

## Insect Apolipophorin III

### PURIFICATION AND PROPERTIES\*

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The hemolymph of adult *Manduca sexta* (tobacco hornworm) contains a 17,000-dalton protein that can associate reversibly with the insect lipoprotein lipophorin. The protein is abundant in the hemolymph of the adult, but is found in larval hemolymph in only small amounts, and does not associate with larval lipophorin. On the basis of its association with adult lipophorin, we have designated the protein apolipophorin III. Apolipophorin III was dissociated from adult lipophorin by guanidinium chloride treatment and isolated by gel permeation and ion exchange chromatography. The unassociated apolipophorin III was also purified from lipophorin-free hemolymph by gel permeation, ion exchange, and lectin chromatography. Both preparations have identical isoelectric points and amino acid composition as well as the following properties. Apolipophorin III is a non-glycosylated polypeptide lacking cysteine and tryptophan. The 17,000-dalton polypeptide dimerizes in solution to a protein of  $M_r = 34,000$ .

from larvae of *Manduca sexta* (Pattnaik *et al.*, 1979; Shapiro *et al.*, 1984), and showed that it resembles other reported lipophorins in its apoprotein composition. Recently we turned to an examination of the lipophorin of the adult moths, and have discovered that this lipoprotein resembles that found in adult locusts, in that it associates with a third apoprotein (apoLp-III<sup>1</sup>) (Shapiro and Law, 1983). This apoprotein occurs also in soluble form as a major component of adult hemolymph. Because of the importance of lipophorin in transport of fat for insect flight and other adult functions, we have isolated and characterized *M. sexta* apoLp-III. We have chosen to designate this apoprotein with a Roman numeral, rather than a letter, to avoid comparison with mammalian apolipoproteins, since we do not yet have a firm basis for such a comparison.

#### EXPERIMENTAL PROCEDURES<sup>2</sup>

#### RESULTS

**Comparison of Larval and Adult Lipophorin**—Fig. 1 shows the SDS-polyacrylamide gel electrophoresis of lipophorin isolated from adult and larval hemolymph. The larval form has the two large apoproteins, apoLp-I and apoLp-II (Shapiro *et al.*, 1984), while the adult lipophorin also has the low molecular weight apolipophorin III.

**Purification**—ApoLp-III was purified both from intact adult lipophorin and from the supernatant obtained following KBr density gradient separation of lipophorin from other hemolymph proteins (Shapiro *et al.*, 1984). We prepared adult lipophorin also by the low ionic strength method of Chino and Kitazawa (1981), and found that the proportion of apoLp-III in this material was similar to that in lipophorin prepared in concentrated salt solutions, demonstrating that the salt does not disrupt the binding of apoLp-III. Indeed, apoLp-III cannot be dissociated from lipophorin in 3 M urea. It was found that 6 M guanidinium chloride was necessary for dissociation. Separation of apoLp-III from intact adult lipophorin was achieved by gel permeation chromatography in 6 M guanidinium chloride. The elution profile of the three

Lipophorin is the sole or major lipoprotein found in the hemolymph of most insects (Chino *et al.*, 1981a). Lipophorins isolated from Lepidoptera (Pattnaik *et al.*, 1979; Chino *et al.*, 1981b), locusts (Chino and Kitazawa, 1971), and cockroaches (Chino *et al.* 1981b; Chino and Kitazawa, 1981) generally contain two apoproteins of rather high molecular weight ( $M_r \sim 250,000$  and  $\sim 80,000$ ). The apoproteins constitute about 60% of the lipoprotein, and the remaining 40% is made up of a mixture of lipids, in which diacylglycerols and phospholipids predominate. In adult locusts, on the other hand, a larger and less dense lipoprotein may be found (Mwangi and Goldsworthy, 1977, 1981; Wheeler and Goldsworthy, 1982, 1983a, 1983b; Van Der Horst *et al.*, 1981). This appears to be a diacylglycerol-loaded form, which serves to transport diacylglycerols from the lipid storage depot in the fat body to the muscles, where fat combustion powers flight. It has been shown that the loaded locust lipophorin associates with a small soluble protein, which has not yet been characterized (Wheeler and Goldsworthy, 1983a, 1983b).

We previously reported the properties of lipophorin isolated

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<sup>1</sup> The abbreviations used are: apoLp-III, apolipophorin III; apoLp-I, apolipophorin I; apoLp-II, apolipophorin II; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PBS, phosphate-buffered saline.

