Reprod. Nutr. Dévelop., 1981, **21** (1), 95-101.

Vitellogenin synthesis in the fat body of the marine crustacean Isopoda, *Idotea balthica basteri*, during vitellogenesis

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Summary. This paper investigates vitellogenin synthesis in the fat body of the female marine crustacean Isopoda, *Idotea balthica basteri*, during vitellogenesis. The fat bodies were incubated in a labelled medium ; one of the samples was treated with an antiserum against vitellogenin and the antigen-antibody complex counted for radioactivity. We assumed this radioactivity to be due to vitellogenin synthesis. It is indicated that vitellogenin may account for most of the protein synthesized by the fat body during vitellogenesis. The first peak of vitellogenin synthesis was observed in the early stages of vitellogenesis (molting cycle period C), but the major increase in that level appeared during stage 2 of vitellogenesis (molting cycle period D, stage D_1). Before laying (and molt), the fat body incorporated less.

Introduction.

It has been shown in several insects that the proteins incorporated in the oocyte yolk (vitellogenins) are synthesized in the fat body (see reviews by Engelmann, 1970 ; Wyatt and Pan, 1978). Meusy (1980) reported that the site of vitellogenin synthesis in crustaceans has not yet been determined. Most studies on the presumed organs of vitellogenin synthesis name the hepatopancreas as the main agent (Ceccaldi and Martin, 1969 ; Besse *et al.*, 1970 ; Wolin *et al.*, 1973). The hemocytes have also been suggested (Kerr, 1968). Since these data were published, we have shown immunoreactive sites for vitellogenin in the fat body and the hepatopancreas of *Porcellio dilatatus*, Crustacea Isopoda Oniscoidea (Picaud and Souty, 1980*a*). Using immunoautoradiography, we then demonstrated that the fat body, incubated in labelled medium, synthesized vitellogenin ; no synthesis was observed in the hepatopancreas (Picaud and Souty, 1980*b*). The latest results in Amphipoda, *O. gammarellus*, have suggested fat body involvement in vitellogenin synthesis (Junéra and Croisille, 1980). The present study quantifies vitellogenin synthesis during the molting cycle. We used the marine crustacean Isopoda, *Idotea balthica basteri*, because it has a short ovarian cycle (18 days ; 20 °C ; 18L : 6D photoperiod) from puberty to death. This cycle is closely correlated with the molting cycle which can be subdivided into periods and stages according to the teguments (general method : Drach and Technigovtzeff, 1967).

Material and methods.

The *Idotea* used in this study were born and reared in our laboratory and originated from a population in Marseille.

The ventral thoracic part of the cuticle with the attached sub-epidermal and perineural fat body was dissected at different periods of the molting cycle, and consequently during the ovarian cycle.

The procedure for incubating the fat body *in vitro* is described in figure 1 (according to Brookes, 1976 : *Leucophaea maderae* and Hagedorn *et al.* : *Aedes aegypti*).

