

## SELECTIVITY OF YOLK PROTEIN UPTAKE: COMPARISON OF VITELLOGENINS OF TWO INSECTS

JOSEPH G. KUNKEL\* and M. L. PAN†

Yale University, New Haven, Connecticut and  
University of Massachusetts at Amherst, Amherst, Massachusetts, U.S.A.

(Received 13 November 1975)

**Abstract**--The vitellogenins of *Hyalophora cecropia* and *Blattella germanica* have similar Stokes' radii (69 and 75.8), sedimentation coefficients (15.9 and 16.8s) and isoelectric points (pH 5.7 and 5.0), and share similar amino acid compositions with vitellogenins of other animals. The two vitellogenins show no immunological crossreaction. *Blattella* oocytes take up their own vitellogenin *in vivo* at a rapid rate and that of *Hyalophora* at a low rate comparable to that of a non-vitellogenic protein. *Hyalophora* oocytes take up their own vitellogenin rapidly *in vitro* and *Blattella* Vitellogenin only at a negligible rate. Molecular weights, shapes and charges of the vitellogenins are similar to non-vitellogenins and form no basis for selective uptake.

### INTRODUCTION

In the development of the yolky eggs of many animal species, certain serum proteins are the major source of yolk. These serum proteins, termed vitellogenins, are synthesized and secreted from other maternal tissues (the liver in vertebrates, and the fat body in insects), and enter the oocytes by pinocytosis (WALLACE and DUMONT, 1968; TELFER and SMIM, 1970) to form the vitellin or yolk protein. This uptake is interesting because it is highly selective; the vitellogenin may be one, or a few out of many serum proteins in the blood. Specialized coated vesicles may be involved in the uptake of the vitellogenins (ANDERSON, 1970).

One test of the selectivity of the uptake mechanism is whether oocytes of one species can take up the vitellogenin of another species. Interspecific uptake of vitellogenins has been observed previously between two species of saturniid moth (TELFER, 1960), between closely related cockroaches (BELL, 1972) and also, interestingly enough, between chickens and alligators (SCHJEIDE *et al.*, 1963). The latter might be considered as evidence that the uptake mechanism is rather non-specific since it can span the classes of reptiles and birds. We present evidence here to the contrary, showing that purified vitellogenins from two species of insects of different orders, Dictyoptera and Lepidoptera, will not support vitellogenesis in each other's oocytes, even though they have very similar gross chemical properties. The results indicate that the selective uptake mechanism involves recognition of subtle differences in the protein structure.

\* Present address: Zoology Department, University of Massachusetts at Amherst, Amherst, Mass. 01002, U.S.A.

† Present address: Department of Zoology, University of Tennessee, Knoxville, Tenn. 37916, U.S.A.

### MATERIALS AND METHODS

#### *Animals*

Cockroaches, *Blattella germanica*, were kept in synchronous culture by controlling their feeding (KUNKEL, 1966), thus providing a source of uniformly aged adult females for use in experiments. Silkworms, *Hyalophora cecropia*, were raised on black cherry foliage in the field; pupae were chilled to terminate diapause: and pharate adults were staged according to SCHNEIDERMAN and WILLIAMS (1954).

#### *Isolation of vitellogenins*

Since the vitellogenins may be modified after uptake into the oocytes (BROOKES and DEJMAL, 1968; WALLACE, 1964), they were isolated from the blood of ovariectomised females, in which the vitellogenins accumulate. The vitellogenins of the two species and two non-vitellogenic serum proteins, I and II, of *Blattella* were purified by combinations of DEAE cellulose chromatography and sucrose gradient centrifugation (PAN and WALLACE, 1974; KUNKEL, in prep.).

