Vitellogenic Blood Protein Synthesis by Insect Fat Body

Abstract. *The female fat body of a moth and a roach incorporated labeled amino acids in vitro into substances precipitable by antibodies formed in response to the sex-limited vitellogenic blood proteins of these species. The fat body of males failed to do so, as did that of females before the appearance of these proteins in the blood.*

The blood of many mature female insects contains proteins that are not readily detected in the blood of males or of immature females (*1–3*). As in the case of the sex-limited blood proteins secreted by the liver of birds and amphibians (*4*), the female-specific insect blood proteins are sequestered by vitellogenic oocytes in the ovaries and are organized into protein yolk bodies. (In view of their function, the term "vitellogenin" is adopted here for such proteins. The term is intended to designate a function and to imply nothing about molecular structure; whether the various insect vitellogenins are structurally similar is not known.)

Although the accumulation of vitellogenin in the blood of ovariectomized insects indicates an extraovarian origin (*1*), the tissue that synthesizes it has not been identified with certainty. The fat body has been implied (*3, 5*), though this view is controversial (*6, 7*). One of the favored criteria has been the presence or absence in fat body extracts of proteins with electrophoretic mobilities similar to those of the vitellogenins in the blood. Since several insect tissues, including the fat body, regularly sequester proteins from the blood (*8*), and since there is no reason to believe that the cells synthesizing a blood protein will necessarily store it in easily detected amounts, the presence or absence of a particular protein in a tissue extract does not constitute conclusive evidence for the site of synthesis.

We followed Shigematsu's approach to the study of the synthesis of blood proteins by the fat body of *Bombyx mori* larvae (*9*) by determining the ability of the tissue to incorporate labeled amino acids into vitellogenin in vitro. After incubation and the addition of carrier proteins, vitellogenin was precipitated from the medium with rabbit antibodies in order to separate it from other proteins that might have been labeled and secreted or lost from the cells.

Two widely different species were studied, the cecropia moth *Hyalophora cecropia* (L.) and the American cockroach *Periplaneta americana* (L.). Either 75 or 150 mg of freshly dissected tissue were rinsed several times with a Ringer solution appropriate for the species and then incubated for up to 4 hours at 25°C by shaking with 1 ml of labeling medium in siliconized, flat-bottomed, tissue-culture tubes. The medium used for the moth tissue was similar to that employed in previous studies of fat body in this species (*10*), except that the ionic composition was modified according to more recent analyses (*11*), and orthophosphite was chosen as the buffer (*12*). The roach medium was like that used for the moth, with the inorganic ion composition taken from analyses of *Periplaneta* blood (*13*). In both cases, 25 μc of ^{3}H-leucine were included per milliliter of medium.

After incubation, the medium was centrifuged free of cells and was mixed with 1.5 times its volume of phosphate-buffered solution (*p*H 7.2) containing 0.15*M* NaCl and a dilution (1:25) of a cell-free blood rich in vitellogenin. To determine the total amount of label released by the cells as protein, we deposited a sample of the incubation mixture on a filter-paper pad and extracted, washed, and determined the radioactivity according to the method of Mans and Novelli (*14*). A second sample was mixed with a rabbit antiserum containing antibodies against vitellogenin. The proportions of carrier blood protein and rabbit antiserum were selected in each case so that, after precipitation of the antigen-antibody complex, the supernatant contained excess antibody. Complete precipitation of vitellogenin was thus assured. The precipitate was washed twice with cold, phosphate-buffered saline and was then transferred to a filter-paper pad; the radioactivity was determined as for the total protein sample.

The antiserums were obtained from rabbits immunized with the proteins of mature eggs. After absorption with pupal male blood, the antiserum to moth eggs exhibited a single zone of precipitation in the antiserum-agar test of Oudin (*1*) when overlayered with pupal female blood. This agrees with earlier analyses which have consistently indicated a single, immunochemically definable vitellogenin in this species (*1*). The absorbed antiserum showed no detectable reaction with larval blood, pupal male blood, or female pupal fat body extracted at the same *p*H and the same ionic strength as the incubation medium. Thus the absorbed antiserum was a selective reagent for the precipitation of vitellogenin from fat body cultures.

