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Extraction Methods and an Investigation of Drosophila Lipids*

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Very many techniques have been described in the literature for extraction of lipids from tissues. A good technique should minimize (ideally, preclude) formation of new covalent bonds and breaking of covalent bonds in the native lipid molecules. Covalent bonds may be formed by autoxidation, which in turn leads to molecular rearrangement, polymerization, and telomerization. Other addition reactions, condensations, and substitutions may occur if heating is used. Lipid molecules may be cleaved by radical processes after primary autoxidation, by hydrolysis, and by enzymic action (autolysis). Dawson (1) has discussed how rapidly autolysis can occur in animal tissues, and Kates (2) has elegantly demonstrated how autolysis of plant phospholipids can be actually stimulated by an extracting solvent.

Theoretically, autolysis can be prevented in four different ways. (*a*) Enzymes can be denatured: alcoholic solvents may or may not (3, 4) do this; acid and heat treatments may themselves injure the lipids. (*b*) Specific inhibitors of the lipolytic enzymes could be used, but this is not feasible until more is known about these enzymes. (*c*) Extraction can be performed at low temperature: this is useful only if conditions permit complete removal of lipids from tissue (5). (*d*) Tissues can be thoroughly dried before extraction. In experimental application all four ways may be imperfect. Pulverization, necessary to permit complete extraction of lipids from most tissues, may be performed at low temperature (5–7) or in presence of alcoholic solvents, but it is usually very difficult to insure that autolysis does not occur *in any part* of the tissue.

In earlier work (8) we extracted lipids from dried, macerated *Drosophila melanogaster* with ether, but later, working with larger quantities of undried flies, we found that most of the phospholipids were autolyzed. Kates' studies (2) led him to suggest *n*-propanol or isopropanol for lipid extraction (isopropanol was his later choice (6, 7)). Attempting to meet the requirements discussed above, we developed a new and relatively simple method of extraction employing *n*-propanol (9), or chloroform-methanol (2:1). The latter proved to be a more useful solvent. The method will be described in detail below, with results of an examination of *Drosophila* lipids.

EXPERIMENTAL

Solvents—Acetone, *n*-butanol, chloroform (containing 0.75% ethanol as preservative), dichloromethane, diethyl ether, and *n*-propanol were of reagent grade, and isooctane of spectrophotometric grade (Merck). 2,2-Dimethoxypropane, provided by the Dow Chemical Company through the courtesy of Dr. G. L. Ellman, was redistilled before use. Its infrared spectrum indicated the presence of methanol and 2-methoxypropene as (unobjectionable) contaminants.

General Methods—Evaporations were carried out at <55° in a rotary ("flash") evaporator, or, in the case of eluted fractions, in a vacuum oven.

Silicic acid (Mallinckrodt, analytical reagent, 100 mesh) was washed twice with methanol, dried at 110–120° in a vacuum oven (15 hours), and stored in a stoppered bottle in a desiccator.

Paper chromatography was carried out on Whatman No. 1 paper.

Ultraviolet spectra were determined with a Cary model 11MS recording spectrophotometer.

Flies—Drosophila melanogaster were cultured at 25° under standard conditions, collected in a bottle about 10 days after eclosion, and killed by storing the bottle in Dry-Ice.[1]

"Fly Powder"—A stainless steel homogenizer (Servall omnimixer) was arranged with the chamber (1 pint) suspended in a bath of 2-methoxyethanol-Dry-Ice. Dry-Ice (about 50 g) was then powdered in it. On removal from storage the flies (about 50 g) were handled very rapidly, as follows: poured on to a sheet of paper, they were separated from any lumps of nutrient accidentally collected, weighed, and transferred to the homogenizer. The homogenizer was then operated intermittently for periods of about 30 seconds, extra portions of Dry-Ice being added to maintain the quantity in the chamber, until a uniform, fine powder was obtained (5 to 10 minutes).[2] Before opening, the chamber was always knocked sharply to dislodge powder from the upper walls.