#### *Labeling of vitellogenins*

For *Blattella* 0.2  $\mu$ Ci of uniformly labeled  $^{14}$ C-amino acid mixture (Schwarz-Mann) was injected daily into each of 58 ovariectomised adult females on days 2 to 5 after the first feeding. On the 6th day after feeding, haemolymph was collected in 0.15 M NaCl buffered with 0.01 M Sodium phosphate, pH 7.2 (PBS). The serum was centrifuged to remove cells and the vitellogenin was then isolated. For *Hyalophora*, 4 diapausing pupae were ovariectomised according to TELFER (1954) and transferred to 25°C to initiate pupal adult development. On day 15 to 16 of the pharate adult

stage, each was injected with 0.1 mCi 3H-leucine (Schwarz-Mann), and held for 4 days to label the vitellogenin. Haemolymph was collected, with a trace of phenylthiourea to prevent melanin formation, centrifuged to remove cells, and pooled. Vitellogenin was then isolated.

After purification, the specific activities of the proteins were determined by dissolving samples in NCS (Nuclear Chicago), counting by liquid scintillation in toluene scintillator (Packard Tricarb 3003), and expressing radioactivity on the basis of protein estimated from absorbance at 280 nm. The amount of radioactive protein taken up by oocytes was estimated in a similar manner, correcting for differences in counting efficiency. Double label counting was performed as described by BUSH (1964).

#### Immunochemical methods

Antisera against the vitellogenins of *Blattella* and *Hyalophora* were obtained by immunizing rabbits with ovariectomized female blood and yolk extracts, respectively, emulsified with Freund's complete adjuvant. Antisera thus prepared were rendered specific for vitellogenins by absorption with larval blood for *Blattella* and with adult male blood for *Hyalophora* antiserum. Figure 1 demonstrates the purity of the vitellogenins as well as the specificity of the antibodies.

Micro-Ouchterlony tests were performed on standard 25 x 75 mm microscope slides covered with 2 ml of 0.5% agarose made in PBS. Wells were cut with a 14 gauge needle and the distance from the peripheral wells to the centre well was 5 mm. Immunoelectrophoresis was performed as described previously (PAN and WYATT, 1971). The Oudin test was carried out as described previously (BECKER *et al.*, 1951).

#### Biochemical analyses

Agarose gel filtration (Biogel A1.5, Bio Rad Labs) was used for determining molecular (Stokes') radii (ACKERS, 1964, 1967). The elution volume ( $V_e$ ) of a protein was determined on a 2.5 x 50cm agarose column. Blue dextran (Pharmacia Co.) was used to determine the void volume ( $V_0$ ), and dinitrophenylalanine was used to determine the internal volume ( $V_i$ ). The column partition coefficient,  $\sigma$ , for a protein was calculated as  $(V_e - V_0)/V_i$ . The values for three calibration proteins of known Stokes' radii (ACKERS, 1964) were determined using multiple independent runs. The inverse error-frequency-complement ( $\text{erfc}^{-1}$ ) transformation of  $\sigma$  (ACKERS, 1967) was calculated from standard tables of the error function. Transformations of  $\sigma$  were plotted against the known Stokes' radii and a straight line was fitted to the data using least squares (Fig. 2). The Stokes' radius of an unknown protein with an associated standard error of estimation was calculated from its elution volume by extrapolation using the standard curve (RAO, 1965).

Amino acid analyses were performed on the purified proteins delipidated by three successive chloroform

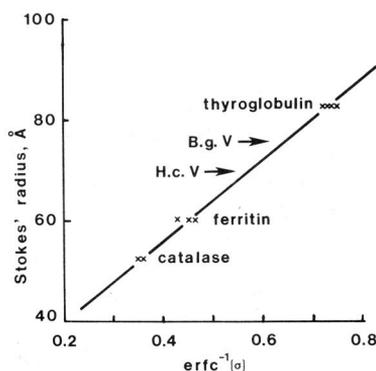


Fig. 2. Gel filtration determination of Stokes' radii of the vitellogenins of silk moth and cockroach. The Stokes' radii of standard proteins were plotted against the inverse error frequency complement ( $\text{erfc}^{-1}$ ) of the column partition coefficient,  $\sigma$ , for each protein, (2.5 x 50 cm, BioGel A1.5). The arrows point to the average elution position for three runs of each unknown: *Blattella* vitellogenin BgV and *Hyalophora* vitellogenin, HcV.