<sup>2</sup> Portions of this paper (including "Experimental Procedures" and Figs. 4-6) are presented in miniprint at the end of this paper. Miniprint is easily read with the aid of a standard magnifying glass. Full size photocopies are available from the Journal of Biological Chemistry, 9650 Rockville Pike, Bethesda, MD 20814. Request Document No. 83M-3383, cite the authors, and include a check or money order for \$3.60 per set of photocopies. Full size photocopies are also included in the microfilm edition of the Journal that is available from Waverly Press.

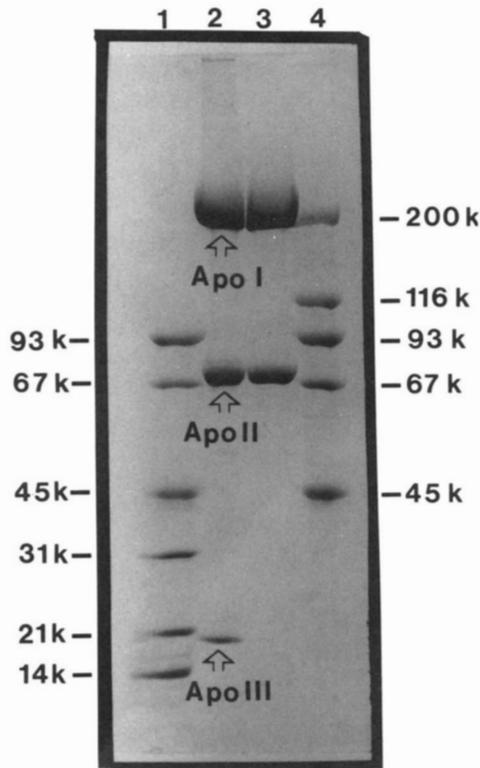


FIG. 1. SDS-polyacrylamide gel electrophoresis of larval and adult lipophorin isolated by KBr density gradient centrifugation. 1 and 4, protein standards; 2, adult lipophorin (10 µg); 3, larval lipophorin (10 µg).

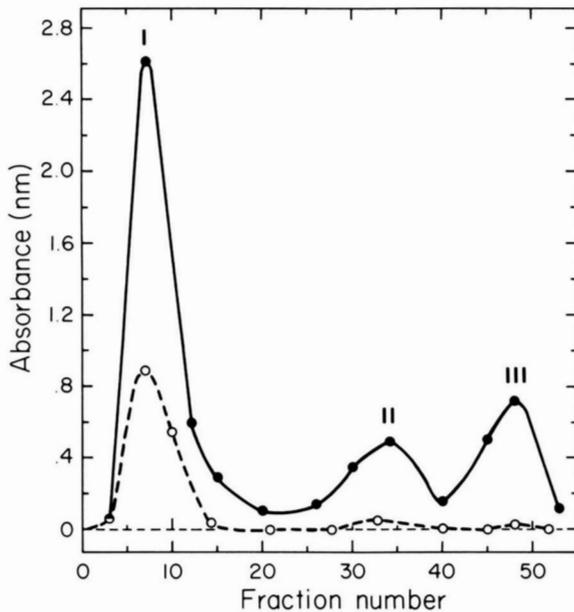


FIG. 2. Separation of apolipophorins. Apoproteins were dissociated by treating adult lipophorin with 6 M guanidinium chloride and passing onto a Sephadex CL-6B column (2.5 × 85 cm) equilibrated with 6 M guanidinium chloride, 50 mM Na phosphate, pH 7.0, at a flow rate of 25 ml/h. Twenty-five ml of sample were applied to the column, and 4.5-ml fractions were collected. ●, absorbance at 230 nm; ○, absorbance at 280 nm.

apolipophorins is shown in Fig. 2.

Isolation of the apoprotein from the KBr subnatant involved gel permeation chromatography on Sephadex G-75, followed by cation exchange on SP (sulfopropyl)-Sephadex

C-25. The contaminating proteins at this stage were shown by reaction with the periodate-Schiff reagent to be glycoproteins, and they could be removed by adsorbing them on a column of concanavalin A-Sepharose. Homogeneous preparations of apoLp-III were obtained from male hemolymph by using these procedures. However, in the female hemolymph an additional protein, microvitellogenin ( $M_r = 31,000$ ), was observed. The female-specific protein could be separated from apoLp-III by an additional chromatographic step on hydroxylapatite (Kawooya and Law, 1983). The products of the various purification steps were subjected to SDS-polyacrylamide gel electrophoresis, and the results are shown in Fig. 3. The two preparations had identical mobilities on native or SDS-polyacrylamide gel electrophoresis. Each showed a characteristic reddish color when stained with Coomassie Blue.