Extraction—A large, sintered glass funnel (diameter 10 to 15 cm, coarse grain), resting loosely in the neck of a conical flask (2-liter), was kept in a −20° refrigerator with a supply of chloroform-methanol (2:1, volume for volume). The fly powder and solvent (100 ml) were mixed on the funnel. A second equal portion of solvent was added when the first had filtered, and so on, until eight portions had filtered. The extract was washed by the Folch method (10), as follows. It was shaken with 0.73% sodium chloride (150 ml), centrifuged (1000 × *g*) at 5° for 10 minutes, and the upper phase removed. The lower phase was rewashed similarly with "pure solvents upper phase" containing 0.29% NaCl, evaporated, and the residue extracted with ligroin (purified, b.p., <60°). The resulting solution was filtered and evaporated, and the ligroin-soluble residue weighed before chromatography on silicic acid.

Soxhlet Extraction—Fly powder residues, after propanol or

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[1] We are grateful to Mrs. T. Sueoka and Dr. E. Glassman, who provided the flies.

[2] Eyes were observed, still whole, in this powder.

chloroform-methanol extraction, were extracted overnight with chloroform in a Soxhlet apparatus, and the malodorous, ligroin-soluble gum so obtained was weighed. About 3% of it could be eluted from silicic acid by 5% methanol in chloroform.

Silicic Acid Chromatography—Silicic acid (50 g), in a slurry with chloroform, was poured into a column (diameter 2.8 cm) with a constriction at its lower end plugged by a minute piece of nonabsorbent cotton. It was then washed with chloroform overnight.[3] Up to 2.5 g of whole *Drosophila* lipid (yellow oil) were then run on in a small volume of chloroform. Development was continued with chloroform, then with increasing percentages (volume for volume) of methanol in chloroform. Meanwhile, the eluate was collected in 10 ml fractions by means of a siphon-delivery fraction collector (Autonomos). In some experiments the rate of flow through the column dwindled after about 200 fractions had been collected, and could not be permanently speeded by the application of air pressure; however, cautious stirring of the top 0.3 cm of adsorbent successfully restored flow. The total period of elution was 150 to 200 hours.

Within 14 hours of delivery each fraction was weighed. Within 24 hours of evaporation, fractions were redissolved in warm chloroform (if insoluble, in methanol), and combined in groups corresponding to sections of the elution curve. Infrared spectra were then determined on these pooled materials which, within a further 48 hours, were used for paper chromatography and hydrolysis (8).

Recovery from columns was usually about 100%. Since the weight of lipid recovered was the sum of 500 to 1000 separate weighings, since late fractions contained traces of silica, and since some nonlipid contaminants (when present) remained adsorbed, recovery could not be defined accurately.

Infrared Spectra—Erley (11) has recently recommended 2,2-dimethoxypropane, with or without an added trace of acid, for drying of samples before infrared analysis. A few drops of 2,2-dimethoxypropane were added to a solution of lipid in chloroform or methanol, and the mixture was evaporated in a rotary evaporator at 50–55° for 15 minutes. No change in spectrum was observed when samples were retreated in this way, with or without exposure to HCl vapor. The efficacy of the treatment has not been evaluated further.

For the spectra, lipids were prepared as films on NaCl plates or as 1 to 5% solutions in chloroform or dichloromethane in NaCl cells (0.10 cm). A Perkin-Elmer model 137 Infracord spectrophotometer (NaCl optics) was used, calibrated to $\pm 0.02\mu$ (± 5 cm^{-1} at 1550 cm^{-1}) with polystyrene, according to manufacturer's instructions.

Spectral data are recorded with conventional notations (12).

Paper Chromatography of Lipids—Development with *n*-propanol-0.25 N aqueous NH_4OH-acetone (4:1:1, volume for volume) distributes most lipids in elongated zones at high R_F and nonlipids at low R_F (8). Tests applied to chromatograms were as follows. Ultraviolet absorption and fluorescence; ninhydrin; phosphorus; Rhodamine 6G (13); 2,4-dinitrophenylhydrazine (13); aniline phthalate (for free sugar) (14); Liebermann-Burchard (15); Dragendorff (16).

Smith's chlorination method for —CONH-compounds (17) was simplified as follows. Chromatograms were dipped (5 minutes) in 15% aqueous NaOCl, washed (10 minutes) in cold, running tapwater, then the color was developed with 0.05 N aqueous KI-saturated *o*-tolidine in 2% aqueous acetic acid (1:1, volume for volume).

