review

Transport of lipids in insects

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Abstract Many insect species are almost completely dependent on lipids for their metabolic needs, although this is usually a function of developmental stage. The primary storage organ is the fat body, which can constitute 50% of the fresh weight of the insect and also acts as the major metabolic center (analogous to the vertebrate adipose tissue and liver). Bathing the fat body (and all other tissues and organs) is the hemolymph, the main functions of which are to transport nutrient substrates to utilization sites and to deliver metabolic wastes to the excretory system.

Although neutral lipids are stored as triglycerides, in times of need they appear to be endergonically released into the hemolymph as diglycerides in the majority of insects thus far studied (particularly silkmoths and locusts). Indeed, diglycerides constitute the largest neutral lipid fraction in the hemolymph of silkmoths, locusts, cockroaches, bugs, etc. In the hemolymph the diglyceride is found as a constituent of specific lipoproteins, and one specific lipoprotein class (lipoprotein I; high density lipoprotein) appears to be necessary for the transport of diglyceride from the fat body cell into the hemolymph. This particular lipoprotein is also involved in the transport of cholesterol from the gut into the hemolymph. Thus, lipoprotein I appears to be the major neutral lipid and sterol transport agent in the insects studied and, in addition, plays a regulatory role in the release of both diglycerides and sterols. Hemolymph lipoprotein II (very high density lipoprotein) may be important in providing protein and lipid to the insect ovary during oogenesis.

Ecdysone, the polyhydroxy steroidal insect molting hormone, is probably carried "free" in the hemolymph, although reports exist of specific hemolymph-binding proteins in some species. The other major insect growth hormone, juvenile hormone, is transported by hemolymph lipoproteins in silkmoths and locusts and by a lower molecular weight hemolymph protein in the tobacco hornworm.

Supplementary key words hormone · ecdysone · juvenile hormone · silkmoth · diglyceride · lipoprotein · fat body · lipid release · hemolymph · sterol

To a great extent, the obvious success of insects on this planet has been their ability to utilize lipids efficiently as substrates for reproduction, embryogenesis, metamorphosis, and flight. In addition, lipids are used as a means of communication (pheromones), for regulation of a variety of physiological processes (hormones), as protection against a desiccating environment (cuticular lipids), and as cell constituents (membranes). In recent years there have been detailed reviews on insect lipids and their metabolism (1, 2) as well as specialized reviews on insect sterols (3) and the endocrine control of insect development (4–6).

Many of the data in this review are from our own studies utilizing saturniid silkmoths such as *Hyalophora cecropia* and *Philosamia cynthia,* and we will briefly review the life history of these insects because aspects of lipid release and transport may be a function of developmental stage. In the case of *H. cecropia,* eggs are normally laid in the spring, embryogenesis takes 8–10 days at 25°C, and the small caterpillar (larva) then escapes from its chorionic confines. After feeding on cherry leaves and growing in mass, the larva synthesizes a new cuticle and sheds its old, restricting exoskeleton (ecdysis). This occurs four times during larval life (five larval stages, or instars), with the older larva being larger but not differing significantly in form from its predecessor. At the end of the summer, the last instar larva encases itself within a cocoon of silk, and about 10 days later it undergoes the first dramatic change in form (metamorphosis) by shedding the old larval cuticle and revealing the hard, brown, immobile pupa. The pupa resides within the cocoon over the winter in a physiologically quiescent state (diapause), protected from the extremes of weather by the cocoon, the heavy cuticle, and a 5 molal concentration of glycerol in the hemolymph. With the onset of rising temperatures in the spring, adult development is initiated within the pupa (pharate pupa; second metamorphic event), and 3 wk later the brightly colored adult moth emerges from the old pupal cuticle (ecdysis) and cocoon and mates, and another cycle begins. It should be noted that, from the time of pupation until adult ecdysis, this insect is essentially a cleidoic system, with only

Abbreviations: TG, triglyceride; DG, diglyceride; FFA, free fatty acid; JH, juvenile hormone; JH I, C_{18} juvenile hormone; JH II, C_{17} juvenile hormone; JH III, C_{16} juvenile hormone; LP, lipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; VHDL, very high density lipoprotein.

the exchange of gasses and water vapor with its environment preventing it from being a completely closed system. Further, the adults do not feed, and they live only a week. Therefore, only the larval stage involves ingestion, and its primary function is substrate accumulation. During the metamorphosis of the pupa to the adult, some tissues are histolyzed while some adult structures develop from embryonic nests of cells (imaginal discs). This is a reorganization *within* the animal, with no exogenous substrate entering the organism, and is therefore an excellent model system for the biochemist and physiologist. The fact that many of the developmental processes are endocrinologically controlled makes the system even more attractive.

This review is concerned with lipid transport in insects, and, although several unique aspects of this phenomenon are known, we are far from understanding the means by which lipids are released from the storage depot (fat body) into the circulatory system (hemolymph) for transport to sites of utilization. It should be mentioned that the insect fat body carries out functions analogous to the vertebrate liver and adipose tissue; i.e., it is both a metabolic center and a storage organ. The insect circulatory system differs from that of higher animals in that it is an "open" system functioning primarily to bathe the internal organs, and in that manner it both removes metabolic wastes and supplies nutrients to all organs and tissues. Unlike the vertebrate, the insect circulatory system generally plays no respiratory role since gas exchange is normally the function of a complicated matrix of tubes (tracheae and tracheoles) reaching from the external environment to individual cells. It should be noted that, although the hemolymph bathes the internal organs in a general sense, individual structures are "protected" from the hemolymph by a basement membrane, the physiology and structure of which are not completely understood.

The fat body constitutes a considerable percentage of fresh weight of the insect (more than 50% in some cases) and is derived from the somatic mesoderm. The amounts of various substrates extractable from the fat body depend on whether carbohydrate or lipid is used as the major energy source for development or other physiological processes (e.g., flight). In the case of *H. cecropia,* the male moth yields more than five times as much lipid as the female, and eight times as much on a fresh weight basis (7). This is partly a result of the fact that lipid is utilized by the female for egg production during the development of the adult, whereas the male conserves lipid during this period in order to have it available as the predominant fuel for flight during adult life (8). It thus appears that development in this insect (and many other lepidopterans) shows a metabolic specialization such that the male is provided with adequate lipid stores for use in the adult stage. These metabolic alterations are reflected in the changing ultrastructure of fat body cells during development. In general, lipid is stored in the fat body in the form of lipid globules composed primarily of triglyceride, and the globules exhibit a laminated structure when examined by freeze-etching electron microscopy (9).

Correlations have been made between the sexual dimorphism in lipid utilization displayed by male and female *H. cecropia* moths and the ultrastructure of their fat bodies during pupal–adult development (10). In the newly molted pupa, the fat body cells of both males and females contain large lipid globules, varying quantities of protein bodies, cytolysomes, glycogen granules, and rough endoplasmic reticulum. After exposure to 6°C for several months, the pupa initiates adult development upon exposure to 25°C for several weeks. At this time the fat body cells "loosen" from one another and float free in the hemolymph rather than being in the relatively compact organ structure characteristic of the diapausing pupa. Among the changes seen in these "free" fat body cells of the female is a depletion of most of the lipid globules by day 8 of adult development. This correlates well with the supposition that lipid is used by the female as a substrate for adult development. The lipid globules, which are normally spherical in the fat body cells of the pupa, take on a ragged appearance, probably as a result of lipolysis (11). During the lipolytic process, one observes the appearance of small lipid globules, many often attached to the mitochondria and some localized within cisternal vesicles of the endoplasmic reticulum. The association of these organelles with the small lipid globules suggests a role for the vesicles and mitochondria in the lipolytic process and perhaps in the intracellular transport of the products of lipolysis. On day 12 of female adult development, very little lipid remains in the fat body, and it is in the form of thin strands bordered by electron-dense proteinaceous material as well as glycogen, giving the appearance of a macrocomplex of lipid, protein, and glycogen. By day 16 of adult development, the adult body has begun to be organized into a relatively compact structure, and a few lipid globules appear. 1 day after adult ecdysis, the cells of the female fat body contain smooth endoplasmic reticulum, many mitochondria, and a few glycogen granules and lipid globules. By contrast, the adult male fat body cells are almost filled with large lipid globules, resulting in the restriction of the cytoplasmic organelles to a small portion of the cell wedged between the lipid globules.

GENERAL ASPECTS OF INSECT LIPID METABOLISM

In general, insects metabolize lipids along routes established for bacteria and vertebrates (1, 2). The major exception is their inability to synthesize sterols from simple precursors, and this will be discussed in a later section of

this review. The synthesis of glycerides by the insect fat body apparently follows the glycerophosphate pathway as in other organisms (12), and fatty acids are broken down via β-oxidation. Thompson (13) recently reviewed the fatty acid composition of seven insect orders and showed that, although changes occur during development and as a result of diet, an insect at any stage usually maintains the general fatty acid pattern of its taxonomical niche. This is particularly true when comparing the ratio of saturated to unsaturated fatty acids. For example, beetles, moths, flies, and bees have a saturated:unsaturated ratio of 0.3–0.44; locusts and cockroaches, 0.76 and 0.87, respectively; and bugs, 9.84. The high figure of the last group reflects their high myristic acid levels. It is of interest that fatty acid methyl esters constitute 3–4% of the neutral lipids in late silkworm (*Bombyx mori*) embryos (14). The major fatty acid methyl ester present was shown to be palmitate, with relatively large amounts of stearate and oleate, and there was noted a tendency toward increased amounts of methyl esters of polyunsaturated fatty acids as embryonic development proceeded. The role of these methyl esters remains obscure, although it has been proposed that they have some growth-regulating properties (15).