We observed that dialysis of apoLp-III solutions against distilled water resulted in loss of the protein through binding to the dialysis membrane. This could be circumvented by dialysis against dilute acetic acid.

**Molecular Weight**—Gel permeation chromatography of apoLp-III on a calibrated Sephadex G-75 column gave an estimated  $M_r = 34,000$ , while chromatography on a TSK 125 high performance liquid chromatography column gave an estimate of  $M_r = 31,000$ . However, SDS-polyacrylamide gel electrophoresis, with or without 2-mercaptoethanol, yielded an estimated  $M_r = 17,000$ . Calculation of a minimum molecular weight, based upon the amino acid composition (see Table I), gave a value of 17,000.

**Carbohydrate**—Bands of apoLp-III on acrylamide gels did not react with the periodate-Schiff reagent or fluorescein isothiocyanate-conjugated concanavalin A, and the protein

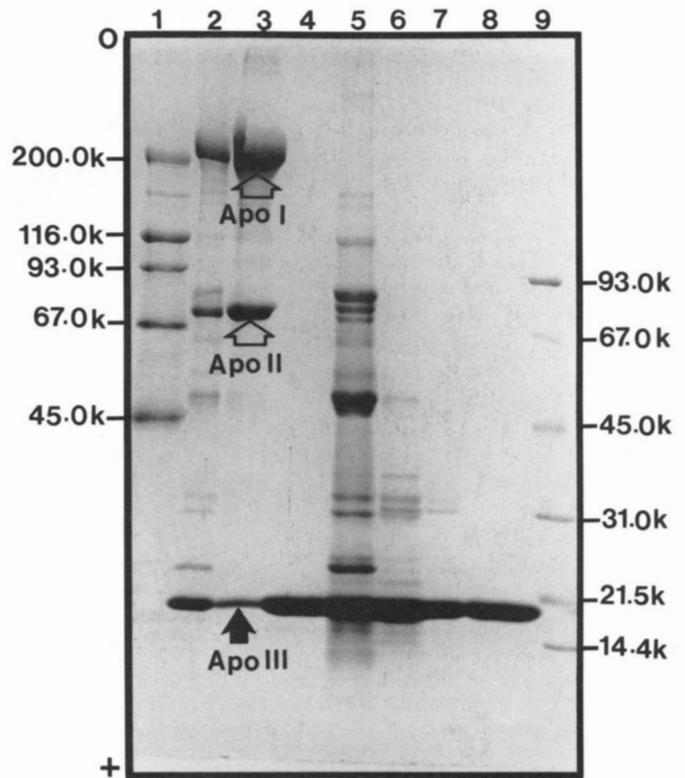


FIG. 3. SDS-polyacrylamide gel electrophoresis of apoLp-III at different stages of purification. 1 and 9, protein standards; 2, crude hemolymph; 3, lipophorin after isolation by KBr density gradient; 4, apoLp-III isolated from lipophorin; 5, subnatant from KBr density centrifugation; 6, after gel permeation chromatography; 7, after ion exchange chromatography; 8, after affinity chromatography.

TABLE I

Amino acid analyses of apoLp-III residues per  $M_r = 17,000$ Data derived from analyses of duplicate samples hydrolyzed for 24, 48, and 72 h in 6 N HCl *in vacuo*, 110 °C

Amino acid	Subnatant-derived protein	Lipophorin-derived protein
Aspartic acid and asparagine	20	19
Threonine	8	8
Serine	13	13
Glutamic acid and glutamine	31	31
Proline	3	2
Glycine	5	6
Alanine	23	24
Valine	10	10
Methionine	2	2
Isoleucine	2	2
Leucine	12	11
Tyrosine	1	1
Phenylalanine	8	8
Histidine	4	4
Lysine	23	22
Arginine	2	2
Tryptophan <sup>a</sup>	0	0
Cystine <sup>b</sup>	0	0
Total	167	165

<sup>a</sup> Duplicate samples hydrolyzed for 24 h in 3 N mercaptoethanesulfonic acid *in vacuo*, 110 °C.

