Archives of Insect Biochemistry and Physiology 42:138–146 (1999)

Equivalence of Riboflavin-Binding Hexamerin and Arylphorin as Reserves for Adult Development in Two Saturniid Moths

M. L. Pan[1]* and William H. Telfer[2]

[1]*Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville*
[2]*Department of Biology, University of Pennsylvania, Philadelphia*

The riboflavin-binding hexamerin (RbH) and arylphorin (ArH) were compared as storage reservoirs for adult development in *Hyalophora cecropia*. The two hexamerins were metabolically labeled with [^{3}H]leucine and [^{35}S]methionine, isolated by column chromatography, and separately injected into pupae whose diapause had been terminated by chilling. By the time of eclosion at least 98% of both hexamerins had been cleared from the hemolymph. Every reproductive and somatic tissue tested contained trichloroacetic acid-precipitable label; consistent differences between the two hexamerins were not detected in the distribution of their label to these tissues. While incorporation of intact hexamerins was not ruled out, hydrolysis and reincorporation of the liberated amino acids were indicated by label in vitellogenin and lipophorin, and by differences in ^{35}S/^{3}H ratios, which ranged from over 1.0 in chorions to 0.4 in wings, as compared with 0.75 in the injected hexamerins. Injection of [^{35}S,^{3}H]RbH from *H. cecropia* into *A. luna*, a species in the same subfamily whose pupae lack this hexamerin, resulted in a pattern of isotope incorporation similar to that yielded by RbH in the donor species. In neither species was there indication of a developing adult tissue that distinguished between RbH and ArH as precursor reservoirs for morphogenesis. This equivalence helps explain how many species of Lepidoptera are able to complete metamorphosis and reproduce without expressing an RbH gene. Evidence is also presented that ArH stored in the fat body protein granules during pupation may be utilized differently from that remaining in pupal hemolymph. Arch. Insect Biochem. Physiol. 42:138–146, 1999. **© 1999 Wiley-Liss, Inc.**

Key words: ***Actias luna; Hyalophora cecropia;*** **storage hexamers**

Abbreviations used: ArH = arylphorin; MtH = methionine-rich hexamerin; RbH = riboflavin-binding hexamerin; PIC = protease inhibitor cocktail; PTU = phenylthiourea; TCA = trichloroacetic acid.

Contract grant sponsor: National Institutes of Health; Contract grant number: GM 32909.

*Correspondence to: M.L. Pan, Department of Ecology and Evolutionary Biology, University of Tennessee, Knoxville, TN 37996-1610. E-mail: mpan@utk.edu

Received 16 March 1999; accepted 1 June 1999

INTRODUCTION

Cladograms based on sequence comparisons show that the four pupal storage hexamers of Lepidoptera are more similar to each other than to any other insect hexamerin thus far sequenced, thus implying descent from a single early lepidopteran protein (Burmester et al., 1998). Differences in composition among the four suggest specializations that might provide a selective advantage for each (Telfer and Kunkle, 1991). But there are also indications of equivalence in function that lead one to wonder why a single hexamerin could not suffice. This, indeed, appears to be the case in *Lymantria dispar*, where arylphorin (ArH), an aromatic acid-rich protein, is the only reported storage hexamer (Karpells et al., 1989). The question has also been raised by the finding in *Actias luna* that reproductive and somatic tissues do not discriminate between ArH and the methionine-rich hexamerin (MtH) as precursor reservoirs during metamorphosis (Pan and Telfer, 1996). Equivalence of the two was unexpected because the tendancy of MtH to be stored in much greater quantities by females than by males had suggested that it has evolved in particular for the support of egg formation (Tojo et al., 1980; Ryan et al., 1985; Bean and Silhacek, 1988).

We report here another attempt to detect differences between hexamerins in the developmental processes they support. This time the comparison is between the riboflavin-binding hexamerin (RbH) and ArH. Cladistic analyses have suggested that the RbH gene separated at an early time in lepidopteran evolution from a gene whose later duplications gave rise to the other three contemporary hexamerins (Burmester et al., 1998). In view of its different history, there was no reason to assume that RbH utilization in metamorphosis would resemble that of the more closely related ArH and MtH.

RbH has an irregular phylogenetic distribution that raises additional questions about its storage functions. *H. cecropia* stores about 140 mg per pupa in its hemolymph, all of which has been consumed by the time of adult eclosion (Pan and Telfer, 1992). But in pupae of *A. luna* and a number of other species of the subfamily Saturniinae, RbH cannot be detected (Telfer and Canaday, 1987). An investigation of whether and how *H. cecropia* RbH would be used if injected into metamorphosing *A. luna* was added to the study to learn more about why this ancient hexamerin is now dispensible in so many moths.

METHODS

Protein Labeling and Isolation

ArH is synthesized throughout the 5th instar in *H. cecropia* (Telfer et al., 1983), but RbH synthesis does not begin until mid-instar, when the caterpillar has reached a weight of 10–12 g (Magee et al., 1994). To be certain that RbH synthesis had begun, hemolymph samples from the males to be labeled were tested for its presence with a monospecific rabbit antiserum. Males testing positive were then injected with 0.1 mCi each of L-[4,5-^{3}H(N)]leucine and L-[^{35}S]methionine (New England Nuclear, Boston, MA), mixed with a small volume of 0.15 M KCl, 10 mM potassium phosphate (pH 6.5), and 5 mM phenylthiourea (PTU). Injected larvae were fed on black cherry leaves (*Prunus serotina*) for 24 h; they were then injected with 0.1 ml of a protease inhibitor cocktail (PIC) and PTU (Telfer et al., 1991), and bled 5 min later.