Fatty acid biosynthesis in insects appears to follow the conventional scheme accepted for mammals and bacteria (1). Detailed studies of fatty acid biosynthesis in *H. cecropia* revealed that although this insect can convert [1-^{14}C]acetate to palmitate, palmitoleate, stearate, and oleate, radioactivity was never detected in either linoleate or linolenate (16, 17). This appears to be characteristic of insects in general and explains their nutritional requirement for linoleate or linolenate.

About 90% of the total fat body lipid is triglyceride, with the remainder being partial glycerides, phospholipids, sterols, etc. (18–23). If neutral lipids are to be released from the fat body into the hemolymph for transport in forms other than as triglyceride, lipase must be present. Although there has been little work on true insect extradigestive (fat body) lipases (22, 24, 25), recent studies on the lipases of the egg of the beetle *Diabrotica virgifera* have revealed a case of "structure-linked substrate latency" (26, 27). That is, lipase activity depended on the means of extraction (freeze–thaw, ultrasound, etc.) and, although the enzyme is "freely active," the substrate is unavailable, perhaps because it is segregated by a membrane system. Thus, regulation of lipolysis could be via membrane disruption. This is consistent with a previous supposition that regulation of lipid utilization in the black fly fat body may be a function of the structure of the lipid globule (9).

Some recent studies by Dutkowski and his colleagues (28–30) on the waxmoth, *Galleria mellonella,* have revealed the most pertinent data thus far on regulation of lipolysis in the insect fat body. In studies with [^{14}C]palmitate they demonstrated that both glyceride synthesis and degradation took place in the fat body and that both activities underwent developmental alterations. Of interest was the finding that lipolytic activity reached a peak just prior to the onset of vitellogenesis in the 6-day pharate adult (12 times the activity of the 1-day pharate adult), presumably so that substrates (FFA) are released and made available to the maturing oocyte. In the late pharate adult (during the last 3 days preceding adult ecdysis), lipolytic activity falls concurrently with an increase in glyceride biosynthesis, perhaps because the stored lipid of the fat body could then be used during adult life as a substrate for flight. Neither the increase in lipolytic activity nor the subsequent increase in glyceride synthesis occurs in ovariectomized females; this suggests that the developing ovaries may control glyceride biosynthesis in the fat body. Indeed, when the fat body from ovariectomized animals was preincubated with ovaries from day 6 pharate adults, the lipolytic activity of the fat body increased significantly. The authors postulate the existence of an ovarian hormone that controls lipid metabolism in the fat body, but neither its nature nor mode of action has yet been investigated. Wigglesworth (31) observed that every lipid droplet in the fat body of *Rhodnius prolixus* has a small (1 μm) disclike area or cap ("catalysome") that shows esterase (lipase) activity under cytochemical analysis; these structures may be control points for lipolysis.

(The reader is referred to Refs. 32 and 33 for data on phospholipid synthesis and correlations between phospholipid and cytochrome *c* synthesis during flight muscle development in silkmoths.)

RELEASE OF LIPIDS FROM FAT BODY

In preliminary experiments in which *H. cecropia* (mainly pupae) were injected with [^{14}C]palmitate and the radioactivity in the glyceride fraction was determined in the fat body and hemolymph several hours later, we (18, 34) first observed that the specific radioactivity of the hemolymph glycerides was far greater (more than 120 times) than that of glycerides in the fat body. Subsequently, it was observed that [^{14}C]palmitate was rapidly incorporated into the glycerides of the fat body when the fat body was incubated with [^{14}C]palmitate in vitro, and that both TG and DG were significantly labeled. These observations suggested two possibilities. First, the hemolymph may have a greater capacity for glyceride biosynthesis when compared with the fat body, and second, DG of extremely high specific activity may be continuously released from the fat body into the hemolymph while the low specific activity TG remains for the most part in the fat body. The second alternative would be a result of the fact that TG is the major glyceride component (about 98%) in the fat body whereas DG is only a minor component. The

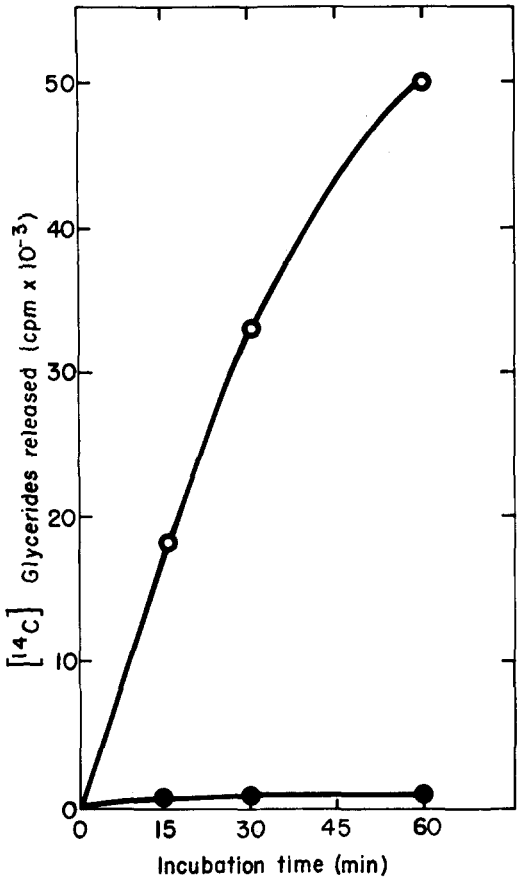


Fig. 1. Time course of ^{14}C-labeled glyceride release from the prelabeled fat body of *H. cecropia*. Prelabeled fat body (170 mg) was incubated (25°C) in medium containing 0.2 ml of hemolymph and 0.8 ml of phosphate saline. ○, DG released; ●, TG released. From Chino and Gilbert (18).

first possibility was immediately ruled out by our observation that isolated hemolymph was not capable of synthesizing glycerides from FFA to any extent. Therefore, the second possibility seemed to be most likely. In order to test this hypothesis, we carried out the following experiments.

The isolated fat bodies from either pupae or adults were incubated with [^{14}C]palmitate after washing the tissues thoroughly with saline. After incubation, the fat bodies containing the labeled TG and DG (prelabeled fat bodies) were reincubated with medium containing pupal hemolymph. After the reincubation, TG and DG in the incubation medium were isolated and assayed for radioactivity. It was clearly demonstrated that DG was specifically and rapidly released from the fat body into the medium, while the amount of the labeled TG found in the medium was extremely small. It should be reiterated that insects have an open circulatory system wherein all the tissues are exposed to the hemolymph. Therefore, incubation of the prelabeled fat body in a hemolymph medium approaches the normal condition, and we consider it to be physiologically satisfactory.

We further examined the nature of this specific release of DG. **Fig. 1** shows the time course of the release of ^{14}C-labeled DG from the prelabeled fat body into the pupal hemolymph medium and demonstrates that the release of DG continues throughout the incubation period. In contradistinction, the release of TG is negligible. The amount of ^{14}C-labeled DG released depends on the quantity of hemolymph added to the incubation medium. As little as 0.01 ml of hemolymph was enough to stimulate DG release, and maximal stimulation was observed with 0.25 ml of hemolymph. Conversely, the amount of prelabeled fat body can also be a limiting factor.

Table 1 summarizes the nature of lipid release from the fat body and reveals the following. (*1*) The release of DG is specific for insect hemolymph, and both adult and pupal hemolymph can stimulate the release of DG. No significant release of DG occurs in media containing saline, serum albumin, or mammalian plasma. (*2*) The presence of TG in all of the incubation media is probably due to the "leaky" nature of the fragile fat body. In fact, when the prelabeled fat body was incubated in undiluted hemolymph (1 ml), the amount of ^{14}C-labeled TG found in the medium was extremely low (see Expt. 2, Table 1). (*3*) FFA is also released from the fat body into the hemolymph. However, this release is not specific for insect hemolymph but is also enhanced by serum albumin. (*4*) The rate of DG release from adult fat body is much higher than that from diapausing pupal fat body, whereas

TABLE 1. Nature of glyceride release from prelabeled fat body of *H. cecropia*

Expt. No.	Incubation Medium	^{14}C-labeled TG Released[a]	^{14}C-labeled DG Released[a]	^{14}C-labeled FFA Released[a]
		%	%	%
1. Adult fat body	0.2 ml adult hemolymph	1.7	37.7	4.9
	0.2 ml pupal hemolymph	1.5	26.1	3.5
	0.3 ml 5% serum albumin	1.0	1.7	15.1
	1 ml phosphate saline	1.1	1.8	2.0
2. Adult fat body	0.3 ml pupal hemolymph	1.4	28.1	
	1 ml pupal hemolymph	0.2	29.1	
	0.5 ml rat plasma	1.2	1.8	
	0.3 ml rat plasma	1.1	1.6	
	1 ml phosphate saline	1.1	1.4	
3. Pupal fat body	0.3 ml pupal hemolymph	1.3	4.5	27.0
	1 ml phosphate saline	1.0	0.7	3.7

Prelabeled fat body was incubated at 25°C for 60 min. Phosphate saline (pH 6.7) was added to give a final volume of 1 ml. Expt. 1: 170 mg of prelabeled fat body containing 54,900 cpm of TG, 242,400 cpm of DG and 61,200 cpm of FFA. Expt. 2: 150 mg of prelabeled fat body containing 89,500 cpm of TG, 229,700 cpm of DG, and 19,100 cpm of FFA. Expt 3: 300 mg of fat body containing 13,900 cpm of TG, 27,500 cpm of DG, and 37,000 cpm of FFA. Modified from Chino and Gilbert (18).