Ninhydrin-positive, Water-soluble Hydrolysis Products—Chromatography with the "I-O" systems of Hardy *et al.* (19) permitted reliable but not rigorous identification of amino acids. Leucine was not distinguished from isoleucine and norleucine, or valine from norvaline. Ethanolamine hydrochloride gave with ninhydrin a diffuse, pale brown zone (R_F, 0.59 in I, 0.58 in O). Most (water-soluble) hydrolysates gave unidentified zones which fluoresced in ultraviolet light. The presence of glutamic acid and arginine in lipids of peaks D and J (Fig. 3) respectively was confirmed by addition of authentic samples to the hydrolysates before paper chromatography.

Detection of Choline—Phosphomolybdic acid (20) and Dragendorff (16) reagents revealed choline chloride (R_F, 0.21 in I, 0.47 in E) after "I-E" chromatography of water-soluble hydrolysates (19).

Detection of Glycerol—A small lipid sample was hydrolyzed with 0.2 ml of 6 N hydrochloric acid. The hydrolysate was poured on to a column (3 × 0.5 cm) of Amberlite IR-4B (OH^-, 200 to 400 mesh), prepared in a medicine dropper plugged with glass wool. The column was washed with 0.2 ml water (eluate rejected), then with a further 0.5 ml. (Stirring with a minute glass rod liberated any bubbles of gas clogging the column.) The 0.5 ml eluate was partially evaporated, chromatographed in *n*-butanol-acetic acid-water (5:1:2), and glycerol (R_F, 0.49) was revealed by silver nitrate spray (21).

Detection of Inositol—The Scherer test was carried out by a modification of Feigl's method (22).

Detection of Sphingosine—Two paper chromatograms (ascending development, 1.5 to 2.5 hours) were run in 2-pentanone-acetic acid (30:2, volume for volume) on each ligroin-soluble hydrolysate. After drying at room temperature (1 hour), one was sprayed with ninhydrin and heated at 110° (5 minutes); the other was sprayed with Clark and Lubs bromothymol blue solution, suspended (5 minutes) in a tank over concentrated NH_4OH, then removed and observed for persistent blue zones. These tests together are useful for detection of sphingosine-like bases. As standard a sample of sphingosine sulfate[4] was used, applied to chromatograms on top of spots of $BaCl_2$.

Recognition of Cerebroside—No sphingosine-like base was detected directly in any fly lipid hydrolysate obtained by the usual method. Peak J lipid (Fig. 3), suspected on infrared evidence of containing cerebroside (but which gave no aniline phthalate reaction on paper, even in presence of excess of H_3PO_4 (23)) was hydrolyzed with 3 N aqueous HCl at 100° (2 hours). This is supposed (24) to liberate quantitatively the hexose of cerebrosides, but much browning occurred, presumably due to simultaneous liberation of amino acids. The hydrolysate was shaken with ligroin (b.p., 65–70°), and a sphingosine-like base was readily detected in the ligroin layer. The aqueous layer was evaporated over KOH under reduced pressure (2 days), and paper chromatography of the residue indicated the presence of glucose or galac-

[3] During the washings, a pale brown "veil" slowly receded down the adsorbent, leaving it uniformly transparent and colorless. If the veil was not removed a spurious peak appeared after the cholesterol peak in the elution curve. The chloroform washings (1.6 liters) from 150 g of silicic acid yielded on evaporation 4.8 mg of a viscous, colorless oil, the infrared spectrum of which indicated long-chain aliphatic material with some carbonyl absorption (1725 cm^{-1}).

[4] Kindly provided by Professor H. E. Carter.

tose. Subjected to paper electrophoresis (8 hours) in 2% aqueous borax (25) the residue gave a zone separable from D-galactose and inseparable from D-glucose.

Characterization of Hydrocarbon and Wax Ester—Extracts obtained by propanol extraction at room temperature deposited colorless crystals if stored at −20°. In one experiment these were filtered off (330 mg from 70 g of flies). They proved to be inhomogeneous, and attempted recrystallization failed. Chromatography on silicic acid afforded three main fractions, each giving a negative Liebermann-Burchard test.