<sup>b</sup> Duplicate samples were performic acid-oxidized, then hydrolyzed for 24 h in 6 N HCl, *in vacuo*, 110 °C.

did not react with the phenol sulfuric acid reagent, and was not retained by a concanavalin A column.

**Antibody Reactions**—Both preparations of apoLp-III were immunogenic. Each antibody preparation gave fused Ouchterlony precipitin lines when diffused against the two apoLp-III preparations. Protein blotting (electrophoretic transfer of larval and adult hemolymph proteins from SDS gels to nitrocellulose followed by incubation with anti-apoLp-III and <sup>125</sup>I-labeled *Staphylococcus* protein A followed by radioautography) showed that larval hemolymph cross-reacted only weakly, while adult hemolymph cross-reacted strongly only at the position corresponding to apoLp-III.

**Apolipophorin III Distribution**—Rocket immunoelectrophoresis was used to quantitate the distribution of apoLp-III between the lipophorin-free subphase and the lipophorin preparation. Similar rocket patterns were obtained using antisera prepared against apoLp-III isolated from lipophorin or the lipophorin-free subphase. A standard curve was constructed from the peak height of rockets *versus* concentration of purified apoLp-III. Unknown samples were appropriately diluted, electrophoresed, and quantitated by comparison of peak height to the standard curve. Adult hemolymph was determined to contain  $17 \pm 5$  mg/ml of apoLp-III. The stoichiometric ratio of apoLp-III to apoLp-I and apoLp-II (which exist in a 1:1 ratio in larval lipophorin (Shapiro *et al.*, 1984)) was estimated as follows. The amounts of apoLp-I and apoLp-II in a sample of lipophorin of density 1.11 g/ml were calculated from the absorbance at 280 nm, a wavelength at which apoLp-III has very little absorbance. An aliquot of this sample was heat denatured and used for quantitative apoLp-III determination by rocket immunoelectrophoresis. From the results, the molar ratio of apoproteins was calculated as 2 apoLp-III per lipophorin particle. The number of apoLp-III polypeptides in the lower density lipophorin particles is obviously much higher as judged by Coomassie Blue staining of gels, but it was not possible to make an estimate with the same techniques because the absorbance due to apoLp-III now becomes significant.

Larval hemolymph contained 0.46 mg/ml of apoLp-III,

none of which was associated with the larval lipophorin.

**Isoelectric Point**—Both preparations of apoLp-III have identical isoelectric points of 6.1.

**Amino Acid Composition**—The amino acid composition of apoLp-III isolated either from lipophorin or lipophorin-free hemolymph is shown in Table I. The values are in good agreement, suggesting that the preparations are identical. The lack of tryptophan is notable; this accounts for the low absorbance of apoLp-III at 280 nm (0.04 absorbance units/mg of protein). The ratio of the absorbance at 230 to 280 nm is approximately 30.

## DISCUSSION

Mammalian blood contains a variety of lipoproteins that serve different lipid transport functions and perform these functions in different ways. Chylomicrons deliver dietary fat to adipose tissue without being taken up by cells, while low density lipoprotein delivers cholesterol esters and other potential membrane components to cells through an endocytotic uptake mechanism involving specific receptors. In general, insects have a single lipoprotein species, which may serve several different functions and may even change its size, density, and apoprotein complement as it alters its function (Chino *et al.*, 1981a; Wheeler and Goldsworthy, 1983a, 1983b; Shapiro and Law, 1983). This lipoprotein has been given the name lipophorin in recognition of its function as a lipid carrier. The hemolymph of the *M. sexta* larva contains a lipophorin with about 60% protein, 40% lipid, and a density of 1.13 g/ml, which is typical of high density lipoproteins (Pattnaik *et al.*, 1979; Shapiro *et al.*, 1984). The particle contains two glycosylated apoproteins. ApoLp-I, a large (245,000 daltons) polypeptide, has some properties that suggest similarity to human apolipoprotein B, although no definitive evidence for a relationship is available. The other apoprotein apoLp-II, is of moderate molecular weight ( $M_r = 78,000$ ) and its properties have not been correlated with those of mammalian apolipoproteins (Shapiro *et al.*, 1984). We do not know the functions of larval lipophorin, but we believe that it may be primarily responsible for the transport of digested fats from the mid-gut to storage depots in the fat body.