[a] Quantity of a particular fraction (TG, DG, FFA) released is expressed as: (cpm in medium fraction/cpm originally in fat body fraction) × 100.

FFA is released from the diapausing pupal fat body at a much higher level than from the adult fat body.

Because the adult fat body is metabolically more active than the diapausing pupal fat body, we postulated that the release of DG may be an endergonic process and the release of FFA is simple diffusion following a concentration gradient. Indeed, when the prelabeled fat body is incubated with hemolymph in the presence of respiratory poisons such as cyanide, azide, or 2,4-dinitrophenol, the release of DG is markedly inhibited while FFA is released at an accelerated rate (**Table 2**). These results may be explained in the following manner. Because glyceride synthesis from FFA is known to require ATP, the addition of inhibitors of cellular respiration to the prelabeled fat body may prevent further glyceride synthesis in the tissue during incubation. This results in an accumulation of FFA in the fat body which in turn accelerates the rate of release of FFA.

It has been suggested by Stevenson (22, 25) that TG is completely hydrolyzed in the moth fat body and that FFA is the form in which lipid is transported from fat body to flight muscle. This is based on experiments that showed that the DG lipase of the flight muscle is not active enough to account for the energy required. However, Stevenson administered the DG to the flight muscle preparation as an emulsion, and we[1] have shown that flight muscle DG lipase activity is much higher when the DG is administered as part of the natural hemolymph lipoprotein complex (see subsequent section.). Further, Stevenson has no reasonable explanation for his corroborating observations that DG is the major hemolymph lipid in the moths that he studied. Recent experiments on the turnover of labeled glycerides in the moth *Spodoptera frugiperda* during both rest and flight indicate that the turnover of the pools of DG during both physiological states is high enough to account for the metabolic rates of the moths (35, 36). The data support the premise that DG is the major transport form of lipids in lepidopterans. Even in moths that feed on nectar during adult life, sugar appears to be converted to lipid that is released from the fat body into the hemolymph as DG (37).

In order to extend our findings on DG release in the silkmoth to other insects, similar experiments were conducted with grasshoppers and cockroaches. As seen in **Table 3,** DG is rapidly released from grasshopper fat body into homologous hemolymph. Indeed, almost 50% of the DG originally present in the tissue is released into the hemolymph. *H. cecropia* hemolymph also markedly stimulates the in vitro release of DG from grasshopper and cockroach fat bodies. Although the data are not shown here, FFA is also released from the fat bodies of these insects.

Prior to our findings on the release of DG from the insect fat body described above, Tietz (38) examined lipid transport in the locust, *Locusta migratoria.* In these pioneering studies she demonstrated that the prelabeled fat body of the locust released glyceride and FFA into the hemolymph and that glyceride release was dependent to a great extent on the presence of hemolymph in the incubation medium. She also observed that the specific radioactivity of the glyceride released into hemolymph was almost 10 times greater than that remaining in the fat body. However, she did not attempt to characterize chemically the nature of the labeled glyceride released. Later Tietz (20) extensively reexamined lipid transport in the locust and essentially confirmed our observations on the specific release of DG from the fat body of *H. cecropia* into hemolymph.

Thus, in representatives of two separate orders of insects (Lepidoptera and Orthoptera), DG and FFA appear to play important roles in lipid transport. This is consistent with the observation that DG is the major glyceride in the hemolymph of these insects whereas TG is present in only minute quantities. (DG is the principal lipid transport form of a crustacean as well [39], and a mixture of DG appears to be the active fraction of an egg-associated pheromone that regulates oviposition in the mosquito *Culex tarsalis* [40]. Of interest is the finding that the fatty acid constituents of the DG were mono- and dihydroxy fatty acids [40].) Because the major glyceride in the fat body is TG (DG is only a minor component), the release of DG from the fat body must depend at least indirectly on the TG pool in the tissue. Tietz (20) has presented data supporting this hypothesis; she observed that the release of DG from the locust fat body occurred concurrent-

TABLE 2. Inhibition of diglyceride release from adult *H. cecropia* fat body by metabolic inhibitors

Expt. No.	Inhibitor	Concentration	^{14}C-labeled DG Released		^{14}C-labeled FFA Released	
			cpm	Relative Value	cpm	Relative Value
		mM				
1.	None		46,200	100		
	KCN	5	10,950	24		
	NaN_3	5	15,220	34		
	2,4-Dinitrophenol	0.2	16,690	37		
2.	None		46,280	100	2,848	100
	KCN	5	15,510	33	7,326	257
	NaN_3	5	19,190	41	4,560	160

Incubation conditions as in Table 1 except that 0.3 ml of pupal hemolymph was used in both experiments (final volume was 1 ml). Inhibitors in the concentration noted were added to the incubation medium prior to addition of the prelabeled adult fat body. Expt. 1: 150 mg of prelabeled fat body containing 153,400 cpm of DG. Expt. 2: 150 mg of prelabeled fat body containing 174,500 cpm of DG and 17,400 cpm of FFA. Modified from Chino and Gilbert (18).

[1] Chino, H., and L. Gilbert. Unpublished observations.

TABLE 3. Nature of glyceride release from prelabeled fat body of adult grasshopper (*Melanoplus differentialis*) and adult cockroach (*Periplaneta americana*)

Expt. No.	Incubation Medium	^{14}C-labeled TG Released	^{14}C-labeled DG Released
		%	%
1. Grasshopper fat body	0.3 ml grasshopper hemolymph	1.2	27.4
	0.3 ml *H. cecropia* pupal hemolymph	1.5	37.4
	1 ml phosphate saline	1.0	1.2
2. Grasshopper fat body	0.4 ml grasshopper hemolymph	0.7	49.5
	1 ml phosphate saline	0.7	1.6
3. Cockroach fat body	0.3 ml cockroach hemolymph	0.6	27.8
	0.5 ml *H. cecropia* pupal hemolymph	0.9	24.8
	1 ml phosphate saline	0.8	1.2

Conditions and notations as in Table 1. Expt. 1: 100 mg of prelabeled grasshopper fat body containing 203,000 cpm of TG and 98,500 cpm of DG. Expt 2.: 50 mg of prelabeled grasshopper fat body containing 55,200 cpm of TG and 141,500 cpm of DG. Expt. 3: 100 mg of prelabeled cockroach fat body containing 95,100 cpm of TG and 48,500 cpm of DG. The grasshoppers used in Expt. 1 were old female adults, and those in Expt 2 were young adults (about 10 days postecdysis). Modified from Chino and Gilbert (18).

ly with the formation of new DG as a result of fat body lipase activity.

Contrary to the above studies on the silkworm and locust, it has been claimed that lipid is released from the cockroach fat body into the hemolymph in the form of TG and FFA (41). This is in obvious disagreement with our finding (18) that DG is released in the same species, although Cook and Eddington (41) found that DG is the major glyceride in the cockroach hemolymph (DG/TG = 10/1). Using essentially the same experimental regimens that we had utilized, they conclude that TG is the major glyceride released from the fat body into hemolymph. They state that during 1 hr of incubation, almost 50% of the TG originally present in the fat body is released into the incubation medium containing hemolymph. If this is correct, the TG in the fat body would have been depleted in about 2 hr. This seems unlikely to occur in vivo because TG is in large supply in the fat body of the cockroach (1, 42). Since the cockroach fat body is very fragile, it is probable that the TG found in the incubation medium is a result of "leaking" from the tissue injured during dissection and incubation. Our original observation that DG is released from the fat body of *Periplaneta americana* has recently been confirmed in experiments in which the incubating fat body was treated with extreme care[2] (see also Ref. 43). Compared with the hemolymph of the silkmoth and the locust, cockroach hemolymph contains an appreciable amount of TG in addition to DG. It is therefore possible that the DG and FFA initially released from the fat body into the hemolymph are resynthesized to TG by hemolymph enzymes. In fact, Cook and Eddington (41) observed that the release of DG exceeds the release of TG during the initial period of incubation. It has also been found that when the adult cockroach is injected with [^{14}C]palmitate, most of the radioactivity in the hemolymph is in DG whereas that associated with TG is very low (ratio of about 10:1).[2] Although the possibility that TG is released from the cockroach fat body has not been completely eliminated, we believe that the release and transport of lipid in this insect is in the form of DG and FFA.

