drosophilia melanogaster (Meig.)	P	TL	43	" "
Calliphora erythrocephala (R.-D.)	A	TL	86	Albrecht, 1961
Chironomus sp.	A	Ceph	45-48	Fast, 1966
"	A	NL	32-35	" "

Legend: L, larva; P, pupa; A, adult; Ceph, cephalin; NL, neutral lipid; TL, total extracted lipid; Satur, saturated fatty acids.

*Approximate value calculated on basis of literature.

larvae were collected primarily during the colder months of the year and were active at temperatures down to 10°C, one would expect a low percentage of saturation. An explanation for this high percentage of saturation might be the supposedly anaerobic environment in which the larvae live. The dehydrogenation of fatty acyl groups by the mitochondrial fatty acid oxidase system generates reduced FAD and reduced NAD. Before these can be reoxidized by the cytochrome system, molecular oxygen must be available. If they are not reoxidized, the available FAD and NAD are quickly used up and no further desaturation can occur. If such is the case, then the adults should have a lower percentage of saturated acids since they live under aerobic conditions. The adults have such a short life span that temperature should not influence their lipids. However, seasonal studies should be made to determine whether there are changes in the larvae. In this study, the larvae were not analyzed according to age. Since the lipid content and composition have been shown to vary with age in other insects, this may apply here also.

Fast (1966) suggested that the high palmitoleic acid content of Diptera is due to a large percentage of ethanolamine

phosphoglyceride in the phospholipid. He demonstrated that when ethanolamine phosphoglyceride was not the major phosphatide, only low levels of palmitoleic acid occurred in the neutral lipids. However, his data show that this was true for all the fractions, not just the neutral lipids. Examination of his data show that when ethanolamine phosphoglyceride was the major phosphatide, the neutral lipids did have high levels of palmitoleic acid. This did not always hold true for the other fractions. This tends to agree with the data for G. barbipes. From the differential elution data (Fig. 3) it appears that ethanolamine phosphoglyceride (cephalin) is the major phosphatide in G. barbipes. Coincident with this there is a high level of palmitoleic acid in the neutral lipid but an exceedingly low level in the phospholipids. The meaning of this relationship is not clear.

Some of the mystery surrounding the fatty acids might be cleared up if a correlation could be established between fatty acid composition, lipid type, and physiological function. This necessitates a good separation of the lipid components so that they can be analyzed individually. In this experiment the sample size was just too large for

the quantity of silica gel and the volumes of eluting fluid used. A large sample size was used because the only means for locating peaks was by weighing portions. Column chromatography was used to fractionate the total mixed lipid sample because exclusive use of TLC had failed to give satisfactory results. The data indicate that a satisfactory resolution of lipid components can be achieved by using more silica gel and larger volumes of eluting fluid, or by application of smaller samples. Using the present results as a guide, one should be able to fairly accurately determine the minimum amount of total mixed lipid that will give detectable peaks for individual components.

CHAPTER V

SUMMARY

This study was undertaken to determine the characteristic lipid content and composition of the chironomid larva Glyptotendipes barbipes (Staeger). The results from column chromatography, thin layer chromatography, and gas chromatography indicate that this larva is unique in its lipid properties. These can be summarized as follows:

(1) The total extractable lipid is two per cent of the wet weight, less than reported for any other dipteran.

(2) The lipid recovered from column chromatography on silica gel consisted of about 72 per cent neutral lipid and 28 per cent phospholipid.

(3) The fatty acid composition of the neutral lipids differed from that of the phospholipids. The neutral lipids had 6.9 times more myristic acid, 3.8 times more palmitoleic acid, and 85 per cent less stearic acid.

(4) The total percentage of saturated fatty acids versus unsaturated fatty acids was about the same for

neutral lipids and phospholipids, with about 61 to 65 per cent saturation.

(5) Cephalin appeared to be a major component of the phospholipid fraction.

Several postulates, among them the effect of an anaerobic environment, are presented as explanation of these facts.

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