After metamorphosis, the hemolymph of the adult moth contains chiefly a lipophorin of density 1.11 g/ml with a smaller amount of a second form of lower density, 1.06 g/ml. Each of these contain not only apoLp-I and -II, but in addition they are associated with variable amounts of a third apoprotein, apoLp-III. The lower density form can be increased markedly by injecting the animal with a synthetic decapeptide, the adipokinetic hormone of the migratory locust, *Locusta migratoria* (Shapiro and Law, 1983). The decrease in density to 1.06 g/ml is accompanied by association of increased amounts of lipid and apoLp-III with the low density lipophorin. This is quite analogous to events in locust hemolymph when adipokinetic hormone is released from the corpus cardiacum into the hemolymph to prepare the animal for flight (Mwangi and Goldsworthy, 1977, 1981; Van Der Horst *et al.*, 1981; Wheeler and Goldsworthy, 1982, 1983a, 1983b).

ApoLp-III can be isolated either from lipophorin or from crude hemolymph. The two preparations are identical in chromatographic and electrophoretic behavior as well as in amino acid composition and isoelectric point. They are immunologically cross-reactive, but there is no immunological cross-reactivity between apoLp-I, apoLp-II, and apoLp-III. Therefore, apoLp-III is not a proteolytic degradation product of apoLp-I or apoLp-II. It should also be noted that apoLp-III, unlike apoLp-I and -II, is not glycosylated. The fact that

apoLp-III from the hemolymph associates rapidly ( $\leq 1$  h) with lipophorin on treatment with adipokinetic hormone (Shapiro and Law, 1983) further indicates that the two polypeptides, that associated with lipophorin and that free in the hemolymph, are identical and distinct from apoLp-I and -II.

The total amount of apoLp-III in adult hemolymph is  $17 \pm 5$  mg/ml. About half of this is free and about half associated with lipophorins, especially with the lowest density (1.06 g/ml) form. The higher density form has two apoLp-III peptides/particle, or an apoprotein ratio of 2 apoLp-III per apoLp-I and apoLp-II.

The monomeric apoLp-III has a molecular weight of about 17,000 as shown by SDS-polyacrylamide gels and by calculation of a minimum molecular weight based upon the single tyrosine residue. Under native conditions, the protein can form dimers, as indicated by gel permeation chromatography. The tendency to dimerize may result from intermolecular interaction of a hydrophobic binding site on apoLp-III. Possibly this site could be involved in the association with the expanded lipophorin surface after lipid loading.

The functions of apoLp-III in the insect lipoprotein is not known. The fact that this apoprotein is present in the larval hemolymph in much smaller amounts suggests that it participates in a function in the adult that is not present in the larva. The use of diacylglycerol to fuel sustained flight is well documented in insects (Weis-Fogh, 1952). Lipophorin is known to participate in the transport of diacylglycerol from fat body to flight muscles (Beenackers *et al.*, 1981). Thus, apoLp-III may function to stabilize the lipid loaded form of lipophorin or as a recognition signal at muscle cells to identify it as a loaded carrier, or both. Although lipophorin is considered to act as a reutilizable shuttle for lipid transport (Chino *et al.*, 1981a), there is no evidence as to whether it enters insect cells in the course of its function (other than the oocyte, which it certainly does enter (Telfer and Kulakosky, 1984)). Further investigation into the function of apoLp-III may shed light on these questions.

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## Supplementary Material To

### Insect Apolipophorin III, Purification and Properties

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## EXPERIMENTAL PROCEDURES

**Animals.** Adult *M. sexta* were from the eggs kindly supplied by Dr. J. P. Reinecke and Dr. J. Buckner, U. S. Department of Agriculture, Fargo, ND. The animals were raised according to Kramer *et al.* (1974).

**Collection of Hemolymph.** An average of 35 adult male animals, 1–3 days after eclosion, were used in each hemolymph preparation. Bleeding procedures and hemolymph collection were performed according to Shapiro and Law (1983). The hemolymph was centrifuged at 12,000  $\times$  g for 5 min to remove hemocytes.

### Purification of the Protein.

Lipophorin was separated from adult hemolymph as described by Shapiro *et al.* (1983), employing ultracentrifugation in a KBr density gradient. The yellow lipophorin band was collected through the side of the tube, one third of the way from the top, by means of a hypodermic syringe. Substant from the KBr density gradient ultracentrifugation was collected from the bottom of the centrifuge tube by means of a hypodermic syringe. It could be identified by its blue color.