ArH and RbH were isolated by column chromatography, using a series of matrices from Bio-Rad (Richmond, CA). Gel filtration on Bio Gel A 0.5M separated the approximately 500 kDa proteins, including primarily the hexamerins and lipophorin, from smaller proteins. Fractions containing the 500 kDa peak were pooled, equilibrated with pH 7.2, 32 mM potassium phosphate, and chromatographed on DEAE-agarose, which removes only ArH from the high molecular weight mix under these conditions (Telfer et al., 1983). The DEAE flow-through fractions were equilibrated with pH 5.35, 35 mM potassium succinate, and passed through a column of CM-agarose, which removed RbH and lipophorin from the mix. Bound ArH was released from the DEAE column with pH 6.6, 64 mM potassium phosphate. RbH was released from the CM column with pH 6.0, 25 mM potassium phosphate (Magee et al., 1994).

The labeled isolates were dialyzed against pH 6.5, 10 mM potassium phosphate in 0.15 M KCl, and then concentrated about 20× in Centricon 30 tubes. Aliquots of each concentrate were set aside for later measurement of radioactivity at the time of adult tissue analysis. At that time, label in various preparatons of RbH varied from 1,500

to 2,075 cpm/μl for ^{3}H and 1,100 to 1,650 cpm/μl for ^{35}S; label in ArH preparations varied from 2,395 to 5,420 cpm/μl for ^{3}H and 1,770 to 2,800 cpm/μl for ^{35}S. Protein contents of the final preparations were in the range of 5–10 mg/ml.

Injections

Injection volumes of 100 μl contained 0.5–1.0 $\times$ 10^{6} cpm of both isotopes. For *H. cecropia* this was only 5–10% of the measured pupal hemolymph volume (Pan and Telfer, 1992), and included at most 2 mg of labeled protein, compared with approximately 230 mg of ArH and 140 mg of RbH in the hemolymph of single pupae. The situation was comparable for *A. luna*, except for the normal absence of RbH from this species.

Treatment and Response of Test Insects

Pupae to be injected with the labeled hexamerins had been chilled for at least 6 months in order to evoke the termination of diapause, and were transferred to 25°C immediately after the injection. The test insects resumed their metamorphosis on schedule: 22 days from apolysis to eclosion for females and 21 days for males of *H. cecropia*, and 13 days for females of *A. luna*. Late in development, the ecdysial lines were coated with paraffin in order to prevent emergence and expansion of the wings, since this greatly reduces the amount of available hemolymph. Tissues were dissected from the adult when the pupal exuvium was crisp and the scales were dry.

Tissue Protein Preparation

Some tissues, including reproductive structures, midgut, and Malpighian tubules, were separated and extracted in their entirety. Where this was not possible, the tissues were sampled instead; sampled tissues included hemolymph (50 μl), fat body (20 mg), thoracic muscle (25 mg), and integument (unexpanded wings from the left side). As a rough approximation, the samples represented about 5% of eclosing adult hemolymph volume, 10% of adult fat body, 25% of thoracic muscle, and 10% of adult cuticle.

Soft tissues were homogenized in measured volumes of PIC. Fifty microliter aliquots of the homogenates were mixed with 0.5 ml ice cold 10% trichloroacetic acid (TCA) and centrifuged 2 h later. Centrifugal pellets were dissolved overnight at 50°C in 0.5 N NaOH, 1% SDS, and 50 mM β-mercaptoethanol. Chorionated eggs were rinsed with phosphate-buffered saline (PBS), blotted, and crushed with mortar and pestle. Yolk was pipetted off and treated as above for soft tissues. The residual chorions were rinsed thoroughly with PBS and solubilized without prior precipitation by TCA. Unexpanded wings from the left side were minced with fine scissors in PIC, homogenized manually in microfuge tubes, and incubated for 2 days at 50°C in 1 ml of the dissolving buffer described above for the other tissues.

Generally, 200 μl of the solubilized preparations were mixed with 4.5 ml Cytoscinct (ICN, Irvine, CA) for scintillation counting. Exceptions were the solubilized preparations of wings and accessory glands, from which only 50 μl were counted, in order to minimize quenching due to the pigment in these two tissues.

Results were calculated as the percentage of injected label that was recovered from each tissue, and are expressed as averages and standard errors (SE). Asterisks are used in the figures to identify the comparisons in which label derived from RbH differs significantly from that derived from ArH ($P < 0.05$ in *t*-tests.)

Label in vitellogenin, lipophorin, RbH, and ArH was measured by precipitating these proteins from hemolymph and yolk extracts with rabbit antisera. Ouchterlony plate tests have already been published that confirmed the specificity of these antisera (Telfer and Pan, 1988). They were also used to establish the volume of each antiserum required to precipitate completely its homologous antigen from the samples of hemolymph and yolk. Precipitation and procedures for washing the precipitates with PBS before dissolving and measuring their isotope contents have already been described (Pan, 1971). Each determination was made on a pool of hemolymph or yolk samples from six females.