(*a*) Colorless material (15 mg), fairly crystalline, very low level of ultraviolet absorption, glycerol not liberated by hydrolysis. Its infrared spectrum showed four prominent bands, at 2930 and 2850 cm^{-1} (s.; C—H stretching), at 1465 cm^{-1} (m.; CH_2 deformation), and at 1370 cm^{-1} (mw.; C—CH_3 deformation), identifying the material as a saturated hydrocarbon (14).

(*b*) Colorless crystals (235 mg), eluted by 5% ether in hexane, apparent $\lambda_{max}^{isooctane}$ 208 mμ, glycerol not liberated by hydrolysis. Its infrared spectrum was simpler than those of triglycerides, strongest bands being at 2935 and 2855 cm^{-1} (C—H stretching), at 1745 cm^{-1} (C=O stretching) and at 1160 cm^{-1} (C—O stretching). It was concluded to be a wax ester.

(*c*) A yellowish oil (60 mg), eluted by ether, yielded glycerol on hydrolysis, and appeared to be impure triglyceride.

Silicic Acid Chromatography of Nonlipids—A mixture containing 1 mg of each of a number of nonlipids was dry-packed on a column of silicic acid (5 g; diameter 1.3 cm), which was then developed in the same way as for lipids. Compounds first appeared in the eluates as follows.

Chloroform (50 ml), 5% methanol (25 ml): none.

Methanol (90 ml), 15%: 2-aminooctanoic acid, 2-pyrrolidone-5-carboxylic acid.

Methanol (90 ml), 15%: cystine, glucose, leucine, proline.

Methanol (190 ml), 25%: phenylalanine, valine.

Methanol (50 ml), 35%: none (except tailing leucine, phenylalanine, proline, valine).

Methanol (40 ml), 50%: alanine, γ-aminobutyric acid, threonine, tyrosine.

Methanol (45 ml): β-alanine, arginine (applied as hydrochloride), aspartic acid, cysteic acid (hydrochloride), glutamic acid, glycine, serine.

Water (25%) in methanol (40 ml): histidine (hydrochloride hydrate), lysine (hydrochloride).

Water (40 ml): cysteine (hydrochloride).

RESULTS AND DISCUSSION

Extraction Method

A batch of flies was halved after powdering with Dry-Ice. One-half was extracted with ether and the other with propanol, in both extractions the temperature being maintained, as far as possible, at 0°. In Fig. 1, elution curves of the lipids obtained showed that the ether-extracted lipid was largely eluted from silicic acid by chloroform alone, whereas the propanol-extracted lipid contained a high proportion of phospholipid which had to be eluted by chloroform-methanol. This proportion of phospholipid was fairly reproducible in other experiments (see below). Thus autolysis during extraction was dramatically revealed by the increase in chloroform-eluted lipid (as free fatty acids, and so forth) at the expense of phospholipid. When dead flies were left

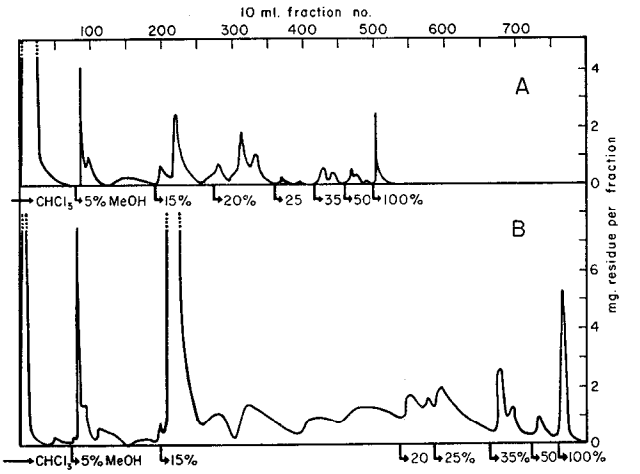

FIG. 1. Silicic acid chromatography of ligroin-soluble lipid obtained by extraction of 45 g of *Drosophila* with (*A*), ether, and (*B*), *n*-propanol. Lipid (2.13 g) was applied to column *A*: 1.90 g of this were eluted by chloroform alone, and 0.14 g by methanolic solvents. Corresponding figures for column *B* were 2.36, 1.36, and 0.99 g. Marks on the abscissae show when changed solvents were introduced.

at room temperature for several days before extraction, autolysis similarly altered the elution curve.