methanol (2:1) extractions, followed by methanol and anhydrous ether rinses and drying under a continuous flow of dry nitrogen. Multiple samples of 2 mg protein were hydrolysed *in vacuo* in 6 N HCl at 110C for 24, 48 and 72 hr and the hydroly-sates were analysed on a Beckman 120C amino acid analyser with automatic integrator using an accelerated technique (SPACKMAN, 1967). Data were analysed by digital computer, extrapolating ammonia, threonine and serine to zero hydrolysis time and valine and isoleucine to infinite time (SPACKMAN *et al.*, 1958). Cystine plus cystine was determined as cys-teic acid by performic acid oxidation (HIRS, 1967). Tryptophan was determined spectrophotometrically by its oxidation with N-bromo succinimide (SPANDE and WITKOP, 1967).

Percent lipid was determined from the chloroform:methanol extracts of the proteins. Carbohydrate content of the extracted proteins was measured by the anthrone reaction (SPIRO, 1966) and expressed as a percentage as mannose (YAMASAKI, 1973; BARZEV *et al.* 1975). Partial specific volumes were calculated from composition data (SCHACHMAN, 1966) assuming specific volumes of 0.640 and 1.093 respectively for the carbohydrate and lipid components. Molecular weights and frictional ratios,  $ff/\rho$ , of the native proteins were calculated from the sedimentation coefficients, Stokes' radii and partial specific volumes (SIEGEL and MONTY, 1966). Subunit mol. wt were determined by SDS gel electrophoresis (WEBER *et al.*, 1972). The sedimentation coefficients were estimated by sucrose gradient sedimentation (MARTIN and AMES, 1961) using human IgG, catalase and cecropia vitellogenin (PAN and WALLACE, 1974) as known sedimenting markers.