RESULTS

Clearing of Labeled Hexamerins From Developing Adult Hemolymph

Labeled and isolated RbH and ArH injected into chilled pupae were both at least 98% cleared from the hemolymph by the time of adult eclosion. On day 18 of the pupal-adult molt of *H. cecropia,* extensive clearing had already been

completed. TCA-precipitable label in the hemolymph had fallen to 13% of that injected into pupae as RbH and about 21% in ArH (Table 1). Clearing continued during the four subsequent days so that just prior to eclosion these figures had dropped to 5.5 and 6.5%, respectively. In pre-eclosion males, residual protein label in the hemolymph was equally low. Actually, labeled hexamerin clearing had been even more extensive than this, for much of the TCA-precipitable label in hemolymph at these stages was in newly synthesized adult proteins. Thus, in day 22, pre-eclosion females, antibodies to RbH precipitated only 33% of total protein label (Table 2); antibodies to ArH precipitated only 9.5% of total protein label. Combining the results in Tables 1 and 2 leads to the calculation that only 2% of label injected into female pupae as RbH and 0.6% of that injected as ArH remained in circulation at eclosion. Labeled hemolymph proteins other than hexamerins included vitellogenin and lipophorin (Table 2), which must have been newly synthesized from hydrolytic products of the hexamerins. These two proteins are known to be synthesized during mid to late pharate adult development (Pan, 1971; Kulakosky and Telfer, 1990), and were presumably in transit to the ovaries, where they accounted for 80–90% of the label that was precipitated by TCA from yolk.

A check on the validity of the antibody-precipitation method was provided by the fact that labeled proteins in the ArH-injected group did not include RbH. In the RbH-injected insects, some label was precipitated by anti-ArH, but the amount was low enough to be considered experimental contamination. This accords with the principle that hexamerins are synthesized in Lepidoptera only during feeding stages (Webb and Riddiford, 1988).

Further indication of a role of hydrolysis in the utilization of transfused hexamerins was the finding that 15–30% of total hemolymph label in the day 18 pooled samples was soluble in 10% TCA. Degradation products of both RbH and ArH were, therefore, circulating and available for incorporation by all tissues.

Distribution of Label in Eclosing Adults

A total of 36 comparisons of RbH and ArH were made as sources of label for adult tissues and secretory products. Half of these entailed measurements of ^{3}H and these are shown in Figure 1. In 16 of these comparisons, the *P* values of the differences between RbH and ArH as sources of label were greater than 0.05, our arbitrarily set threshold for significance. The two exceptions (asterisks in Fig. 1) were accessory glands and fat bodies of females, both of which incorporated a significantly greater fraction of ^{3}H from ArH than from RbH. However, the amount of ^{3}H in the accessory glands was extremely low, less than 1% of the total injected; in fat body, from which 20 mg samples were measured, the amount of label was also small. In an analysis of ^{35}S distribution (not shown), none of the 18 comparisons had *P* values < 0.05, including accessory glands and fat body. The developing adult tissues tested, therefore, did not manifest preferences for either RbH or ArH as reservoirs of precursors for morphogenesis.

TABLE 1. Protein Label Remaining in the Hemolymph of Pharate Adult *H. cecropia* That Had Been Injected as Pupae With [^{3}H, ^{35}S]-RbH or -ArH

	Day 18 female (n = 6)		Day 22 female (n = 9)		Day 21 male (n = 5)	
Source of label	Measured in 50 µl[a] Ave. ± SE	Calculated for whole insect[b]	Measured in 50 µl[a] Ave. ± SE	Calculated for whole insect[b]	Measured in 50 µl[a] Ave. ± SE	Calculated for whole insect[b]
^{3}H from RbH	0.50 ± 0.08	12.8	0.23 ± 0.02	5.70	0.25 ± 0.15	4.86
^{35}S from RbH	0.51 ± 0.07	13.1	0.22 ± 0.02	5.46	0.27 ± 0.17	5.28
^{3}H from ArH	0.80 ± 0.11	24.0	0.35 ± 0.07	6.54	0.18 ± 0.01	4.78
^{35}S from ArH	0.59 ± 0.08	17.7	0.26 ± 0.05	6.34	0.14 ± 0.02	3.75

[a]Amounts of label are expressed as percentages of the approximately 10^{6} cpm injected into the hemolymph at the pupal stage.

[b]Label remaining in the hemolymph was calculated for whole insects by multiplying the amount in 50-µl samples times total hemolymph volume (in µl), and dividing by 50. Hemolymph volume averages 26% of body weight (in mg) in day 18 females, 23% in pre-eclosion, day 22 females, and 31% in pre-eclosion, day 21 males (Pan and Telfer, 1992).

TABLE 2. Labeling of Specific Hemolymph and Yolk Proteins in Females Injected With [^{3}H]-RbH and -ArH

Sample and source of label[a]	Label precipitated by[b]			
	Anti-vitellogenin	Anti-lipophorin	Anti-RbH	Anti-ArH
Day 18 hemolymph				
^{3}H from RbH	21.3	3.8	41.9	0
^{3}H from ArH	7.8	1.2	0	27.9
Day 22 hemolymph				
^{3}H from RbH	14.9	5.5	32.8	2.1
^{3}H from ArH	15.7	6.3	0	9.5
Day 22 Yolk				
^{3}H from RbH	62.3	18.9		
^{3}H from ArH	71.7	23.6		

[a]Female pupae were injected with labeled RbH or ArH and bled and dissected on the days shown in the left-hand column. Precipitations were from the pooled samples of 6 females.
[b]Antibody-precipitated label is expressed as a percentage of the label that was precipitated from an equal volume of the sample by 10% trichloroacetic acid.