### a) From the subnatant:

The volume was reduced to approximately 2.0 ml by ultrafiltration through a YM10 Amicon membrane (Amicon Corp., Lexington, MA). Gel permeation was performed at 4°C on a 2.5 cm  $\times$  1 m Sephadex G-75 column equilibrated with PBS (0.10 M Na-phosphate, 0.15 M NaCl, 0.05 M EDTA and 0.02 percent (w/v) NaN<sub>3</sub>, pH 7.0). Fractions (3.2 ml each) were collected and the absorbance of each fraction was read at 230 and 280 nm with a Perkin-Elmer Lambda 3 Spectrophotometer. A typical elution profile is shown in Fig. 4. ApoLp-III was located by subjecting aliquots of fractions to SDS-PAGE (Fig. 4, bottom). The appropriate fractions were pooled and the volume reduced to 1.0 ml by ultrafiltration. The ultrafiltrate was dialyzed against 10 mM Na-succinate, pH 5.4, and applied to an SP-Sephadex C-25 (Pharmacia) cation exchange column (8 ml bed volume) equilibrated in the same buffer at 25°C. The protein was eluted from the column with 0.1 M NaCl, pH 6.0. The elution profile is shown in Fig. 5.

SDS-PAGE was again used to locate fractions containing apoLp-III from the SP-Sephadex column (Fig. 5). These were pooled and dialyzed against buffer (0.02 M Tris-HCl pH 7.4, 0.5 M NaCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub> and 0.02 percent (w/v) NaN<sub>3</sub>) and loaded on to a concanavalin A-Sepharose (Pharmacia) column (8 ml bed volume) equilibrated in the same buffer at 25°C. ApoLp-III was eluted with buffer while contaminating glycoproteins could be removed by elution with 20 ml of 0.5 M  $\alpha$ -methyl-D-mannoside (Fig. 5).

### b) From adult lipophorin:

Apoproteins were dissociated from intact lipophorin in 6M guanidinium chloride and separated by gel permeation chromatography, as previously described (Shapiro *et al.*, 1984). The elution pattern is shown in Fig. 2. The fractions containing apoLp-III were combined and desalted on Sephadex G-25 (1.5  $\times$  40 cm) equilibrated in 0.1 M acetic acid. SDS-PAGE showed that contaminating proteins were still present, and the apoLp-III was further purified by chromatography on SP-Sephadex, as described above.

### Polyacrylamide Gel Electrophoresis.

a) Native PAGE was performed on 4–30 percent acrylamide gradient gel slabs. The resolving gel (0.375 M Tris-HCl, pH 8.8) was overlaid by a stacking gel containing 0.125 M Tris-HCl, pH 6.8. The electrode buffer contained Tris-glycine pH 8.3 (0.025 M Tris; 0.192 M glycine).

b) SDS-PAGE was performed on 4–15 percent gradient gel slabs (1.5  $\times$  18  $\times$  20 cm separating gel, with 1 cm deep stacking gel) made with a BRL gradient maker. The gels were either stained with Coomassie Blue R-250 for proteins or with periodate-Schiff reagent (Kapitany and Zebrowski, 1973) or with fluorescein isothiocyanate conjugated concanavalin-A (Furlan *et al.*, 1979) for glycoproteins. SDS-PAGE was performed according to Laemmli (1970).

**Isoelectric Focusing.** Isoelectric focusing (Wrigley, 1971) was performed on 5 percent gel slabs (125  $\times$  100  $\times$  0.8 mm). The gels were prepared from a mixture of 3.35 ml acrylamide (29.1 percent); 3.35 ml N,N-methylene bis-acrylamide (0.9 percent); 2.35 ml glycerol (8.7 percent v/v); 0.3 ml 2 percent fresh ammonium persulfate; 10  $\mu$ l N,N,N',N'-tetramethyl-ethylenediamine and 1 ml of Bio-Lyte 5/7 (Bio-Rad). The CTL casting system (Bio-Rad) was used to cast the gel slabs. Samples (15  $\mu$ g of protein) were applied to the gels in duplicate by means of filter paper wicks and subjected to electrophoresis at a constant power of 3 watts for 2 1/2 hrs. At the end of each run, the gel was sliced into half. One half was further subjected to isoelectric focusing while the other was placed in the fixative (4 g