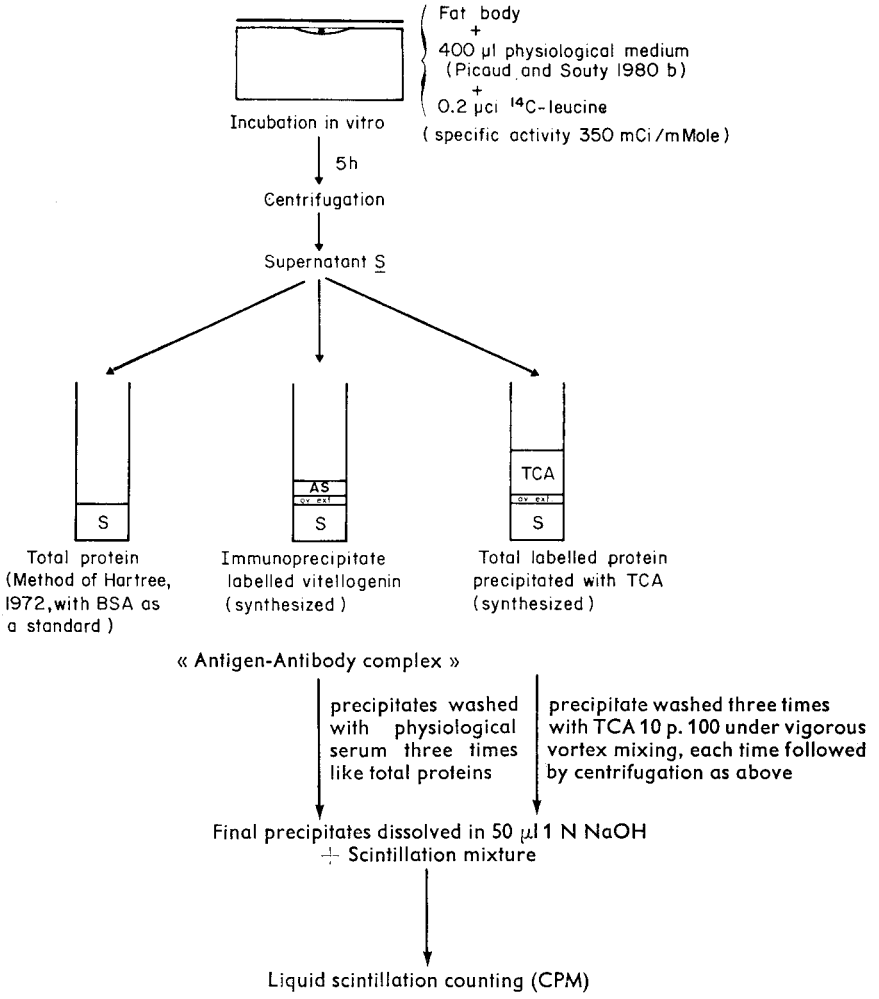

FIG. 1. — *Methods.*

S = Three samples of 100 µl.

AS = antiserum (40 µl). Amount of As was determined by microtests (immunoprecipitates). AS added, incubated at 30 °C for 1 h and then at 4 °C overnight.

Ov. ext. = Ovarian extract (20 µl) ; TCA = Trichloroacetic acid (120 µl).

The preparation of the specific antibodies for the vitellogenin used in this study has been previously described (Picaud and Souty, 1980*a*).

Cold vitellogenin, or more exactly, its immunologically identical substance, i. e. lipovitellin, was furnished by an ovarian extract 1 : 40 in physiological saline.

Male fat bodies (period D) were incubated under the same conditions to compare the incorporation rates of ^{14}C-leucine.

Several assays were made for each period.

Results.

The results are presented as counts/min/100 μg of protein according to the method of Hartree (1972) (table 1).

The male fat bodies incorporated less than the female ones. They never presented as high a rate of incorporation as period D female fat bodies, and did not even reach the lower rate observed during previtellogenesis.

Vitellogenin, synthesized by the fat body during vitellogenesis, is shown in figure 2; the first peak of synthesis was observed during stage C_2, but a highly significant increase coincided with stage D_1''', followed by a drop as laying occurred.

Results concerning x/y (table 1), during stage 1 and a part of stage 2 of vitellogenesis, demonstrated that vitellogenin accounted for most of the increase in protein synthesis. At the end of stage 2, vitellogenin represented 60 to 65 p. 100 of the protein synthesized by pubescent female fat bodies.

Discussion.

This study shows vitellogenin production by the fat bodies of pubescent females at different periods of the molting cycle, concomitant with oocyte development. During previtellogenesis, vitellogenin accounts for about 73 p. 100 of the total protein synthesized. This is not surprising as vitellogenin is transported in the hemolymph before deposition in the oocytes. Using electron microscopy (Souty, 1980), we previously reported that micropinocytotic penetration of the exogenous yolk occurs at an early stage of vitellogenesis (molting cycle stage C_1) and that the most significant increase in synthesis takes place during period D, coinciding with a considerable increase in oocyte length (200 to 500 μm) ; synthesis then decreases from stage D_2 to laying. We have previously shown that micropinocytotic uptake was completely lacking at the end of stage D_1 (Souty, 1980).

The results obtained *in vitro* in this study confirm those realized *in vivo* by Meusy *et al.* (1974) studying the crustacean Amphipoda, *Orchestia gammarellus*.

During stage 1 of vitellogenesis, most of the increased protein synthesis is for vitellogenin production. This coincides with a period of tegument stability. However, during stage 2 of vitellogenesis, there is increased synthesis of protein other than vitellogenin, and it is logical to suppose that this protein may play a part in molting preparation.

TABLE 1

Vitellogenin synthesis by the fat body during molting cycle in females and males (SE : standard error)

FEMALES :

Ovarian cycle	Previtellogenesis		Stage 1 of vitellogenesis			Stage 2 of vitellogenesis				Laying
	Oocytes									
Molting cycle period	Period A	Period B	Period C			Period D				Molt
			Stage			Stage				E
			C_1	C_2	C_3	D_0	D_1'	D_1''	D_1'''	D_2	
Diameter (μm)	50	80-90	90-100	100-140	140-180	180-200	200-400			400-500	
Cycle (days)	1	1	7-8			7					2
Number of assays ..	5		5	6	5	6	6	4	6		5
Vitellogenin											
Antibody precipitate cpm/100 μg protein ± SE ..... (x)	366 ± 149		1 069 ± 419	2 907 ± 930	2 388 ± 1 042	2 588 ± 565	7 179 ± 1 678	8 944 ± 2 700	3 528 ± 1 050		2 254 ± 914
Total protein											
TCA precipitate cpm/ 100 μg protein ± SE (y)	499 ± 115		1 124 ± 494	4 097 ± 1 265	2 960 ± 780	3 273 ± 557	7 185 ± 1 576	13 705 ± 4 461	5 959 ± 1 481		3 142 ± 959
Vitellogenin/Total protein x/y	0.73		0.95	0.71	0.81	0.79	1	0.65	0.59		0.72

MALES :

Number of assays ..						8				
x.....						78 ± 20				
y.....						170 ± 42				
x/y						0.46				

The low incorporation rate in males, which was always less than in females at all stages studied, may be imputed to insufficient washing.