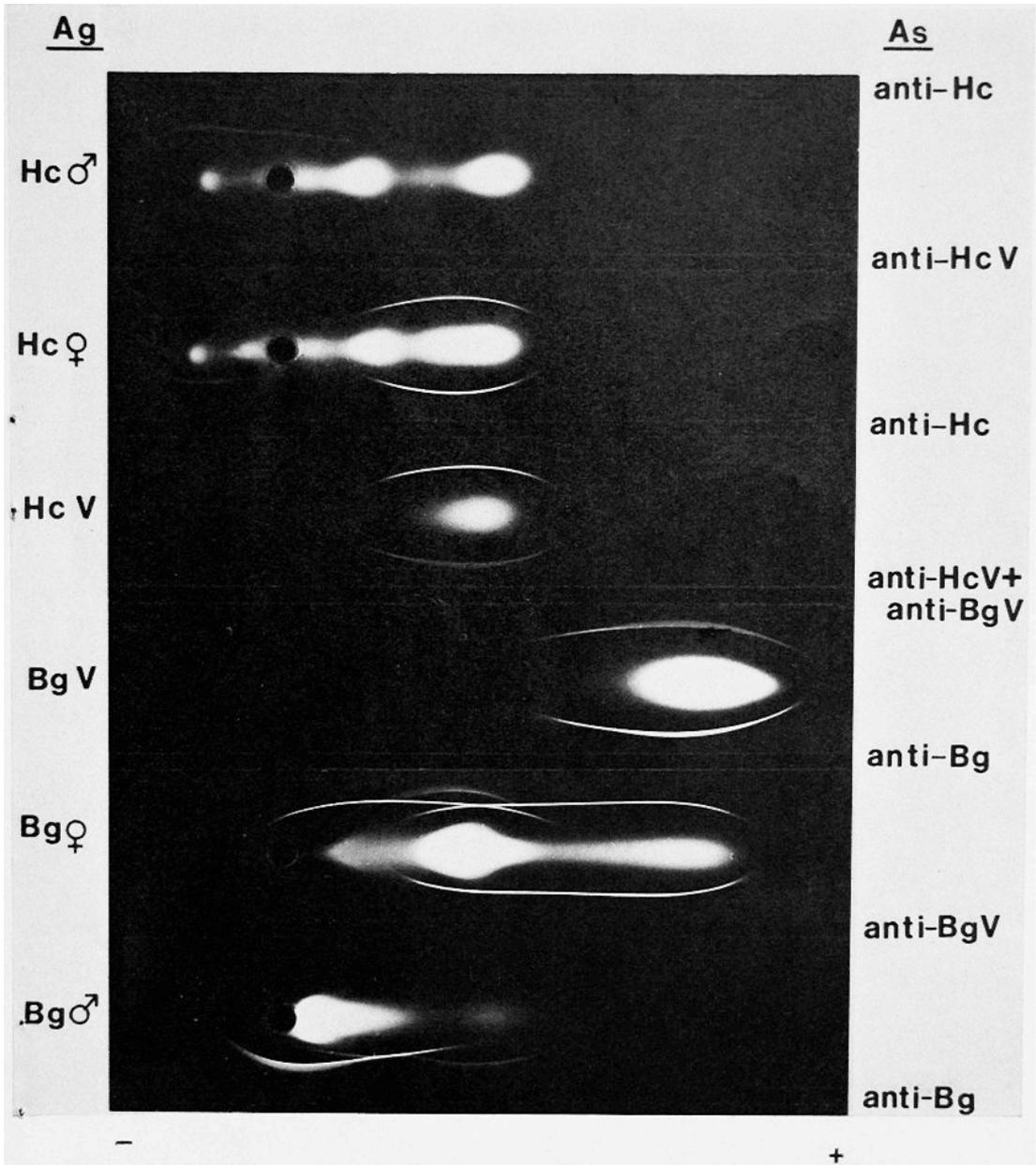


Fig. 1. Immunoelectrophoresis of purified vitellogenin, V, male sera and ovariectomised female sera of *Blattella*, Bg and *Hyalophora*, Hc. The antisera used were: a complex antiserum against silk moth yolk, anti-Hc; the same antiserum adsorbed making it specific for vitellogenin, anti-HcV; a complex antiserum against *Blattella* ovariectomised female serum, anti-Bg; the same antiserum adsorbed to make it specific for vitellogenin, anti-BgV. Illustrated is a photographic composite of two slides; on one slide the proteins were stained immediately after electrophoresis, and in a duplicate the antisera were added and immunodiffusion was allowed to occur before washing and staining.