Sexual Comparisons

Male reproductive tissues contained very little label compared to yolk and chorion, which accumulated over 20% of the label from hexamerins injected into females (Fig. 1). Lacking the need to produce eggs, males were able to increase the distribution of label to somatic tissues, and in particular to the integument. In males, the two wings chosen to represent integument incorporated an average of 5.4% of injected label from the two hexamerins (n = 9, SE = 0.7) compared with 3.1% in females (n = 17; SE = 0.3) (P = 0.002). Total body integument is about ten times as massive as two wings, and therefore might have incorporated 54% of injected label in males, compared with only 31% in females. This is the sort of difference that would be expected if label

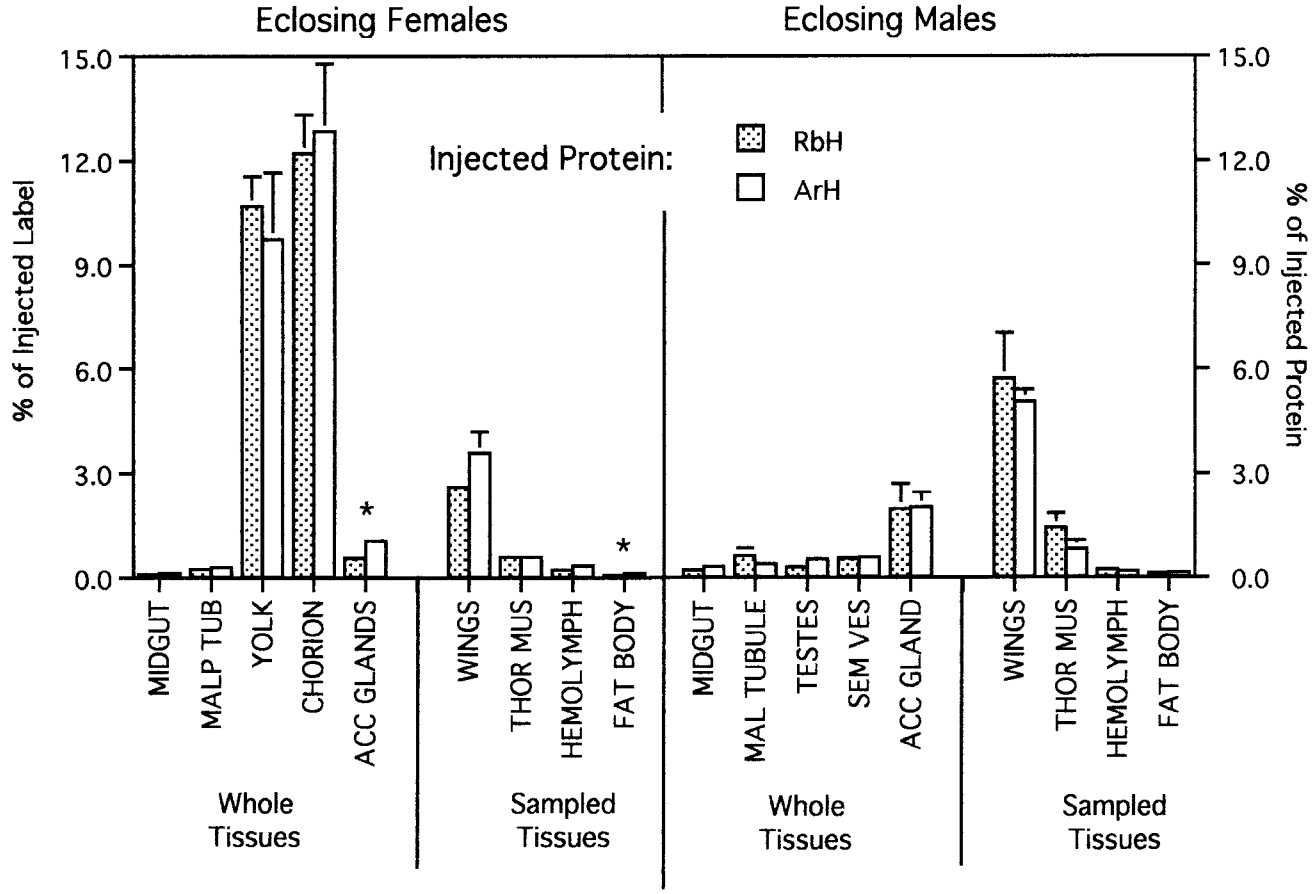

Fig. 1. Comparison of RbH and ArH as storage sites for [^{3}H]-precursors that are incorporated into TCA-precipitable components of pharate adult *H. cecropia*. Labeled hexamerins were injected into pupae; tissues were dissected and extracted at the time of eclosion but before wing expansion. Bars show averages and standard errors for 9 females and 5 males. Standard errors are in some cases too small to be visualized. Asterisks indicate comparisons where the probability that the averages for RbH- and ArH-derived label represent variants of a single population is less than 0.05.

released from ArH and RbH goes into a general pool that is competed for by both reproductive and somatic tissues.