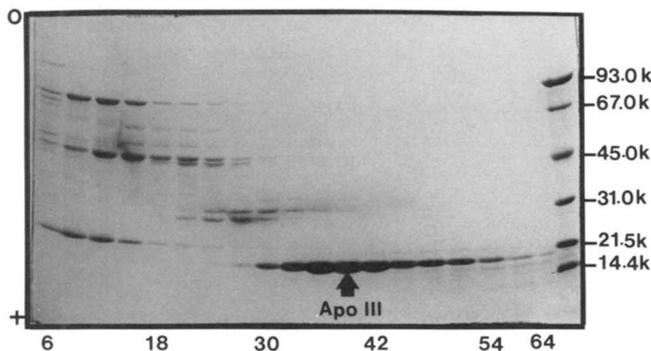
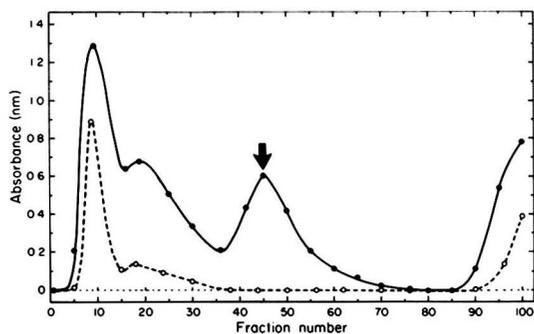
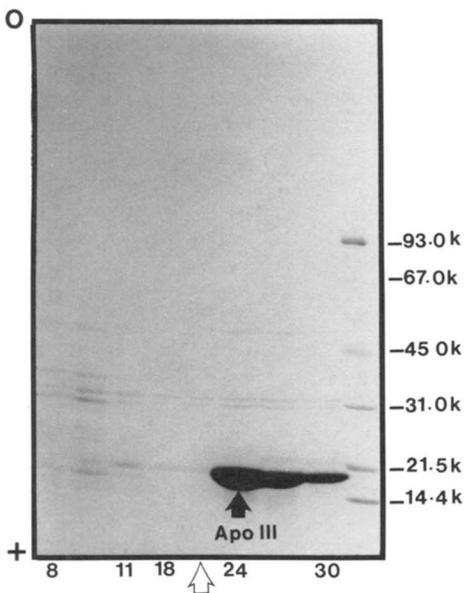
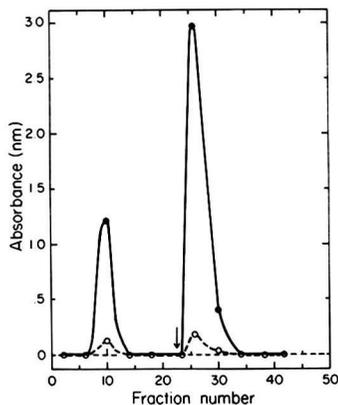


Fig. 4. Gel permeation chromatography. Top: Chromatography of subnatant from KBr density gradient centrifugation on Sephadex G-75 (see text). Closed circles, absorbance at 230 nm; open circles, absorbance at 280 nm. Bottom: SDS-PAGE of selected fractions from the gel permeation chromatography shown at the top.



sulfosalicylic acid, 12.5 g trichloroacetic acid, H<sub>2</sub>O up to 100 ml). ApoL-III bands were visible (due to precipitation) within 10 minutes of fixation. The distance of the bands from the cathode was measured. An identical distance was measured on the other half of the gel, and the pH measured at that point by means of the MI-465 miniature glass electrode (Microelectrodes Inc., New Hampshire).

**Molecular Weight Estimation.** The molecular weight of apoL-III was estimated by gel permeation chromatography at 4°C using a Sephadex G-75 column calibrated with protein standards, and by high performance gel permeation chromatography on a Bio-Rad TSK 125 column (0.15 M Na<sub>2</sub>SO<sub>4</sub>, 0.02 M Na<sub>2</sub>PO<sub>4</sub>, 0.02 percent NaCl, pH 6.8 buffer) and a Varian 5000 chromatograph with UV 50 detector. The monomeric molecular weight was estimated from its electrophoretic mobility relative to that of Bio-Rad protein standards on SDS-PAGE.