When Martin (44, 45) reported that TG is the major hemolymph lipid of *Pyrrhocoris apterus,* we thought that the hemipterans might be an exception to the rule that DG is the major lipid constituent of insect hemolymph. In *Pyrrhocoris,* however, much of the hemolymph TG may have been contained in lipid-filled cells (adipocytes) and free-floating lipid droplets. In fact, fat body cells may have been released into the hemolymph. Just recently, Thomas (23) has demonstrated that in another hemipteran, *Oncopeltus fasciatus,* whose hemolymph does not contain these lipid inclusions, the principal neutral lipid is DG (48% of the neutral lipid). Further, as in the silkmoths, DG is the major lipid released from the fat body of *Oncopeltus* into the hemolymph, where it then becomes associated with hemolymph lipoproteins. The orders Lepidoptera and Hemiptera are widely separated in evolutionary time, and if both orders utilize DG release as a physiological mechanism, the phenomenon is likely to be typical of a large variety of insects.

A different mechanism of lipid transport has been proposed by Wlodawer and colleagues (46–48) for the waxmoth, *Galleria mellonella.* They have shown that when prelabeled fat body is incubated in vitro, FFA is rapidly released into the hemolymph medium; they suggest that the released FFA is then synthesized into TG in the hemolymph. However, these authors did not examine the actual quantity of lipid released during incubation. Further, in their experiments the specific radioactivity of the DG of the prelabeled fat body was relatively low, and the release of ^{14}C-labeled DG may have been overlooked. Even if their hypothesis is true, it cannot be extrapolated to other insects. The waxmoth is a highly specialized insect in that the larva feeds on beeswax and probably has a unique system for the utilization of long-chain fatty acids and higher alcohols. It would not be surprising if the mechanism of lipid transport in this particular insect differs from that proposed for other species.

[2] Chino, H. Unpublished observations.

FAT BODY–HEMOLYMPH INTERRELATIONSHIPS DURING LIPID RELEASE

To study the relationship between the fat body glycerides and those of the hemolymph, Beenakkers and Gilbert (49) determined the fatty acid composition of the glycerides during the pupal and adult stages of *H. cecropia.* They demonstrated that the hemolymph lipid concentration increased threefold from pupa to the end of adult development and that the DG fraction constituted about 90% of the hemolymph lipid and contained relatively large amounts of palmitate and oleate. Of interest here was the finding that there is a significant difference in fatty acid composition between hemolymph and fat body glycerides. This, together with data from double-labeling experiments, suggests that glyceride release does not occur in a random manner but is restricted to those neutral lipids with a specific fatty acid composition. The alternative explanation of the data is that two different glyceride pools exist in the fat body, only one of which is freely accessible to the hemolymph. The differences in fatty acid composition between the fat body glycerides and those of the hemolymph support the notion of compartmentalization (chemical and/or structural) in the fat body. This supposition would be in agreement with studies of vertebrate adipose tissue in which it is presumed that there is an active compartment in which glycerides are in direct interchange with the fatty acids of the medium and a storage compartment where the bulk of cellular lipids is located (50). Under this view, TG in the storage compartment of the fat body is hydrolyzed and the products are transported to the active compartment where glycerides are resynthesized so that they have a fatty acid pattern appropriate for entry into the hemolymph. Although the data favor such a mechanism for *H. cecropia,* they do not prove it. Recent studies on the locust do support this supposition, however (51).

We still do not fully understand the reason for the high rate of release of DG from fat body to hemolymph, but it may be a consequence of a retarded rate of triglyceride synthesis in the active compartment. The two-compartment theory is consistent with other data from studies discussed previously indicating that DG release is an endergonic process, and it may be that the energy required is needed for glyceride synthesis in the active compartment.

HORMONAL CONTROL OF LIPID MOBILIZATION

When a locust is flown for 2 hr the lipid content of the hemolymph is increased three- to fourfold, and this is reflected in increased hemolymph lipoprotein lipid (21, 52, 53). Supporting the observations discussed previously that DG is the major lipid transport form in some insects was the finding that it was the DG fraction that increased in the hemolymph lipoproteins of the locust during flight. In studies primarily aimed at investigating the hormonal control of carbohydrate metabolism by the corpora cardiaca (neurohemal organs attached to the brain), it was noted that these structures contained a substance that could stimulate lipid utilization by the cockroach fat body (54). That an adipokinetic hormone was released from the corpora cardiaca was demonstrated in the locust because aqueous extracts of corpora cardiaca injected into the locust caused time-dependent elevations in hemolymph lipid (predominantly DG) similar to the change occurring during flight (55). Preliminary studies (effects of heat, proteases, etc.) suggested that the active factor is a peptide and its site of action is the fat body, where it presumably causes the formation and release of DG into the hemolymph during flight when the metabolic demands of the insect increase dramatically. Recent experiments suggest that the adipokinetic hormone is synthesized in the corpora cardiaca rather than originating in the brain and only being stored in the corpora cardiaca (56). Although the mechanism of action of the corpora cardiaca factor is not yet known, it may act via cyclic AMP (57).

In contradistinction to the proposed adipokinetic hormone (hyperlipemic factor) in the locust, recent studies by Downer and Steele (43, 58, 59) on the cockroach suggest the occurrence of a hypolipemic factor. Injection of aqueous extracts of the corpus cardiacum–corpus allatum complex (CC–CA) appeared to result in a general decrease in the neutral lipids of the hemolymph and an increase in the fat body, and the data suggest a stimulation of the uptake of lipid from hemolymph into the fat body. The major changes were in TG and DG, and the authors postulate that DG is as important as a vehicle for lipid uptake into the fat body as it is for lipid release from the fat body. Although CC–CA complexes were utilized, dilution experiments suggest that the corpus cardiacum is the site of storage of this hypolipemic factor. As for the physiological significance of the new hormone, it may be important after feeding, when the substrate concentration of the hemolymph increases and must be shunted into the fat body for storage. There is no evidence to suggest that the hypolipemic factor activates a hemolymph lipase that may hydrolyze the hemolymph glycerides resulting in the uptake of free fatty acids by the fat body. Indeed, the data almost rule out this possibility.

How can one reconcile the existence of a hyperlipemic hormone in the locust and a hypolipemic hormone in the cockroach, both originating from the corpus cardiacum? Are the differences due to variations in experimental regimens of the investigators, to the occurrence of different

factors in the corpus cardiacum, or to different responses between the two insect species? Downer (60) showed that injection of cockroach corpus cardiacum extracts elicited a hyperlipemic response in the locust and, conversely, that injection of locust corpus cardiacum extracts into the cockroach resulted in a hypolipemic response. It appears, therefore, that the same or similar substances are released from both the cockroach and locust corpora cardiaca but that the insects respond differently. These preliminary observations suggest that the chemical nature of the hormone did not change during evolution but that the site of action or response did change as the insects' major metabolic pathways took different routes. The cockroach is primarily a utilizer of carbohydrate for locomotion (54) whereas the locust can utilize lipid almost exclusively as a fuel for flight (52). It is reasonable to assume that fine regulatory controls for the two catabolic processes may have evolved utilizing the same regulatory hormone.

It was of interest to determine whether any vertebrate hormones with known adipokinetic properties were effective in stimulating the release of lipid from the insect fat body. A series of experiments was conducted with fat bodies from pupal and adult *H. cecropia* and from the cockroach *P. americana* that had been prelabeled with [^{14}C]palmitate (57). The results indicated that the hormones utilized fall into four groups: (*1*) ACTH had no effect on the insect system; (*2*) insulin had a slight effect in inhibiting the release of DG and FFA; (*3*) thyroxine, growth hormone, and gonadotropin clearly stimulated the release of DG and FFA; and (*4*) epinephrine had a dual effect of suppressing the release of DG and stimulating the release of FFA. These data are among the first to demonstrate a specific metabolic effect of a vertebrate hormone on an insect, analogous to the effect it exerts in the vertebrate (adipose tissue) system. The only aspect of this work with possible physiological significance for the insect is that with epinephrine, because there are several reports in the literature suggesting the presence of epinephrine and/or norepinephrine in insects (57). In the case of the vertebrate adipose tissue, epinephrine stimulates fatty acid release presumably via lipase activation, and the same event may take place in the cockroach fat body. Since cyclic AMP also stimulates the release of FFA from the cockroach fat body, the epinephrine effect may manifest itself through cyclic AMP.