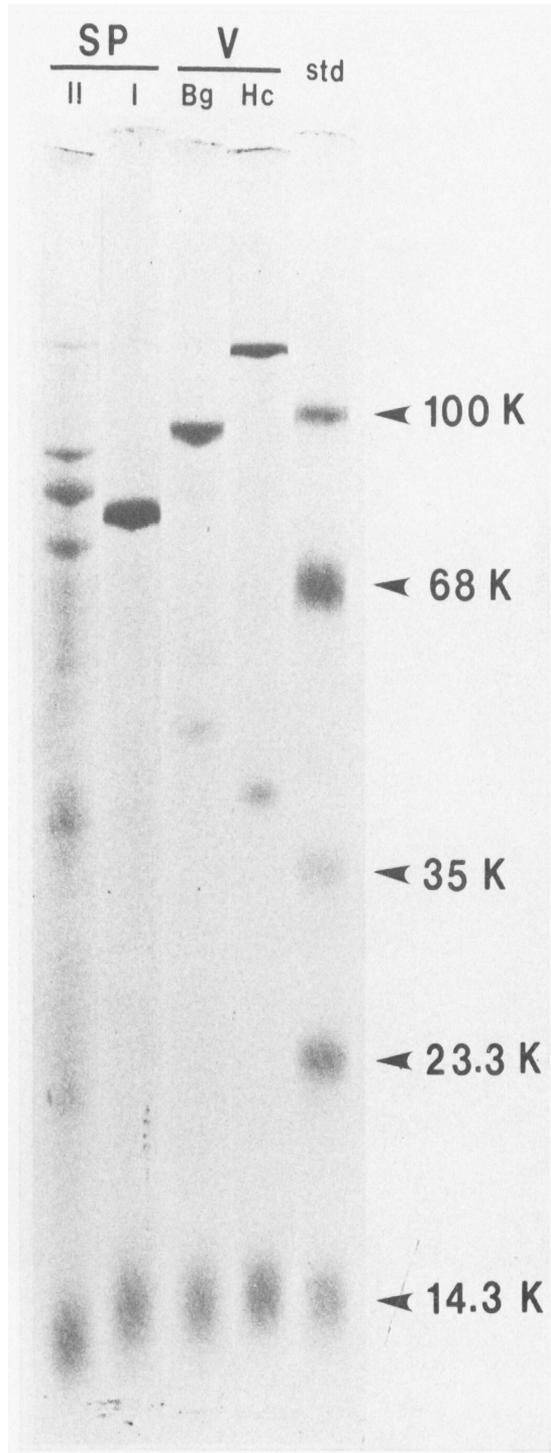


Fig. 4. Subunit composition of purified serum proteins determined by SDS acrylamide gel electro-phoresis (7.5 % acrylamide, 0.4 x 8 cm gels, Coomassie Brilliant Blue stain; WEBER *et al.*, 1972) SP = non-vitellogenic serum proteins I and II of *Blattella*. V , vitellogenins of *Blattella*, Bg and *Hyalo-phora* Hc. STD = a gel with five known molecular weight protein standards, top to bottom: phosphorylase a, bovine serum albumin monomer, pepsin, trypsin and lysozyme. Each of the gels contains an equivalent amount of lysozyme as an internal mobility standard.

**RESULTS**

*Physical properties of the vitellogenins*

Physical and chemical properties of the two vitello-genins and the two *Blattella* serum proteins are given in Table 1. The Stokes' radii of the vitellogenins of *Hyalophora* and *Blattella*, although similar, are significantly different ( $P < 0.001$ ). The two other major serum proteins in adult *Blattella*, I and II, have similarly large Stokes' radii but *Bg* II despite its large size has a low sedimentation coefficient due to its high (53%) lipid content. The similar size of the two vitellogenins is also indicated by their mobility in sucrose gradients (Fig. 3). They form a single absorbance peak sedimenting at about 16s, but the  $^{14}\text{C}$  labelled *Blattella* protein is seen to sediment at about 16.8s, slightly faster than the  $^3\text{H}$  labelled *Hyalophora* protein whose sedimentation coefficient, 15.9s, has been determined in the analytical ultracentrifuge (PAN and WALLACE, 1974). Based on their Stokes' radii, partial specific volumes and sedimentation coefficients, the calculated molecular weights and frictional ratios of the four proteins suggest that they are all similarly large and spherical (Table 1).

Despite similarities in native molecular weight, unique subunit structure is revealed by SDS acrylamide gel electrophoresis, Fig. 4. Both vitellogenins have a large and a small subunit. The *Blattella* vitellogenin has variable minor bands which may be artefacts of how long the protein had been in the bloodstream of the ovariectomised female. Molecular weights of the subunits were estimated by running suitable known mol. wt standard proteins in the same gels with the unknowns and extrapolating on a mobility vs log mol. wt plot of the standard proteins (Table 1). Since molar ratios of the subunits cannot be adequately determined from the stainability of the gels it is not possible to accurately estimate the number of each subunit in the native 16s vitellogenins. Since *Blattella* serum protein I has only one subunit it was possible to calculate that there are six subunits in the native protein. This along

with its sedimentation coefficient and amino acid composition (Table 2), suggest that it is similar to the protein calliphorin (MUNN *et al.*, 1972). *Blattella* serum protein II has an apparently heterogeneous subunit composition. A serum protein with similar properties has been described in