$^{35}S/^{3}H$ in the Tissues of Eclosing *H. cecropia*

Differences between adult tissues in the incorporation ratios of ^{35}S and ^{3}H are shown in Figure 2. The tissues are arranged along the horizontal axis in this figure according to decreasing $^{35}S/^{3}H$ ratios in RbH-injected individuals (open squares); the ratios from ArH-injected individuals (closed circles) were then added in the same sequence. The ratios differed widely from the values of 0.75 in the injected hexamerins, ranging from less than 0.4 in wings to over 1.1 in chorions. This is in accord with findings in two earlier investigations that developing adult tissues synthesize proteins from precursors released by digestion of storage hexamers from calliphorin (Levenbook and Bauer, 1984) and ArH (Wu and Tischler, 1995). In females, the difference between chorion (on the left) and wings (on the right) accords with the respective modes of molecular cross-linking in these two products: high cys content facilitates disulfide bridge formation in the hardening of the chorion in moths (Kawasaki et al., 1971) in contrast to the better known phenol oxidase-based cross linking in cuticular sclerotization.

Utilization of *H. cecropia* RbH and ArH by *A. luna*

The distribution of label derived from *H. cecropia* RbH and ArH injected into female *A. luna* is shown in Figure 3. The distributions were similar to those obtained for the two hexamerins in *H. cecropia* females (Fig. 1), with highest labeling in the yolk, chorion, and wings. As an amino acid reservoir, therefore, RbH appears to be replaceable by ArH, and presumably also MtH, in those species that have lost or stopped expressing their RbH genes.

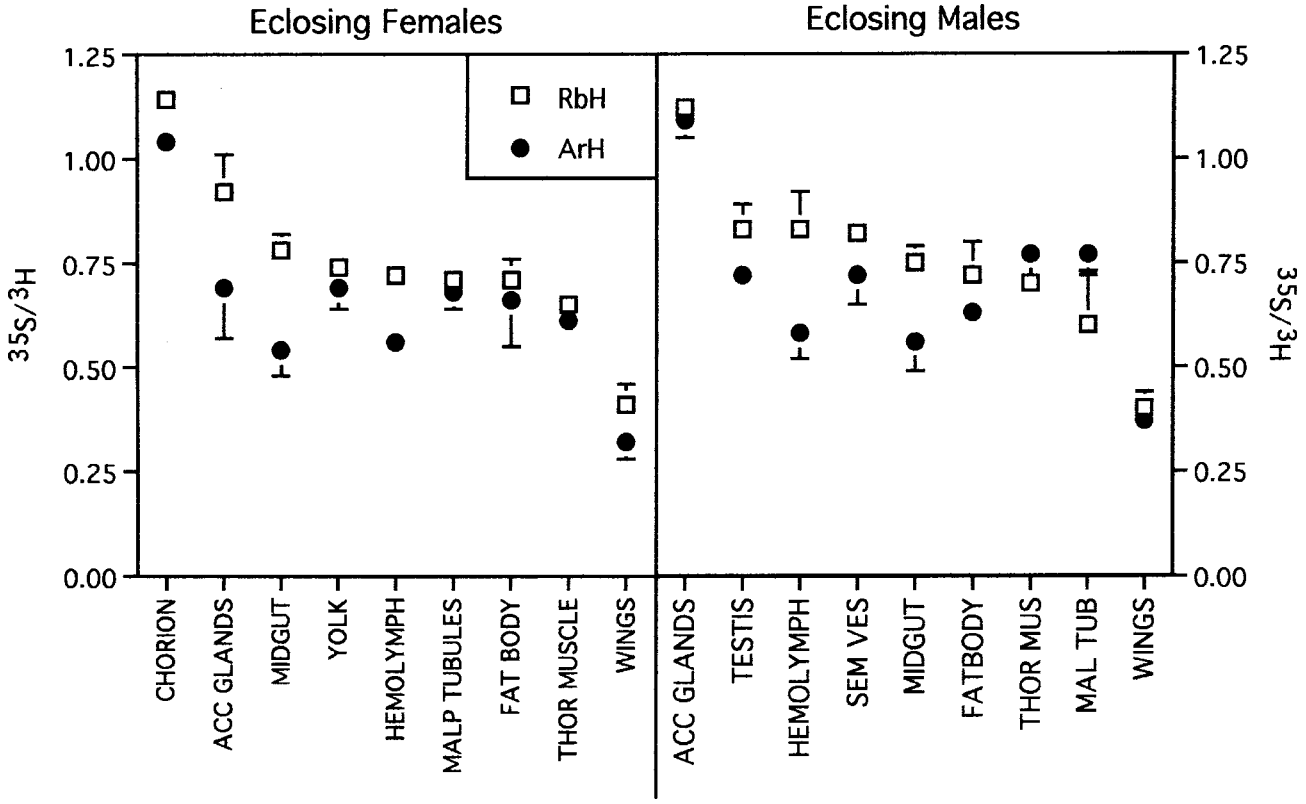

Fig. 2. Comparison of $^{35}S/^{3}H$ in adult tissues from individuals injected as pupae with labeled hexamerins. Tissues are arranged on the horizontal axis in decreasing order of the $^{35}S/^{3}H$ ratios generated by injection of labeled RbH (open squares, positive error bars). Tissues from ArH-injected animals are arranged in the same sequence (closed circles, negative error bars). The ratios of both injected probes were 0.75. Values are averages for 9 females and 5 males. Standard errors, where greater than the diameter of the points, are indicated by up-error bars for RbH and by down error bars for ArH.