Powdering of the flies with Dry-Ice is an unobjectionable method of fragmentation, and can be done efficiently and easily. It was desirable to extract from the powder as much as possible of the total ligroin-soluble lipid[5] at low temperature. Repeated washing with propanol at −20° yielded no more than 80%, but subsequent washing at room temperature raised this figure to over 99% and completed a useful extraction. Chromatographic results suggest that in this procedure different lipids tend to be extracted sequentially.

A model experiment demonstrated that the elution behavior of a variety of nonlipids parallels that of phospholipids. During silicic acid chromatography of propanol-extracted fly lipids (and blood lipids (26)) free amino acids and sugars appeared predictably in specific eluates. For our study of chemically bound amino acids this was an unsatisfactory finding, only avoidable by removal of nonlipids before chromatography. From published results the Folch washing procedure (10, 24, 27) seemed most attractive for this purpose, the more so if chloroform-methanol (2:1, volume for volume) could replace propanol as the extracting solvent. Unlike propanol, it did not completely dehydrate fly powder at −20° (it did so at room temperature) but it did extract over 99% of the lipid without warming to room temperature (Fig. 2). By subjecting one-half of an extract thus obtained four times to the simple washing procedure (10), and the other half four times to the procedure with sodium chloride, we extended the findings of Folch *et al.* to *Drosophila* lipids. In either procedure the third and fourth washings were devoid of ninhydrin-positive nonlipids. Ligroin-soluble lipid recovered from

[5] We arbitrarily chose to study only lipid which, after extraction and before chromatography, was soluble in purified ligroin (b.p., <60°). Such material may contain small quantities of nonlipids (free amino acids, sugars, salts, and so forth) and larger quantities of lipids which are insoluble in ligroin when purified. "Total lipid" means the total extractable by neutral solvents, and represents the sum of lipid extracted by the chosen method plus that subsequently obtained by Soxhlet extraction.

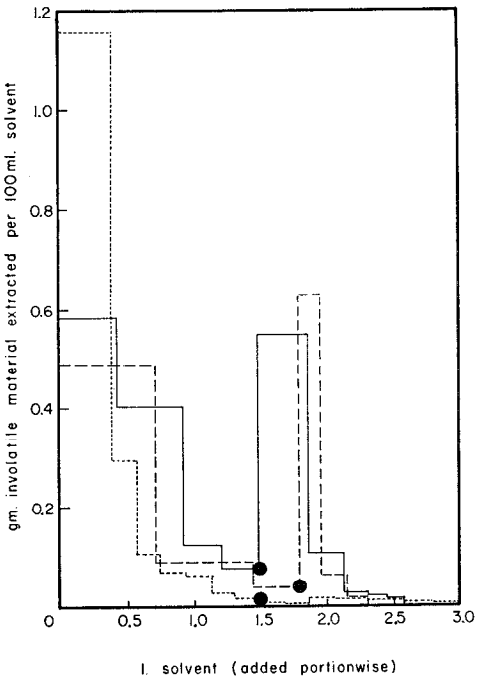

FIG. 2. Course of extraction from fly powder. Figures were calculated for 100-g flies, different batches of which were used in each experiment. ——, *n*-propanol, total involatile residue; – – – –, *n*-propanol, ligroin-soluble portions of involatile residues; ------, chloroform-methanol (2:1, volume for volume), total involatile residue (material extracted at room temperature was mainly insoluble in ligroin). The beginning of extraction at room temperature is marked with a dot.