Several studies have suggested that juvenile hormone (product of the corpus allatum) may also affect lipid metabolism by inhibiting lipid synthesis in the fat body and possibly by stimulating the incorporation of hemolymph lipids into the developing ovary (42). Experiments utilizing [^{14}C]acetate suggested that juvenile hormone stimulates turnover in the cockroach (61), but it was without effect in the locust (62–64). Data on the changes in lipid content during the reproductive cycle of the cockroach *Leucophaea maderae* in both fat body and ovaries revealed that the amount of lipid in the ovaries increases during oogenesis as the quantity of fat body lipid decreases (42). This occurs at the same time that the corpus allatum is secreting juvenile hormone at a maximal rate; the juvenile hormone at this stage acts as a gonadotropic hormone (i.e., oogenesis does not go to completion in the absence of juvenile hormone). In vitro studies with labeled precursors in the presence or absence of active corpora allata suggest that the juvenile hormone stimulates the incorporation of palmitate into ovary glycerides and phospholipids and may inhibit incorporation into the fat body. If indeed the hormone did act as postulated above, more substrate would have been made available for yolk deposition in the developing oocyte. Whether the juvenile hormone has an adipokinetic effect on the fat body of this insect has not yet been investigated. In the locust, it appears that the fat body of allatectomized animals has a greater capacity for lipid synthesis but that this capacity is strongly affected by feeding activity (65).

ROLE OF HEMOLYMPH LIPOPROTEINS IN DIGLYCERIDE TRANSPORT

In our early experiments (18) with *H. cecropia* hemolymph as a medium for the prelabeled fat body, we observed that the DG released from the fat body into the hemolymph became associated with a specific hemolymph lipoprotein as judged by paper electrophoresis. This observation together with other relevant data led us to postulate that DG in the cells of the fat body may be specifically taken up by a hemolymph lipoprotein and then carried to the site of utilization. Indeed, we observed that boiled hemolymph lost the capacity for stimulating DG release from fat body but dialyzed hemolymph retained sufficient activity to promote DG release. This observation strongly supported our premise that specific hemolymph lipoproteins were important in the release and transport phenomena.

Since that time we have independently purified and analyzed these hemolymph lipoproteins from several species of silkmoths (66–69). As will be seen subsequently, there are two major classes of lipoproteins in the hemolymph of these insects. On the basis of function, Chino and his colleagues (66, 67) have termed them "diglyceride-carrying lipoproteins I and II," while Thomas and Gilbert (68, 69) used the terms high density lipoprotein (HDL) and very high density lipoprotein (VHDL) because of their density as judged in the ultracentrifuge and because these designations have been used by individuals working with mammalian lipoproteins (70, 71). Diglyceride-carrying lipoprotein I is identical with the insect HDL, and diglyceride-carrying lipoprotein II is the same as the VHDL. (There may also be a low density lipoprotein in at least

TABLE 4. Purification scheme for hemolymph lipoproteins I and II

Step	Fraction	Protein	Total ^{14}C-labeled DG Recovered	Specific Activity of ^{14}C-labeled DG	Recovery of LP-I
		mg	*cpm*	*cpm/mg protein*	%
1	Original prelabeled hemolymph	168.0	157,950	940	100
2	70% ammonium sulfate precipitate	125.5	143,910	1,150	91.3
3	Supernate from centrifugation after dialysis of precipitate from step 2	124.0	137,115	1,110	86.7
4	Precipitate resulting from bringing supernate in step 3 to 0.002 M phosphate, pH 6.5 (mixture of LP-I and LP-II)	12.7	60,330	4,750	38.2
5	Fraction not adsorbed to DEAE-cellulose column (purified LP-I)	3.4	45,480	13,780	28.8
5′	Fraction eluted from column with 0.15 M KCl in 0.04 M phosphate buffer (purified LP-II)	7.5	1,970	263	

Starting material was 5 ml of pupal hemolymph from *P. cynthia* prelabeled with [^{14}C]palmitate. Modified from Chino, Murakami, and Harashima (67).

one species.) Because the insect hemolymph lipoproteins do more than carry diglyceride and may not be exactly analogous to the mammalian blood lipoproteins, we will designate the two major classes as lipoprotein I (LP-I) and lipoprotein II (LP-II) in this review. It is LP-I that accepts the labeled DG from the fat body, and, although LP-II has DG associated with it, it does not accept ^{14}C-labeled DG from the fat body.

In their studies on hemolymph lipoprotein purification, Chino et al. (66, 67) used pupae of the silkworm *Philosamia cynthia* as experimental animals because this species was shown to be essentially the same as *H. cecropia* insofar as lipid release is concerned. Prelabeled hemolymph was used as starting material for lipoprotein purification and was prepared in the following manner. Pupae were injected with [^{14}C]palmitate and the hemolymph was collected. This hemolymph contained lipoprotein having ^{14}C-labeled DG because, as discussed previously, the injected [^{14}C]palmitate is first incorporated into the DG and TG of the fat body and the DG is then rapidly released into the hemolymph, where it becomes firmly associated with a specific lipoprotein. Throughout the purification procedure, the radioactivity of the ^{14}C-labeled DG was taken as a measure of lipoprotein purity.

The method developed for purifying these lipoproteins (67) involves fractionation by ammonium sulfate, precipitation at low ionic strength (pH 6.5), and DEAE-cellulose column chromatography (**Table 4**). The specific precipitation at low ionic strength is a critical and most efficient step (see step 4, Table 4) in that both LP-I and LP-II are precipitated together, resulting in a mixture of LP-I and LP-II essentially free from other contaminating hemolymph proteins. (There are more than 20 electrophoretically separable proteins in the hemolymph of this insect [72].) The separation of the two lipoproteins from one another by DEAE-cellulose column chromatography revealed that purified LP-I is heavily labeled with ^{14}C-labeled DG, whereas only a trace amount of this labeled lipid is associated with LP-II.

Purified LP-I and LP-II are both essentially homogeneous, as judged by ultracentrifugation, electrophoresis, and electron microscopy. **Fig. 2** reveals the morphology of purified LP-I and LP-II under the electron microscope, and it is evident that both LP-I and LP-II are globular. Furthermore, their granular appearance seen after negative staining suggests the presence of a substructure. From the electron micrographs it was calculated that the diameters of the molecules are 13.5 ± 0.6 nm for LP-I and 10.0 ± 0.5 nm for LP-II. The molecular weights as determined by sedimentation equilibrium are approximately 700,000 (LP-I) and 500,000 (LP-II). Utilizing these molecular weights and the partial specific volumes (0.87 for LP-I and 0.75 for LP-II, assuming a spherical shape), calculations showed that the molecular size of the lipoprotein is proportional to the diameter of the molecule.

The chemical compositions of LP-I and LP-II are given in **Table 5.** Lipid constitutes 44% of the weight of LP-I and approximately 10% of LP-II, and DG is the major (and possibly only) glyceride present in both lipoproteins. In this connection, it should be pointed out that there may always be some turnover of LP-I DG (i.e., loading of DG from the fat body and unloading at sites of utilization), so that the DG content and molecular weight of LP-I probably depend on the physiological state of the fat body. A significant amount of free cholesterol is present in both lipoproteins, but neither cholesteryl ester nor FFA was detected. The phospholipid content is relatively high in both lipoproteins, and phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin are the major components. The yellow color of the major hemolymph lipoproteins of these saturniids is apparently due to the presence of 3-hydroxy-3′-keto-α-carotene. In addition, the hemolymph yields all-*trans*-β-carotene, neo-β-carotene, all-*trans*-lutein, and neolutein (73). The yellow color of LP-I and

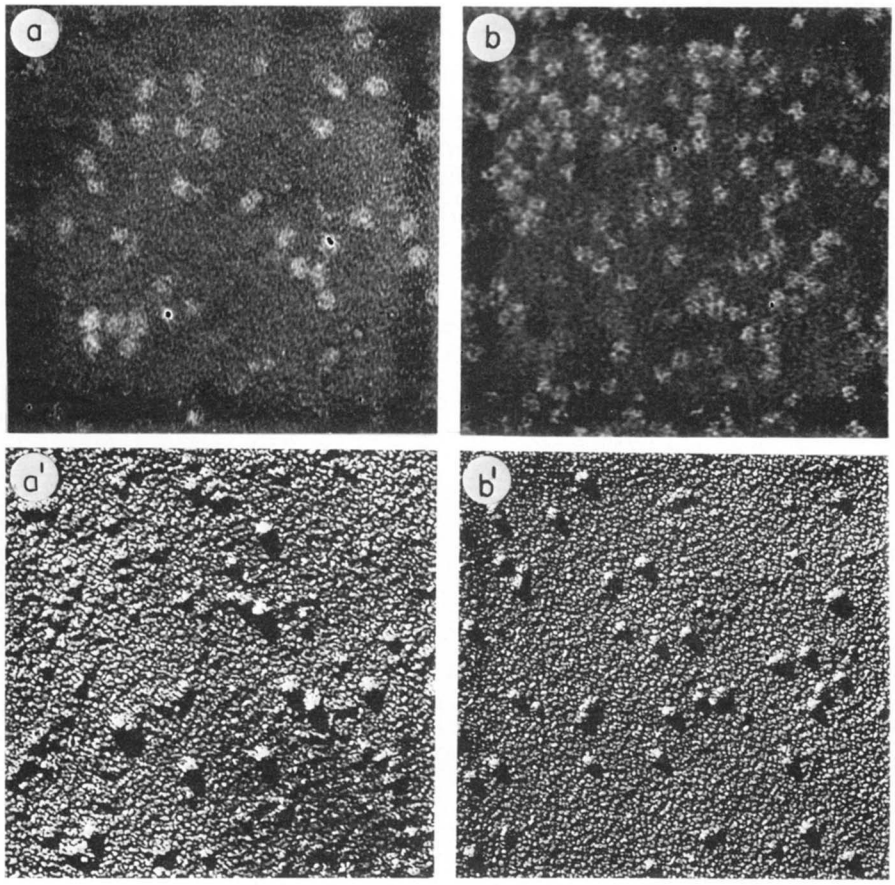


Fig. 2. Electron micrographs of LP-I and LP-II. *a*, LP-I (negatively stained; × 250,000); *a'*, LP-I (shadowed; × 125,000); *b*, LP-II (negatively stained; × 250,000); *b'*, LP-II (shadowed; × 125,000). From Chino et al. (67).