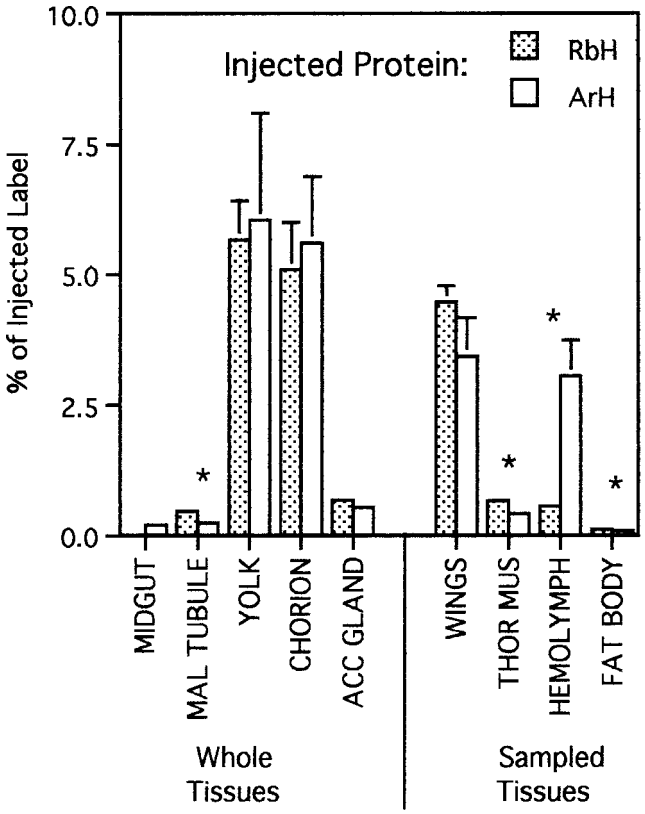

Fig. 3. Comparison of RbH and ArH from *H. cecropia* as storage sites for [^{3}H]-precursors that are incorporated into TCA-precipitable components of pharate adult *A. luna*. Labeled hexamerins were injected into pupae; tissues were dissected and extracted at the time of eclosion but before wing expansion. Bars show averages and standard errors for 11 females injected with RbH and 8 females injected with ArH. Standard errors are in some cases too small to be visualized. Asterisks indicate comparisons where the probability that the averages for RbH- and ArH-derived label represent variants of a single population is less than 0.05.

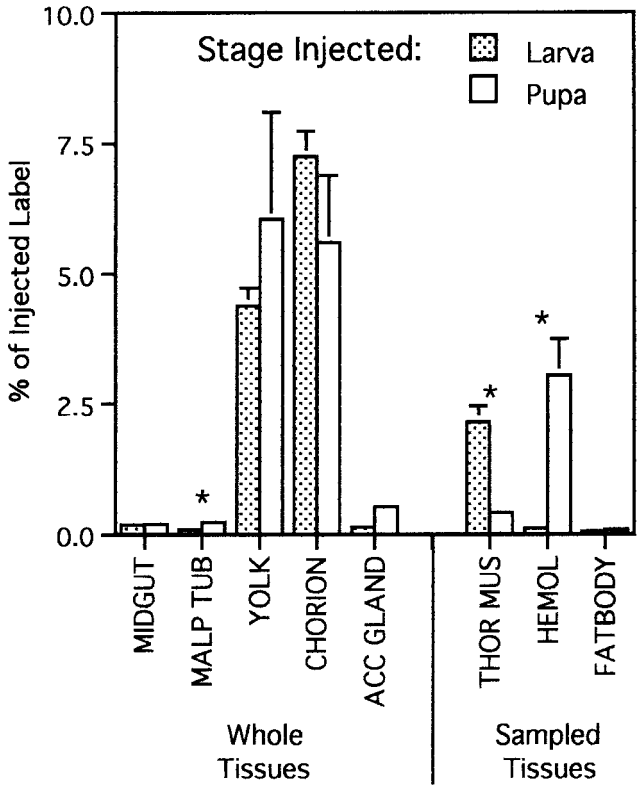

Fig. 4. Comparison of ArH as a storage site for [^{3}H]-precursors that are incorporated into TCA-precipitable components of pharate adult *A. luna* females. Results from injections of *A. luna* ArH into caterpillars are from Pan and Telfer (1996); those from injections of *H. cecropia* ArH into pupae are from Figure 3. Bars show averages and standard errors for 5 females injected as caterpillars and 8 females injected as pupae. Standard errors are in some cases too small to be visualized. Asterisks indicate comparisons where the probability that the averages for RbH- and ArH-derived label represent variants of a single population is less than 0.05.

A second issue raised by the *A. luna* experiment concerned the mode of ArH storage. ArH from *H. cecropia* hemolymph transfused into *A. luna* caterpillars was stored during the subsequent larval-pupal transformation, as it is in the donor, in both fat body granules and hemolymph (Pan and Telfer, 1992). But in the present study, labeled ArH was injected into pupae that had completed protein granule deposition many months earlier. If stores in hemolymph and protein granules are utilized at different times or in different ways, therefore, tissue distribution of ArH-derived label should vary in the two studies.