The antiserum to roach eggs, after absorption with adult male blood, produced two zones of precipitation when overlayered with the blood of adult females, so that apparently there are two immunochemically defined vitellogenins in this species. As with vitellogenin from cecropia, extracts of fat body

Table 1. Release of ^{3}H-leucine-labeled vitellogenins to an incubation medium by tissues of *Hyalophora cecropia* (L.), as measured by precipitation of radioactive material by specific antibody.

Stage of development	Antibody-precipitated label in 0.04 ml	
	Radioactivity (10^3 disintegrations per minute ± S.D.)	Fraction of perchloric acid–insoluble label (%)
	Female fat body	
Mature feeding larva	0.28 ± 0.08	0.2
Initiation of spinning	0.25 ± 0.07	0.3
Initiation of molting	1 ± 1	0.8
Day 3 of molting	138 ± 51	54
Day 5 of molting	152 ± 43	69
Pupal ecdysis (7th day)	50 ± 27	51
Day 2 after ecdysis	25 ± 10	67
Day 4 after ecdysis	3 ± 3	59
	Female midgut	
Pupal ecdysis	0.02 ± 0.01	0.3
	Female wing sacs	
Pupal ecdysis	Negligible	< 0.1
	Male fat body	
Day 5 of molting	0.51 ± 0.13	0.2
Pupal ecdysis	0.17 ± 0.08	0.2
	Male midgut	
Pupal ecdysis	0.04 ± 0.01	0.3

Table 2. Release of ^{3}H-leucine-labeled vitellogenins to an incubation medium by tissues of *Periplaneta americana* (L.), as measured by precipitation of radioactive material by specific antibody.

Stage of development	Antibody-precipitated label in 0.8 ml	
	Radioactivity (10^3 disintegrations per minute ± S.D.)	Fraction of perchloric acid–insoluble label (%)
	Female fat body	
Newly emerged adult	0.19 ± 0.02	0.95
Ootheca forming	1047 ± 40	83
Day 1 after ootheca forming	891 ± 52	77
Day 2 after ootheca forming	891 ± 98	81
Day 3 after ootheca forming	841 ± 176	78
Day 4 after ootheca forming	446 ± 15	62
	Female midgut	
Ootheca forming	2.24 ± 0.28	1.60
	Male fat body	
Mature adult	2.36 ± 0.43	2.00

at the *p*H and ionic strength of the culture medium produced neither band. This result confirms the electrophoretic evidence that vitellogenin from *Periplaneta* is not readily detectable in the fat body (*7, 15*).

An additional difference between the two animals was the time in the life cycle at which the vitellogenins appeared. Vitellogenin from cecropia appears in the blood during the larval-pupal molt and is stored there at maximum concentration until yolk formation commences during the pupal-adult transformation (*1*). In contrast, the two vitellogenins from *Periplaneta* appear together approximately 5 days after the emergence of the adult, when yolk deposition begins.

Incubation of cecropia fat body, taken from animals between day 3 of the larval-pupal molt and day 4 after pupation, led to the appearance in the medium of a labeled protein precipitable by perchloric acid and antibody (Table 1). On day 5 of the molt, the label precipitable by antibody was at a maximum, accounting at that time for 69 percent of the total protein label.

After a 30- to 40-minute lag at the outset of the incubations, label precipitated by both perchloric acid and antibody increased linearly with time; both were completely inhibited by $5 \times 10^{-3}M$ cycloheximide (*16*). Therefore, the demonstrated radioactivity did not result from direct adsorption of free amino acid to carrier protein or from inadequate washing procedures. The lag period presumably encompassed the time required for synthesis and secretion as well as for cellular adaptation to the medium.

The labeling of vitellogenin in vitro resembled the synthesis and secretion of this protein in vivo in that the activity was limited to female fat body at appropriate stages of metamorphosis (Table 1). Thus, fat body from caterpillars or from females initiating the larval-pupal molt, prior to the appearance of vitellogenin in the blood, did not significantly promote the appearance of antibody-precipitated labeled protein in the incubation medium, although label insoluble in perchloric acid appeared in copious amounts. Neither midgut nor wing sacs at the time of pupation incorporated labeled leucine into vitellogenin. Finally, and what is most convincing, fat body from pupating males was also inactive.

Parallel results were obtained with *Periplaneta* (Table 2). Between 62 and 83 percent of the label secreted as protein into the incubation medium by the fat body of egg-producing females was precipitated by homologous antibody to vitellogenin. Neither male nor newly emerged adult female fat body exhibited this capacity, nor did the midgut of mature females, though all of these tissues gave rise to labeled proteins in the medium.