We incubated the fat bodies in a labelled medium and quantified the vitellogenin released by the fat body during a molting cycle. The high rate of incorporation into released vitellogenin coincided with the second stage of vitellogenesis, i.e. rapid oocyte growth. It would be interesting to compare this secreted vitellogenin with the intra-

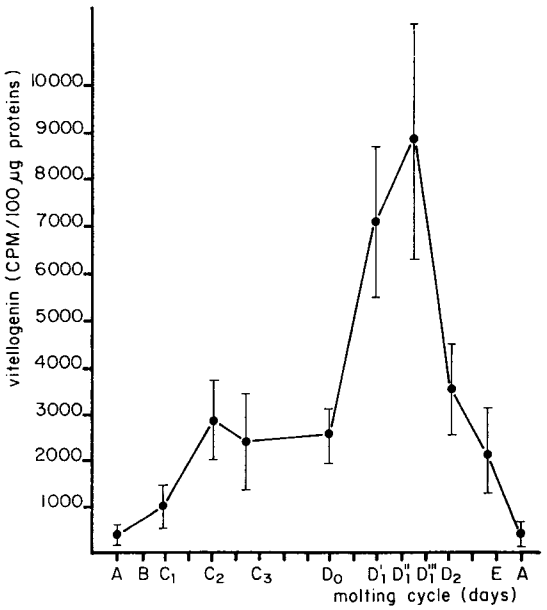

FIG. 2. — *Relationship between the incorporation rates of ^{14}C-leucine by incubated fat bodies and the ovarian cycle (or molting cycle) of* Idotea balthica basteri.

cellular vitellogenin produced by fat body homogenization. Koeppe and Ofengand (1976) studying *Leucophaea maderae* showed that « the amount of vitellogenin in the fat body reaches a steady state after about 3 h of incubation, while that in the medium increases approximately linearly with time after a lag of about 2 h. After 5 h of incubation, 45 p. 100 of the total protein made is vitellogenin. Of the total secreted protein, 75 p. 100 is vitellogenin and the secreted vitellogenin is approximately 80 p. 100 of the total vitellogenin synthesized ». If applied to *Idotea*, these data would prove that our estimation of the amount of vitellogenin released does correspond to a large quantity of true synthesized vitellogenin. On the other hand, the rate of vitellogenin in regard to total protein would be over-estimated if a large part of other protein fractions remains in the fat body.

In view of these results in *Idotea*, we can only draw a parallel between the different peaks of vitellogenin synthesis and the variations of ecdysteroid titers in the hemolymph of several crustaceans during the molting cycle. Results obtained in the Isopoda, *Ligia oceanica*, by radio-immunological methods (Maissiat, 1978) are especially interesting : the principal peak occurred in males at the D_1''' stage of the molting cycle. This point must be clarified in *Idotea* females.

Conclusion.

This study demonstrates that the fat body synthesizes vitellogenin in *Idotea balthica basteri*. We have tried to present quantitative results on vitellogenin synthesis by the

fat body during vitellogenesis (or molting cycle). We also wished to show the relations between the present results and the previous electron microscopic study of oocyte development during vitellogenesis. A parallel is drawn between the different rates of vitellogenin synthesis and the ecdysone titers in the hemolymph during the molting cycle (Maissiat, 1978). It is thus necessary to determine if ecdysone intervenes in vitellogenin synthesis.

Reçu en juin 1980.
Accepté en septembre 1980.

Résumé. La synthèse de la vitellogénine par le tissu adipeux a été étudiée chez les femelles d'*Idotea balthica basteri* (Isopode marin) au cours de la vitellogenèse. Les tissus adipeux ont été incubés dans un milieu contenant un précurseur radioactif. Les immunoprécipités ont été obtenus par addition d'un immunsérum spécifique anti-vitellogénine, et leur radioactivité, mesurée au compteur à scintillation, traduit le taux de synthèse de la vitellogénine marquée. Au cours de la vitellogenèse, la vitellogénine représente la plus grande part des protéines synthétisées par le tissu adipeux. Un premier pic de synthèse est observé dès la première phase de la vitellogenèse (période C du cycle de mue), mais un deuxième pic, beaucoup plus marqué, apparaît pendant la 2e phase de la vitellogenèse (période D du cycle de mue, étape D_1). Avant la ponte (et l'exuviation), le tissu adipeux offre un taux d'incorporation faible.

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