In Figure 4 the distributions of label in these two experiments are compared. High variances in the results for yolk and chorion place limits on the interpretation. Nevertheless, the differences are sufficient to suggest meaningful consequences. Thus, thoracic muscle labeling drew preferentially on ArH stored in the fat body. There was a tendency in this same direction in the chorion. By contrast, adults developing with labeled ArH stored in fat body had extremely low levels of TCA-precipitable label in hemolymph, and a tendency toward lower labeling in yolk protein.

DISCUSSION

Comparing the Storage Functions of RbH and ArH

As in the earlier comparison of storage functions of MtH and ArH (Pan and Telfer, 1996), no

evidence was found for differences between RbH and ArH in their support of adult development in *H. cecropia*. While several of the 9 tissues compared incorporated a larger share of ^{3}H from one hexamerin than from the other ($P < 0.05$; asterisks in Fig. 1), significance was not repeated for that tissue in both sexes, and was not found in any of the measurements of ^{35}S labeling. Tissue-specific utilization, while not ruled out, was simply not revealed by the approach taken in this study.

RbH-derived label partitioned between adult female tissues of *A. luna* (Fig. 3 dotted columns) in a pattern essentially the same as in *H. cecropia* (Fig. 1). ArH-derived label was also very similar in the two species. There was a high level of label in the hemolymph of eclosing *A. luna* females that had been injected as pupae with ArH from *H. cecropia* (Fig. 4), but this resulted not from new synthesis but from inefficient clearing of the injected probe. We showed this earlier when ArH from *H. cecropia* was found by species-specific antibody detection to compete poorly with *A. luna* ArH for the hemolymph clearing mechanism during adult development (Pan and Telfer, 1992).

As an amino acid reservoir for metamorphosis, RbH appears from these results to be replaceable by hemolymph ArH, and this suggests how many species of Lepidoptera were able to dispense with it. While RbH is an abundant hemolymph protein in *H. cecropia*, the concentration of ArH is nearly twice as high (Pan and Telfer, 1992) and there is an equal or greater amount of the latter stored in the fat body, along with the MtHs, the principal fat body hexamerins. Thus, failure to produce RbH during the last larval instar might not seriously diminish the amino acid reserves available for adult developoment. Since the riboflavin-storing function of this protein can be served by lipophorin (Miller and Silhacek, 1992; Magee et al., 1994), its only known ligand-binding function is also replaceable. The question then becomes not so much how other species get along without RbH, but how its persistence can be accounted for in those species that still produce it.

Incorporation of Hydrolytic Products

A distinction between the incorporation of free amino acids and intact hexamerins was not a primary goal of the present work. But as with calliphorin utilization in *Calliphora vicina* (Levenbook and Bauer, 1984), hydrolytic products appear to be the primary mode of transferring isotope from the injected hexamerin to adult tissue proteins. In the present study, this is especially clear in egg formation; over 8% of injected ^{3}H from both RbH and ArH found its way into vitellogenin and lipophorin, the two major yolk proteins, and around 12% into the chorion (Fig. 1), whose proteins are known to be synthesized by the follicular epithelium (Paul and Kafatos, 1975). The hydrolysis model has also been proposed for *Bombyx mori* (Ogawa and Tojo, 1981) and autogenous forms of *Aedes atropalpus* (Wheeler and Buck, 1996), in which production of vitellogenin, the principal protein of adult females, is matched by a decline in stored hexamerins, the principal proteins of pupae. Hydrolysis is further indicated in the present study by variations in $^{35}S/^{3}H$ ratios from 0.4 in wings to over 1.0 in chorions in females and accessory glands in males (Fig. 2). That a substantial fraction of label circulating in the hemolymph of day 18 females was soluble in 10% TCA is also consistent with this model.

The selective incorporation of aromatic-rich hexamerins into cuticle that occurs in Diptera (Scheller et al., 1980; Konig et al., 1986; Tsakas et al., 1991; Chrysanthis et al., 1994) has not been detected in our experiments with Saturniids. MtH and ArH yielded similar fractions of their label to cuticle in *A. luna* (Pan and Telfer, 1996). And in the present study, the percentages of label from RbH and ArH incorporated into cuticle, in this case the wings, was similar, despite the fact that RbH does not have the high aromatic amino acid content expected for a role in sclerotization (Magee et al., 1994).

Fat Body Versus Hemolymph Storage

A final question concerned differences between the utilization of labeled ArH in pupal hemolymph, as occurred in the present study, and that stored in both fat body and hemolymph (Pan and Telfer, 1996). If the pattern of differences shown in Figure 4 is borne out, it will indicate that products made available from the two ArH stores have different destinations. A useful way to explain this difference in terms of cell biology would be to envision hydrolytic products from storage granules being released to the hemolymph for general distribution, while synthesis of proteins within the fat body utilized primarily amino

acids released locally by lysosomes from newly endocytosed ArH. Such a model would be broadly applicable because bimodal storage probably occurs in a wide variety of insects (e.g., Martinez and Wheeler, 1993; Chrysanthis et al., 1994).

LITERATURE CITED

Bean D, Silhacek D. 1988. Changes in titer of the female-predominant storage protein (81k) during larval and pupal development of the wax moth, *Galleria mellonella*. Arch Insect Biochem Physiol 10:333–348.