In both species the specificity of incorporation with regard to sex and to stage in the life cycle were consistent with well-established occurrences of vitellogenin in vivo (*1–3*). Our conclusions are in agreement with results obtained by other methods (*17*) for the wood roach, *Leucophaea maderae*, and for the moth, *Antheraea polyphemus*. Taken in conjunction with the evidence that the fat body in a variety of insects plays a metabolic role in egg production (*18*), the similarity in our data from two widely divergent species suggests that insect vitellogenins in general may prove to be secreted by the fat body. Failure to detect the protein in tissue extracts indicates either a change in solubility characteristics of the protein at the time of secretion or a fairly prompt secretion following its synthesis.

M. L. PAN, WILLIAM J. BELL
WILLIAM H. TELFER
Department of Biology, University of Pennsylvania, Philadelphia 19104

References and Notes

1. W. H. Telfer, *J. Gen. Physiol.* **37**, 539 (1954); *Annu. Rev. Entomol.* **10**, 161 (1965).
2. L. Hill, *J. Insect Physiol.* **8**, 609 (1962); K. K. Thomas and J. L. Nation, *Biol. Bull.* **130**, 254 (1966); F. Engelmann and D. Penney, *Gen. Comp. Endocrinol.* **7**, 314 (1966).
3. G. C. Coles, *J. Exp. Biol.* **43**, 425 (1965).
4. P. J. Heald and P. M. McLachan, *Biochem. J.* **94**, 32 (1965); D. Rudack and R. A. Wallace, *Biochim. Biophys. Acta* **155**, 299 (1968).
→ H. Laufer, *Ann. N.Y. Acad. Sci.* **89**, 490 (1960); A. K. Minks, *Arch. Neer. Zool.* **27**, 175 (1967).
6. T. F. Roth and K. R. Porter, *J. Cell Biol.* **20**, 313 (1964).
7. K. G. Adiyodi and K. K. Nayar, *Biol. Bull.* **133**, 271 (1967).
→ M. Locke and J. V. Collins, *J. Cell Biol.* **36**, 453 (1968).
9. H. Shigematsu, *Nature* **182**, 880 (1958); G. M. Price and T. Bosman, *J. Insect Physiol.* **12**, 741 (1966).
10. E. Stevenson and G. R. Wyatt, *Arch. Biochem. Biophys.* **99**, 65 (1962).
11. J. W. Micheda and R. E. Thiers, *Proc. Int. Congr. Zool. 16th* **2**, 39 (1963).
12. H. E. Robertson and P. D. Boyer, *Arch. Biochem. Biophys.* **62**, 396 (1956); M. M. Martignoni and R. J. Scallion, *Biol. Bull.* **121**, 507 (1961).
13. K. van Asparen and I. van Esch, *Arch. Neer. Zool.* **11**, 342 (1956).
14. R. J. Mans and G. D. Novelli, *Arch. Biochem. Biophys.* **94**, 48 (1961).
15. K. G. Adiyodi, *J. Insect Physiol.* **13**, 1189 (1967).
16. H. L. Ennis and M. Lubin, *Science* **146**, 1474 (1964).
17. M. Blumenfeld and H. A. Schneiderman, *Biol. Bull.* **135**, 466 (1968); V. J. Brookes, *Develop. Biol.*, in press.
18. I. W. Pfeiffer, *J. Exp. Zool.* **99**, 183 (1945); W. W. Doanne, *J. Exp. Zool.* **146**, 275 (1961); L. Hill, *J. Insect Physiol.* **11**, 1605 (1965); M. F. Day, *Biol. Bull.* **84**, 127 (1943).
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Transplantation of Pluripotential Nuclei from Triploid Frog Tumors

Abstract. *Renal tumors were produced by injection of a cell fraction of a tumor into triploid tadpoles of* Rana pipiens *before they began feeding. Triploid tumor cells were dissociated and transplanted into activated and enucleated eggs. Pluripotency of the implanted nuclei was evidenced by the formation of swimming triploid tadpoles.*

Diploid nuclei of renal tumor cells, when transplanted to enucleated eggs of the common leopard frog *Rana pipiens*, result in a low yield of tadpoles (*1*). These experiments suggest that pro-