LP-II is of invaluable aid in following the lipoproteins during column chromatography. The amino acid analyses of LP-I and LP-II demonstrate the presence of more lysine in LP-I and more glutamic acid in LP-II (**Table 6**). LP-II is therefore more acidic than LP-I. This finding agrees with the observation that LP-II migrated at a greater rate than LP-I during gel electrophoresis.

The data suggest that LP-I has the capacity to take up DG from the fat body. Indeed, the experimental results clearly reveal that LP-I can efficiently take up ^{14}C-labeled DG from prelabeled fat body whereas LP-II cannot (**Table 7**). It was also observed that the uptake of DG from the fat body by LP-I was markedly inhibited by respiratory inhibitors such as cyanide, as was observed in the inhibition of DG release from the fat body into the hemolymph. Since no significant binding of ^{14}C-labeled DG with LP-I occurred when emulsified ^{14}C-labeled diolein was simply incubated with purified LP-I, the uptake of DG by LP-I from the fat body seems to require a specific interaction between the tissue cells and LP-I. LP-I therefore appears to have a regulatory role (perhaps by carrying a hormone; see subsequent section on hormone transport).

LP-I thus appears to play a vital role in lipid transport. LP-II, on the other hand, can accept lipids from LP-I but its physiological role is not well understood. In many insects, a protein presumably synthesized in the fat body is later incorporated into maturing oocytes and is requisite for completion of normal oogenesis. In the cockroach, this "vitellogenin" appears to be a lipoglycoprotein (74).

Thomas and Gilbert (69) found that LP-II (VHDL) was in high concentration in the hemolymph of *H. gloveri* female pupae but just barely detectable in male pupal hemolymph. During ovarian maturation the concentration of hemolymph LP-II decreases, and in the newly emerged female adult its concentration is about 20% that of the pupa. If the ovaries are removed from the pupa and the animal allowed to develop, the concentration of LP-II in hemolymph in the almost fully developed adult is even greater than it was in the pupa. These findings suggest that LP-II is a vitellogenin and moves from the hemolymph to the maturing ovary during development. Further studies revealed that LP-II contains a carbohydrate moiety, and therefore it too is a lipoglycoprotein. LP-II thus appears to be well suited for the transport of protein, carbohydrate, and lipid to the maturing oocytes as prospec-

TABLE 5. Chemical analysis of hemolymph lipoproteins from *P. cynthia* pupae

Component	LP-I Amount	LP-I % Weight[a]	LP-II Amount	LP-II % Weight[a]
	mg		*mg*	
Protein	31.08	56.0	58.80	90.3
Total lipids	25.03	44.0	6.32	9.7
1. Triglyceride	<0.30	<1.2	ND	
2. Diglyceride	14.10	56.3	2.18	34.5
3. Monoglyceride	ND		ND	
4. Cholesterol	3.30	13.2	0.80	12.7
5. Cholesteryl ester	ND		ND	
6. Phospholipid	6.46	25.8	3.13	49.5
7. Sum of 1–6	24.16	96.6	6.11	96.7

Starting material was LP-I and LP-II purified as in Table 4. Modified from Chino et al. (67).

[a] Lipid fractions (1–7) are expressed as percentages of total lipid. ND, not detectable.

TABLE 6. Amino acid composition of *P. cynthia* hemolymph lipoproteins

Amino Acid	LP-I	LP-II
	mmoles/mole amino acids recovered	
Asp	126	118
Thr	49	59
Ser	69	78
Glu	104	149
Pro	47	50
Gly	67	48
Ala	63	75
Val	74	66
Met	5	4
Ile	58	48
Leu	90	66
Tyr	28	44
Phe	48	33
His	28	32
Lys	107	90
Arg	37	40

From Chino et al. (67).

tive components of the yolk. When LP-II is extracted from the hemolymph and yolk and the mixture is analyzed by analytical centrifugation, a single peak is discerned ($S_{20,w} = 16$), suggesting further that the hemolymph and yolk LP-II may be identical. LP-I is in low concentration in the yolk and changes only slightly in concentration in the hemolymph during oogenesis in contrast to LP-II, which is selectively accumulated by the oocyte. Studies with labeled fatty acids and analysis of the lipid components of the yolk and hemolymph lipoproteins lend further credence to the postulate that, in *H. gloveri* at least, LP-II may have the role of providing substrate to the developing egg, where it is used as both an energy source and structural material during subsequent embryogenesis.

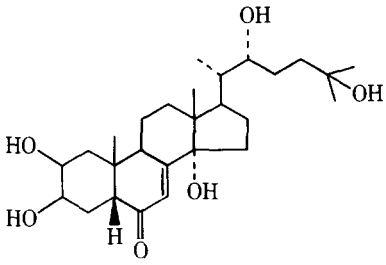
The isolation and characterization of hemolymph lipoproteins from the pupae of *H. cecropia* and *H. gloveri* have been achieved by Thomas and Gilbert (68, 69), who employed density gradient ultracentrifugation techniques that had been developed to isolate mammalian plasma lipoproteins. Although there is general agreement on the characteristics of LP-I and LP-II in our two laboratories, there were some differences. The data of Thomas and Gilbert (68) suggest a lower molecular weight for LP-I and show that LP-I contains 48% lipid and LP-II contains 6% lipid. DG constitute about 70% of the total lipid in LP-I and 45% in LP-II. In addition, *Hyalophora* hemolymph lipoproteins contain significant quantities of sterol ester (although much less than the amount of free sterol), as well as hydrocarbons, which are a known constituent of insect hemolymph (1), FFA, and phosphatidylserine. The other lipids noted earlier for *Philosamia* were also present in addition to those stated above. It should also be noted that, although there are only two major lipoprotein classes, when one separates hemolymph by gel electrophoresis, as many as eight proteins stain with lipid crimson, suggesting the presence of numerous lipoprotein species (72). These patterns appear to change during development, and each lipoprotein may in fact be composed of several protein components, some of which may possess enzymic activity (72).

Very recently, Peled and Tietz (75, 76), utilizing sucrose gradient centrifugation, have attempted to isolate the lipoprotein responsible for DG transport from the hemolymph of the locust. Hemolymph was treated with cold ethanol (−20°C) to precipitate the protein fraction containing the lipoprotein. The isolated lipoprotein was yellow because of the presence of carotenoid pigments (mainly β-carotene), as we had observed for the silkmoth LP-I and LP-II, and the major glyceride present was DG. The estimated molecular weight was 350,000 and its chemical composition (including amino acid analysis) was similar to that of silkmoth LP-I. Although the isolated yellow lipo-

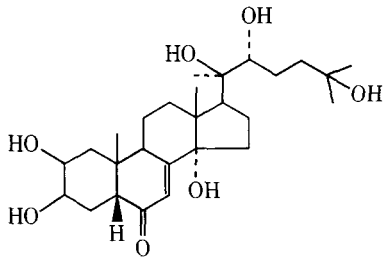
TABLE 7. Uptake of fat body diglyceride by LP-I and LP-II in vitro

Incubation Medium	Protein Used for Assay	^{14}C-labeled DG Taken Up	Specific Uptake
	mg	*cpm*	*cpm/mg protein*
0.08 ml hemolymph	3.16	9,660	3,060
Mixture of LP-I and LP-II (from step 4, Table 4)	0.44	5,930	13,500
Purified LP-I	0.44	11,310	25,700
Purified LP-II	0.44	570	1,295

120 mg of prelabeled fat body from adult *H. cecropia*, containing 61,530 cpm of ^{14}C-labeled DG, was incubated at 28°C for 60 min. All tubes were brought to 1 ml final volume by adding phosphate saline. Modified from Chino et al. (67).



α-Ecdysone



β-Ecdysone

Fig. 3. Structures of α- and β-ecdysone. See Refs. 3–6 for details.

protein could promote DG release from the locust fat body, the specific activity was lower than that of the original hemolymph. It was further shown that full activity was restored by the addition of a non-lipid-associated hemolymph protein that by itself showed little activity. Contrary to these observations, the capacity of purified *P. cynthia* LP-I to promote DG release was eight times higher than that of the original hemolymph (see Table 7). It is possible that the treatment of the hemolymph with cold ethanol as the first step in the isolation procedure could have partially denatured the protein moiety of the lipoprotein and resulted in some loss of ability to stimulate the release of DG.