**Amino Acid Analysis.** Duplicate samples were hydrolyzed in 6N HCl at 110°C in vacuo for 24, 48, and 72 hr. Cysteine and cystine were determined as cysteic acid after performic acid oxidation (Hirs, 1976). Tryptophan was determined by amino acid analysis preceded by mild hydrolysis in 3M mercaptoethanesulfonic acid (22 h, 110°C) (Penke et al., 1974). Analyses were performed on a Drexel 0-330 amino acid analyzer using the standard column and three-buffer system suggested by the manufacturer, with an additional sodium citrate buffer (0.2 M Na<sup>+</sup>/pH 7.4) added for tryptophan elution after the B buffer. Peaks were directly integrated on a Hewlett-Packard 338CA integrator.

**Antibodies and Immunology.** Purified, lyophilized apoL-III (0.5 mg) from either lipophorin and from lipophorin-free hemolymph was dissolved in 0.5 ml H<sub>2</sub>O, emulsified in 1.5 ml Freund's complete adjuvant and injected intramuscularly into all four limbs of New Zealand White rabbits. After 1 month animals were boosted with 0.5 mg apoL-III emulsified in 0.75 ml Freund's incomplete adjuvant. Blood was collected by heart puncture three weeks later and sera stored at -70°C.

Double radial immunodiffusion (Ouchterlony, 1968) was performed in 1.8 mm thick 1 percent agarose in PBS and stained with Coomassie Blue.

Protein blotting (Towbin et al., 1979; Burnette, 1981) was performed on antigens separated by SDS-PAGE. Separated proteins were electrophoretically transferred to nitrocellulose, incubated with specific antisera followed by [<sup>125</sup>I]Staphylococcus protein A. Cross-reactivity was detected by radioautography.

Rocket immunoelectrophoresis was performed according to Meeke (1973) using a barbital-glycine/tris buffer (20 mM barbital, 192 mM glycine, 10 mM Tris; pH 8.6) and 0.6 percent agarose with medium electroendosmosis (SeaKem ME agarose, FMC Corp.). Purified apoL-III standards and unknown samples were applied (3 µl per well) and electrophoresis was performed for 6.5 h at 120 V. Samples were placed in a boiling water bath for 1 min prior to electrophoresis to dissociate apoL-III from lipophorin. This heat treatment did not alter the peak height or the rocket pattern of standard apoL-III.

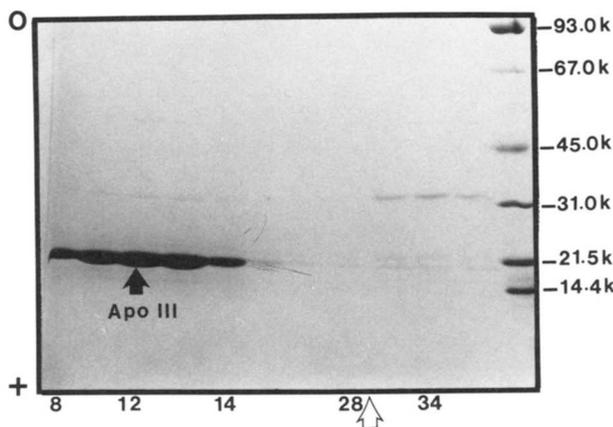
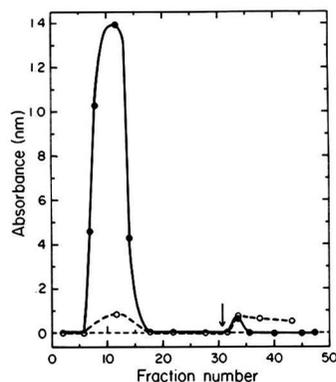


Fig. 6. Lectin affinity chromatography. Top: Pooled fractions from the ion exchange column (Fig. 5) were applied to a column of concanavalin A-Sepharose and eluted with buffer (see text). At the arrow, buffer containing  $\alpha$ -methyl-D-mannoside was added. Closed circles, absorbance at 230 nm; open circles, absorbance at 280 nm. Bottom: SDS-PAGE of selected fractions from the concanavalin A-Sepharose column shown at the top.

Fig. 5. Ion exchange chromatography. Top: Pooled fractions from gel permeation chromatography (Fig. 4) were concentrated and applied to a SP-Sephadex C-25 column at pH 5.4 (see text). At the arrow, sodium chloride containing buffer, pH 6.0, was added (see text). Closed circles, absorbance at 230 nm; open circles, absorbance at 280 nm. Bottom: SDS-PAGE of selected fractions from the ion exchange chromatography shown at the top.