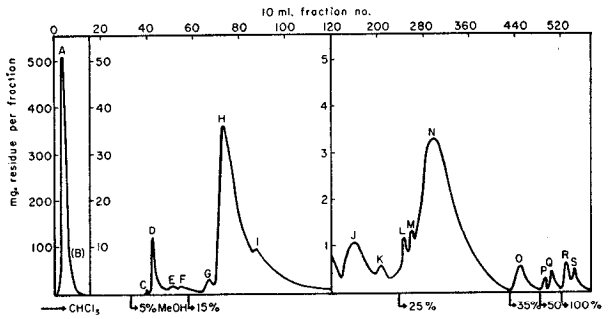

FIG. 3. Silicic acid chromatography of *Drosophila* lipids, extracted by chloroform-methanol and washed. Marks on the abcissa show when changed solvents were introduced. In this particular experiment (*cf.* Fig. 2, succeeding paper) peak B was not resolved from peak A. 40.5-g flies yielded 2.20 g of ligroin-soluble lipid, and the weights of lipid eluted by chloroform and by 5, 15, 25, and 35 to 100% methanol in chloroform were respectively 1.344, 0.044, 0.534, 0.274, and 0.027 g. Total phospholipid thus weighed 0.879 g, and total lipid recovered after chromatography 2.223 g.

the four washings (upper phases) amounted to 2% of the total lipid in the simple procedure, and only 0.2% in the procedure with sodium chloride.

In the method adopted *Drosophila* were powdered with Dry-Ice, and the powder was washed eight times with chloroform-methanol at −20°. The lipid extract obtained was subjected to two Folch washings with brine, evaporated, and the ligroin-soluble residue was chromatographed on silicic acid. This method is reproducible, and in principle is recommended for most chemical investigations of lipids. Biological materials other than *Drosophila* require slightly different handling. For example, blood may be mixed directly with the solvent at −78° (26). The method appears to have advantages over others currently proposed (*e.g.* (5, 28)).

TABLE I

Distribution of lipid components among fractions eluted from silicic acid

Tests are recorded as positive by fraction letter and weakly positive by (w).

Component	Peaks (Fig. 3)
Cholesterol	A(w), B, D*E*, G*
Choline (by Dragendorff)	N
	H, N, O
Ethanolamine	H, I, J(w), K(w), L(w), M(w)
Glycerol	A, B, C, D, E, F, G, H, I, J(w), K(w), L(w), M, N
Inositol	G, H, I, J, K, L, M, N
Phosphorus	D, E, F(w), G, H, I, J, K, L, M, N, O, P + Q(w), R + S
Plasmalogen	A(w), D(w), E, H, I, M, N
Ninhydrin test	H, I, J, K, L, M
—CONH— test	D(w), E(w), F(w), G, H, I, J, K, L, M(w), O(w), P + Q(w), R + S

* Brown color rather than typical blue-green for cholesterol.

Drosophila Lipids

So far as we are aware, no detailed study of *Drosophila* lipids has been published. With the adopted method we obtained elution curves with 19 peaks, as shown (A to S) in Fig. 3. The pattern of elution and the quantitative and chemical data (Fig. 3, Tables I and II) were reproduced satisfactorily in several experiments on different batches of flies. The total, ligroin-soluble lipid content of whole flies (6.1 ± 0.3% by weight) was fairly constant in all batches. Neglecting minor quantities obtained by Soxhlet extraction of residues from the main extraction, the ratio of the weight of lipid (containing phosphatidylethanolamine) eluted by 15% methanol in chloroform to the weight eluted by chloroform was similarly constant (0.38 ± 0.02). Phosphorus was entirely absent from fractions eluted by chloroform but was present in almost all subsequent fractions and, on this basis, weights of phospholipid and nonphospholipid could be calculated. Whole flies contained 2.2 ± 0.1% by weight of phospholipid.

Infrared spectra of lipids of peaks A to O were examined, and all showed characteristic lipid absorptions at 2950–2910 (s.), 2870–2850 (s.), 1735–1725 (s.), 1455–1450 (ms.), and 1365 cm^{-1} (m.). Correlation with published data, the most useful of which can be found in (12, 29–32), confirmed the identifications listed in Table II. Lipids of peaks B to O showed absorption bands in the region 3500–3100 cm^{-1} (mw. to ms.), assigned to O—H and N—H stretching vibrations, and lipids of peaks G to O an unassigned band at 2700 cm^{-1} (w.). Lecithin fractions (peak N) all absorbed at about 3400 cm^{-1}, even after treatment with 2,2-dimethoxypropane and acid (see "Experimental"). This supports the view (33) that the monohydrated formula for lecithins indicated by elementary analysis cannot be explained by simple hydration.