The literature on mammalian plasma lipoproteins is voluminous; it has been reviewed often (70, 71) and will not be discussed in detail here. From ultracentrifugation analysis it is apparent that the insect LP-I and LP-II are comparable to the mammalian high density and very high density lipoproteins, respectively, insofar as density is concerned (68). One of the major differences is that the insect hemolymph lipoproteins are rich in DG, whereas TG is the primary glyceride of the mammalian plasma lipoproteins. Perhaps the major difference concerns the physiological role of the lipoproteins in the two circulatory systems.

Although it has been suggested in the past that mammalian lipoproteins (α- or β-lipoproteins) can stimulate the release of TG from the liver and that apoprotein in the blood can combine with lipid in the liver to form lipoprotein, it is more generally accepted that both the lipid and protein moieties of the mammalian lipoproteins are synthesized within the cells of the liver and released as lipoprotein (71). In contrast, the insect hemolymph lipoprotein (e.g., LP-I) efficiently takes up lipid (DG) from the fat body, a process that seems to take place at the surface of the fat body cells. In other words, in mammals "vehicle" (protein) and "load" (TG) are synthesized together within the cell, whereas in insects the "vehicle" in the hemolymph loads DG at the cell surface and acts as a shuttle system. This idea has recently been supported by studies showing that, although insect hemolymph lipoproteins are synthesized by the fat body, the process of DG release from the fat body is not dependent on lipoprotein synthesis (75, 77). In light of the above, we propose that insect hemolymph lipoprotein (e.g., LP-I) is synthesized predominantly in the larval fat body and is released into the hemolymph. It there functions both as a carrier of lipid during the insect's life (with little turnover) and as a regulatory agent in eliciting the release of lipid (DG) from the fat body into the hemolymph when needed. LP-I may be the first example of a lipoprotein isolated in a pure form that functions in this manner. It is in reality a "lipid-carrying protein." The intricate mechanisms involved in "loading" and "unloading" of this lipoprotein are still a matter of conjecture.

STEROL TRANSPORT

Insects lack the ability to synthesize sterols from acetate and thus require them in the diet if normal development and reproduction are to proceed (1, 3, 78). Once the insect has ingested the required sterol (predominantly cholesterol in carnivorous insects and β-sitosterol in phytophagous species), it does have the requisite enzymes for sterol esterification, reduction of double bonds, a variety of oxidative transformations, hydroxylation, conjugation, dealkylation, and side-chain cleavage (3). In addition to utilization of sterols as constituents of cellular and subcellular membranes, insects utilize a sterol as their molting hormone and, in fact, appear to have made use of steroid hormones long before mammals evolved.

Cholesterol (or β-sitosterol) is the obvious primary substrate for molting hormone biosynthesis, and β-ecdysone is the true insect molting hormone (**Fig. 3**). Because insects must periodically shed their exoskeletons in order to grow, the molting hormone that initiates this process is a crucial regulatory agent. The prothoracic glands of the insect synthesize and secrete α-ecdysone (79, 80), which can be considered a prohormone, and it is converted to β-ecdysone by a number of other tissues, including the fat body (5). Although α-ecdysone ([20*S*]-2β,3β,14α,22*R*,25-pentahydroxy-5β-cholest-7-en-6-one) may have unique functions of its own, we will consider it to be primarily a precursor for β-ecdysone. In the field of transport, both α- and β-ecdysone should be considered because α-ecdysone

must make its way from the prothoracic glands to other tissues for conversion, and β-ecdysone must be transported from these conversion sites to organs and tissues that are controlled by the molting hormone but cannot hydroxylate α- to β-ecdysone (e.g., imaginal discs).

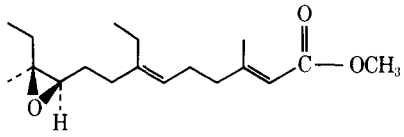
In 1970, three papers (81, 82, 85) appeared dealing with the possible transport of α-ecdysone (no labeled β-ecdysone was then available) in the hemolymph. When [^{3}H]ecdysone was injected into the bug *Pyrrhocoris apterus,* some of the recovered hemolymph proteins were labeled, and this label was attributed to the α-ecdysone (81). Preliminary examination suggested that the ecdysone was reversibly bound, that there were several binding sites, and that several association constants existed for these protein–ecdysone complexes. Similar studies and results were obtained with *Drosophila hydei,* in which the [^{3}H]ecdysone was found to be reversibly bound to three hemolymph proteins (82; see also 83). If indeed ecdysone is transported in the hemolymph as a protein conjugate, it may be for the purpose of protection from degradative enzymes or to aid in its transit out of and into tissues. The large number of hydroxyl groups distributed in the molecule (Fig. 3) renders it appreciably soluble in water (α-ecdysone ~ 1 mg/ml; β-ecdysone ~ 5 mg/ml), and there is no obvious necessity for a carrier protein for solubility purposes. Even in the case of Emmerich's (81, 82) studies, the author states that the binding is weak and susceptible to weak salt solutions.

In the case of silkmoths, there is no evidence that either α-ecdysone or β-ecdysone binds to specific macromolecules in the hemolymph (84). Although ecdysone is relatively water-soluble, it is a sterol. Since sterols are carried as components of lipoproteins in many organisms, we investigated the possibility that the silkmoth hemolymph lipoproteins may be involved in ecdysone transport (85). Pupae were injected with α-[^{3}H]ecdysone, and larvae were fed an artificial diet containing the labeled hormone. Hemolymph was removed and significant quantities of α-ecdysone were detected. However, when the pupal and larval hemolymph lipoproteins were extracted and purified, no significant binding of ecdysone to either lipoprotein class was noted. Gel filtration analysis showed that none of the other hemolymph proteins exhibited significant radioactivity. However, it should be noted that during the fractionation of the lipoproteins most of the fed or injected [^{3}H]ecdysone was found to be associated with the protein fraction that precipitated with 70% ammonium sulfate. This observation leaves open the possibility of relatively weak (unspecific?) binding between one or more hemolymph proteins and ecdysone, and it is possible that these bonds are broken during the fractionation procedures (e.g., gel filtration) by the relatively high salt concentrations used. Thus, although the data support the premise that ecdysone is transported as "free" hormone, the possibility that it exists in a "bound" form has not been ruled out. However, the solubility of the ecdysones in aqueous media and the fact that injected hormone rapidly finds its way to all tissues of the insect (84) lead us to believe that specific hemolymph binding proteins are not involved.

Although the problem of ecdysone transport has not yet been elucidated, more is known about cholesterol transport. Cholesterol must find its way from the digestive tract of the insect to all cells engaged in mitotic activity, as it is an important membrane constituent, and to the prothoracic glands, where it serves as a substrate for ecdysone biosynthesis. Chino and Gilbert (86) demonstrated that ingested cholesterol is absorbed from the gut wall into the hemolymph of silkmoth larvae (*Philosamia cynthia*), where it is transported as a constituent of the hemolymph lipoproteins. Indeed, more than 95% of the [^{14}C]cholesterol released from the gut into the hemolymph is associated with hemolymph LP-I and LP-II.

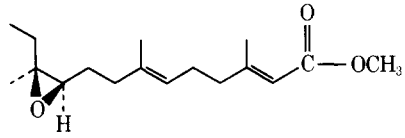
Using isolated midguts from larvae fed on [^{14}C]cholesterol, we were able to obtain preliminary information on the role of the hemolymph lipoproteins in eliciting the release of cholesterol. When the isolated midguts containing [^{14}C]cholesterol were incubated in an isotonic saline solution and the medium was analyzed, no ^{14}C was noted in the medium. However, the addition of a small amount of hemolymph to the incubation medium stimulated the release of significant quantities of cholesterol, and it was quickly bound to the hemolymph lipoproteins. When similar experiments were conducted with either LP-I or LP-II in the medium, it was demonstrated that LP-I had a highly stimulatory effect on the release of [^{14}C]cholesterol from the isolated midgut preparation but LP-II had at most a slight effect. This is consistent with our previous studies, which showed that LP-I had more endogenous cholesterol than LP-II (67, 68). However, because LP-II has some cholesterol associated with it but does not take up cholesterol directly from the medium, cholesterol transfer between the two lipoprotein classes was a strong possibility. By incubating LP-I containing labeled cholesterol with cold LP-II, we were able to demonstrate that this was the case and that, although LP-II was incapable of binding "free" cholesterol, it could accept the sterol from the other major hemolymph lipoprotein class. This is similar to the situation in humans, where the VLDL has the capacity to take up cholesteryl esters from other serum lipoproteins in vitro (87), and supports the premise that cholesterol is at the surface or close to the surface of the lipoproteins (88).