Burmester T, Massey H, Zakharkin S, Benes H. 1998. The evolution of hexamerins and the phylogeny of insects. J Mol Evol 47**:**93–108.

Chrysanthis G, Kaliafas A, Mintzas A. 1994. Biosynthesis and tissue distribution of four major larval serum proteins during development of *Ceratitis capitata* (Diptera). Insect Biochem Mol Biol 24:811–818.

Karpells S, Leonard D, Kunkel J. 1989. Cyclic fluctuations in arylphorin, the principal serum storage protein of *Lymantria dispar,* indicate multiple roles in development. Insect Biochem 20:73–82.

Kawasaki H, Sato H, Suzuki M.1971. Structural proteins in the silkworm egg-shells. Insect Biochem 1:130–148.

Konig M, Agrawal O, Schenkel H, Scheller K. 1986. Incorporation of calliphorin into the cuticle of the developing blowfly, *Calliphora vicina*. Wilhelm Roux's Arch Dev Biol 195:296–301.

Kulakosky PC, Telfer WH. 1990. Lipophorin as a yolk precursor in *Hyalophora cecropia*: uptake kinetics and competition with vitellogenin. Arch Insect Biochem Physiol 14:269–285.

Levenbook L, Bauer A. 1984. The fate of larval storage proteins during adult development of *Calliphora vicina*. Insect Biochem 14:77–86.

Magee J, Kraynack N, Massey HC, Jr, Telfer WH. 1994. Properties and significance of a riboflavin-binding hexamerin in the hemolymph of *Hyalophora cecropia*. Arch Insect Biochem. Physiol 25:137–157.

Martinez T, Wheeler DE. 1993. Identification of two storage hexamers in the ant *Camponotus festinatdus*: accumulation in adult queenless workers. Insect Biochem Molec Biol 23:309–317.

Miller S, Silhacek D. 1992. Binding of riboflavin to lipophorin and a hexameric protein in the haemolymph of *Heliothis virescens*. Insect Biochem Mol Biol 22:571–583.

Ogawa K, Tojo S. 1981. Quantitative changes of storage proteins and vitellogenin during the pupal-adult development in the silkworm, *Bombyx mori* (Lepidoptera:Bombycidae). Appl Ent Zool 16: 288–296.

Pan ML. 1971. The synthesis of vitellogenin in the *cecropia* silkworm. J Insect Physiol 17:677–689.

Pan ML, Telfer WH. 1992. Selectivity in storage hexamerin clearing demonstrated with hemolymph transfusions between *Hyalophora cecropia* and *Actias luna*. Arch Insect Biochem Physiol 19:203–221.

Pan ML, Telfer WH. 1996. Methionine-rich hexamerin and arylphorin as precursor reservoirs for reproduction and metamorphosis in female luna moths. Arch Insect Biochem Physiol 32:149–162.

Paul M, Kafatos FC. 1975. Specific protein synthesis in cellular differentiation. II. The program of protein synthetic changes during chorion formation by silkmoth follicles, and its implementation in organ culture. Dev Biol 42:141–159.

Ryan R, Keim P, Wells M, Law J. 1985. Purification and properties of a predominantly female-specific protein from the hemolymph of the larva of the tobacco hornworm, *Manduca sexta*. J Biol Chem 260:782–787.

Scheller K, Zimmerman H-P, Sekeris C. 1980. Calliphorin, a protein involved in the cuticle formation of the blowfly, *Calliphora vicina*. Z Naturforsch 35c:387–389.

Telfer WH, Canaday D. 1987. Storage proteins of saturniid moths: interspecific similarities and contrasts. Biol Chem Hoppe-Seyler 368:571–572.

Telfer WH, Kunkle JG. 1991. The function and evolution of insect storage hexamers. Ann Rev Entomol 36:205–228.

Telfer WH, Pan ML. 1988. Adsorptive endocytosis of vitellogenin, lipophorin, and microvitellogenin during yolk formation in *Hyalophora.* Arch Insect Biochem Physiol 9:339–355.

Telfer WH, Keim P, Law JH. 1983. Arylphorin, a new protein from *Hyalaphora cecropia*: comparisons with calliphorin and manducin. Insect Biochem 13:601–613.

Telfer WH, Pan ML, Law JH. 1991. Lipophorin in developing adults of *Hyalophora cecropia*: support of yolk formation and preparation for flight. Insect Biochem 21:653–663.

Tojo S, Nagata M, Kobayashi M. 1980. Storage proteins in the silkworm, *Bombyx mori*. Insect Biochem 10:289–303.

Tsakas S, Katsoris P, Bourtzis K, Marmaras V. 1991. Incorporation of arylphorins (LSP-1) and LSP-2 like protein into the integument of *Ceratitis capitis* during pupariation. Insect Biochem 21:507–515.

Webb B, Riddiford L. 1988. Regulation of expression of arylphorin and female-specific protein mRNAs in the tobacco hornworm, *Manduca sexta.* Dev Biol 130:682–692.

Wheeler DE, Buck N. 1996. A role for storage proteins in autogenous reproduction in *Aedes atroppalpus*. J Insect Physiol 42:961–966.

Wu M, Tischler M. 1995. Utilizaion of [^{14}C]phenylalanine derived from arylphorin or free amino acid in *Manduca sexta* pharate adults. Arch Insect Biochem Physiol 28:257–272.