Nearly all phospholipid fractions contained chemically bound amino acids (see Table II). Glutamic acid predominated in

TABLE II

Nature of Drosophila lipids separated by silicic acid chromatography

Peak	Lipids identified*	Amino acids residues present										
		Ala	Arg	Asp	Cys	Glu	Gly	Leu	Lys	Phe	Pro	Ser	Val
A	Hydrocarbon, wax ester, cholesterol ester, triglyceride												
B	Cholesterol												
C													
D	Glycerophosphatidic acid	+		+		+	+	+			+	+	+
E	Phosphatidylglycerol (?)			+		+							
F		+		+	+	+	+	+		+	+	+	+
G	Inositol phospholipid	+				+	+					+	
H	Phosphatidylethanolamine, ethanolamine plasmalogen, phosphatidylserine		+			+	+				+	+	
I	Inositol phospholipid	+		+	+	+	+				+	+	+
J	Glucose-containing cerebroside, glycerophospholipid	+	+	+		+	+	+			+	+	+
K	Inositol phospholipid, lysophosphatidylethanolamine, lysophosphatidylserine (?)	+	+			+	+					+	
L		+		+		+	+	+		+	+	+	+
M		+		+		+	+	+		+	+	+	+
N	Phosphatidylcholine, choline plasmalogen	+	+			+	+	+		+	+	+	+
O		+	+				+	+		+	+	+	+
P + Q													
R + S	Phosphatidopeptide (?)	+	+						+		+	+	

* Substances listed are constituents present and do not necessarily make up the entire fraction. Because of fractionation controlled by fatty acid components it is possible that one or two lipids are major constituents of more than one fraction. For example, the material of fractions L and M could not be distinguished by infrared spectroscopy.

† It is not known to which lipid constituents the amino acids are bound.

peak D lipid. Except in fractions in which phosphatidylserine was obviously present, their concentration was less than one-tenth mole per kilogram of lipid. Minor qualitative variations in bound amino acid content were observed in different experiments; in some, peaks O to S afforded a greater variety of amino acids. It is clear from the distribution of bound amino acids that no one component exists in a very large quantity and none has yet been obtained in an amount sufficient for characterization. Although properly prepared fractions always have the amino acids and phospholipids occurring together it remains an open question whether they are bound together in the same molecule. It is further worthy of note that in preliminary experiments we have observed that addition of C^{14}-labeled glutamic acid or arginine to frozen fly powder results in an appreciable binding to lipid during extraction. Some spontaneous combination or exchange is therefore indicated.

Neglecting the uncharacterized amino acid-containing lipids, the identifications listed in Table II conform with results of silicic acid chromatography generally accepted in the literature. The material eluted in peak D (P, 4.4%), was acidic, and when promptly subjected to paper chromatography it gave one lipid zone, of high R_F, containing phosphorus. But if its chloroform solution were left exposed to the atmosphere for several days the residue could only be redissolved with great difficulty. Paper chromatography of the redissolved material revealed a lipid zone of high R_F, but phosphorus only at low R_F, streaking from the origin. This suggests autohydrolysis of free glycerophosphatidic acid (34, 35). Elution from silicic acid before phosphatidylethanolamine is consistent with the behavior of synthetic glycerophosphatidic acid,[6] as also is the high R_F value (37, 38). Numerous recent reports make it clear that this evidence does not exclude polyglycerophosphatidic acids. Fractions apparently containing such materials have been reported (13, 39, 40) as ninhydrin-negative yet containing nitrogen; this is consistent with our finding of amino acids after hydrolysis.

SUMMARY

1. The general problem of extractions of lipids without degradation is discussed and details of a satisfactory procedure are presented.

2. Among lipids obtained from *Drosophila* were hydrocarbon, wax ester, cholesterol ester, triglyceride, cholesterol, glycerophosphatidic acid, phosphatidylglycerol (?), inositol phospholipids, phosphatidylethanolamine, ethanolamine plasmalogen, phosphatidylserine, cerebroside, lysophosphatidylethanolamine, lysophosphatidylserine (?), phosphatidylcholine, choline plasmalogen, and lipids containing bound amino acids.

3. Twelve different bound amino acids and perhaps peptides were found in lipid fractions, but no fraction contained more than 0.1 mole of amino acid per kg of lipid.

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