Although we have demonstrated that LP-I "stimulates" the midgut to release cholesterol, which then binds to the lipoprotein, the mechanism has not yet been investigated. Since cholesterol is not taken up by the lipoproteins in the absence of the midgut, one can assume some special physi-



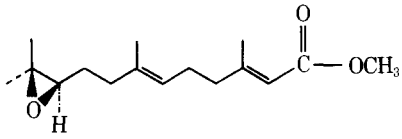
JH I, or C_{18} JH

(source is the silkmoth *Hyalophora cecropia*)



JH II, or C_{17} JH

(sources are the silkmoth *Hyalophora cecropia* and the tobacco hornworm moth *Manduca sexta*)



JH III, or C_{16} JH

(sources are the tobacco hornworm moth *Manduca sexta* and the grasshopper *Schistocerca vega*)

Fig. 4. Structures of the juvenile hormones. See Refs. 4–6 for details.

cal and/or chemical interactions between the lipoproteins of the hemolymph and the surface of the midgut. Because insects are dependent on an exogenous source of sterol in their diet, interference with the mechanisms by which lipoproteins load sterols at the gut surface may be a reasonable approach to pest control.

JUVENILE HORMONE TRANSPORT

The juvenile hormone (JH) is a unique molecule in terms of both its presumed action and its structure (5). It is responsible for maintaining larval characteristics in the immature insect and thereby prevents precocious maturation. It is the only hormone described to date possessing an epoxide function (**Fig. 4**). Although the initial chemical studies elucidating the structure of the C_{18} JH were conducted on extracts of whole animals, recent work by Dr. K. Judy and his colleagues (5) utilizing corpora allata in organ culture have definitively shown that these glands are the source of the juvenile hormones so far characterized.

A number of studies have revealed that methionine is the source of the ester methyl carbon of JH (5), and just recently it was demonstrated that the 12,000 *g* supernatant fraction of *Manduca sexta* corpus allatum homogenates catalyzes the formation of labeled JH from [*methyl*-^{3}H]-*S*-adenosyl-L-methionine, suggesting the presence of an enzymic alkylating mechanism in the corpus allatum (89). The major portion of the JH molecule appears to be derived from terpenoid precursors. Utilizing *M. sexta* corpora allata in vitro, high resolution liquid chromatography, and microchemistry, Schooley and his colleagues (90) demonstrated the efficient incorporation of acetate, mevalonate, and propionate into JH II. Labeled JH III resulted from incubation of corpora allata in the presence of labeled acetate and mevalonate but not propionate. Their data suggest that the isoprenoid skeletal units of JH arise from mevalonate and homoisoprenoid units derived from one propionate and two acetates (possibly through homomevalonate). JH III appears to be derived from 3 moles of mevalonate (9 moles of acetate), and the biosynthetic route is probably similar to the accepted terpenoid pathway. JH II, on the other hand, arises from 2 moles of mevalonate plus 2 moles of acetate and 1 of propionate. In their view (90), the two acetates and one propionate must be responsible for the homoisoprenoid unit.

Although the above studies are of interest to the lipid biochemist, we are primarily concerned here with the transport of this presumably lipoidal hormone. The first experiments on this subject by Whitmore and Gilbert (91) were based on the assumption that the hormone would be transported as a component of one or more hemolymph lipoproteins or would at least be bound to some hemolymph macromolecule.

When ^{14}C-labeled JH I (C_{18} JH) was injected into silkmoth pupae (*H. cecropia, H. gloveri, Antheraea polyphemus*) or incubated with hemolymph in vitro and the hemolymph was chromatographed 4–12 hr later on Sephadex G-100, a large peak of labeled protein was eluted. Subsequent analysis revealed that more than 95% of the label was attributable to the JH. Gel electrophoretic analysis of the *H. gloveri* pupal hemolymph revealed the presence of 25 protein bands, 6 of which were classified as lipoproteins on the basis of staining with lipid crimson. When hemolymph was incubated in vitro with the ^{14}C-labeled JH, all of the lipoproteins were radioactive, but when the labeled hormone was injected into the insect, all the recoverable label from the hemolymph proteins due to JH was associated with one HDL (LP-I). It could be argued that the above observation was a result of overloading the insect with JH or due to the method of application and that another constituent of the hemolymph is the true carrier. However, Whitmore and Gilbert (91) extracted the HDL and other hemolymph proteins from *H. gloveri* that had not been treated in any way and bioassayed the extracts for JH activity. Only the HDL extract from a developmental stage known to have a high endogenous titer of JH (adult male) yielded positive results in the three bioassays utilized. The other proteins gave negative results, as did the lipoprotein from a stage known to be devoid of endogenous JH (pupa). This strongly suggests that a specific hemolymph lipoprotein is the natural vehicle for the transport of JH from the corpora allata to the target tissue. Preliminary calculations showed that there is enough of this lipoprotein in the hemolymph to carry sev-

eral hundred micrograms of JH, although only a few micrograms can be extracted from the insect.

We have discussed the fact that the hemolymph lipoproteins carry several lipids, predominantly DG, as well as cholesterol. The above data reveal that, at least in the case of silkmoths, the hemolymph lipoproteins are also involved in the transport of JH. We as yet do not know how specific the lipoprotein is for JH; i.e., will JH analogs or mimetic substances also bind? Perhaps the configuration and lipoidal nature of the lipoprotein simply allows the JH to partition out of the aqueous portion of the hemolymph into the more hospitable environment of the lipoprotein. The fact that several other hemolymph lipoproteins do not appear to carry JH under natural conditions suggests some degree of specificity. It should be noted that the HDL may have endocrinological functions apart from carrying the JH. For example, it may act at the surface of the corpora allata to stimulate the entrance of JH into the hemolymph as it appears to do in the case of the entrance of cholesterol from the gut into the hemolymph and the release of DG from the fat body. Alternatively, the JH–lipoprotein complex may be important as a protective device for the hormone against degradative enzymes known to exist in the hemolymph (5).

The studies on the silkmoth have been confirmed by similar experiments on adult female locusts (92). Here also, by utilization of gel chromatography, electrophoresis, and isoelectric focusing, one specific lipoprotein (lipoprotein VI; there are six hemolymph lipoproteins in *Locusta migratoria*) appears to be the carrier of injected ^{3}H-labeled JH I. In contradistinction to the reports concerning the silkmoths and the locust, recent studies (see below) suggest that lipoproteins may not function as JH carriers in some insects and, indeed, that the JH is not as water-insoluble as originally believed. These observations point out once again the futility of attempting to extrapolate data obtained from one species to the more than one million diverse species of insects.

On the basis of Whitmore and Gilbert's observations (91) that when hemolymph containing JH is subjected to Sephadex chromatography free JH was eluted near the inclusion volume, Kramer et al. (93) postulated that much of the unbound JH forms a true solution and that most of this unbound JH in the hemolymph exists as monomers. Indeed, using ^{3}H-labeled JH I they demonstrated that aqueous solutions in concentrations as great as 5×10^{-5} M could be prepared. When C_{18} JH was injected into tobacco hornworm (*Manduca sexta*) larvae, Kramer and his colleagues (93) were able to detect binding to a hemolymph protein by the use of Sephadex chromatography and electrophoresis. In contrast to the studies on the silkmoths and locust, the primary binding protein in larval *M. sexta* appears to be relatively small (mol wt 34,000), with a dissociation constant of 2.99×10^{-7} M and a concentration in the hemolymph of 7.7×10^{-6} M when treated as a simple thermodynamic equilibrium. Independently, Goodman and Gilbert[3] have also studied JH transport in *M. sexta* and have confirmed the results of Kramer and his colleagues (93). The physiological role of this relatively small binding protein is presently being investigated.

CONCLUSIONS AND PROSPECTS

Although studies on lipid transport (serum lipoproteins) in mammals appear to have entered the "exponential phase" of growth some time ago, research on insect lipid metabolism in general, and on lipoproteins in particular, remain in the "latent phase." The observations presented in this review are largely the product of only a few laboratories, and a great deal remains to be accomplished. For example, the exact reason for the insect's lack of ability to synthesize sterols from acetate remains enigmatic. Essentially nothing is known about the protein chemistry of the apoproteins constituting the hemolymph lipoproteins, and even the data presented here on the lipid constitution of the lipoproteins are somewhat conflicting. The mechanisms by which a lipoprotein elicits DG release from the cells of the fat body and sterol from the cells of the gut are completely unknown, and these unique phenomena are certainly worthy of further study by the lipid biochemist.

Insects are well suited to their ecological niches due no doubt to physiological accommodations to evolutionary forces over millions of years. The advantages of utilizing DG rather than TG as the major lipid constituent of their hemolymph remain conjectural. Since juvenile hormone mimetic substances are soon to be utilized for insect control, it is important that we learn more about their mechanism of action. It is reasonable to assume that if binding to hemolymph proteins is requisite for the action of the hormone, investigations concerning the mechanism of binding may allow us to develop means of interfering with the binding and thereby obstruct the normal development of the insects.

In addition to being excellent experimental organisms that yield milliliters of hemolymph for study and have short life cycles, well-known genetics, etc., insects are man's most fierce competitors for control of this planet (e.g., crop damage, vectors of disease, etc.). It is imperative that investigators probe insect physiological mechanisms in search of vulnerable points so that control can be approached from a rational point of view. Further study of lipid utilization and transport may offer such opportunities.

[3] Goodman, W., and L. I. Gilbert. Unpublished observations.

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