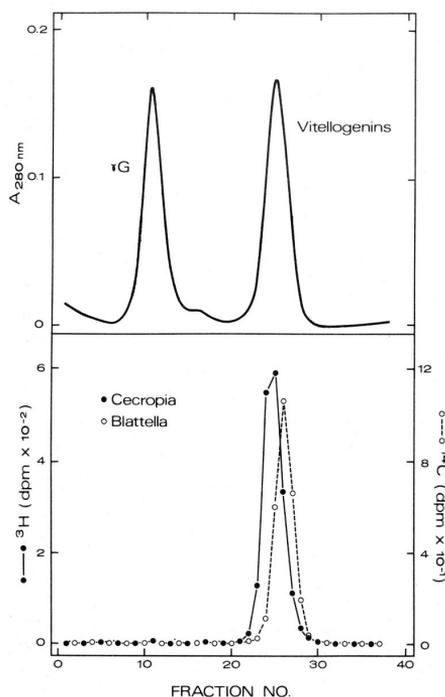


Fig. 3. Relative sedimentation of  $^3\text{H}$ -*Hyalophora* vitellogenin and  $^{14}\text{C}$ -*Blattella* vitellogenin. Human IgG was used as a 7S sedimentation marker. Sedimentation is from left to right, 5 to 25 $\times$ 5[ $\mu$ ], sucrose gradient in PBS (International SB283 rotor, 41,000 rev/min, 15 hr), fractions were differentially counted in Bray's solution (BRAY, 1960).

Table 1. Physical and chemical properties of vitellogenic and non-vitellogenic serum proteins

Vitellogenins	pI	%	%	-	Stokes'	MW§	MW		
					radius	native	SDS		
		CHO	lipid	Vξ	(Å)	$S_{20,w}$	( $\times 10^3$ )	$f/f_0$ §	( $\times 10^3$ )
<i>Blattella germanica</i>	5.0	4.5	15.7	0.784	75.0 $\pm$ 1.6'	16.8	659	1.27	100
<i>Hyalophora cecropia</i>	5.7	1.0	9.43'	0.758	69.4 $\pm$ 1.6'	15.9	516	1.29	52
<b>Non-vitellogenins</b>									
<i>Bg</i> I	5.5		0.0	0.731	59.5	19.0	476	1.16	120
<i>Bg</i> II	6.9		53.2	0.926	63.0	5.4	511	1.10	43

\* mean  $\pm$  standard error of the mean for three replicates.

t from PAN and WALLACE (1974).

ξ calculated from amino acid, CHO and lipid composition (Schachman, 1957).

§ calculated from partial specific volume, Stokes' radius and sedimentation coefficient. (SIEGEL and MONTY, 1966).

**Table 2. Protein composition expressed as mole per cent**

i	m=	Vitellins*								Non-vitellins	
		Bg 1	Lm 2	Lo 3	Hc 4	Ld 5	Gg 6	Rp 7	Xl 8	BgI 9	BgII 10
Asp		12.18	14.76	11.13	9.68	9.97	11.08	8.88	8.55	12.01	10.90
Thr		5.97	5.65	4.82	5.15	5.36	5.54	4.79	6.35	3.35	6.09
Ser		9.55	8.15	7.57	9.24	9.70	7.06	8.55	7.70	3.51	6.30
Glu		10.97	11.30	12.71	14.32	12.81	11.08	10.95	13.68	11.66	11.22
Pro		4.47	4.75	6.03	4.42	5.19	3.60	4.96	5.12	5.81	3.62
Gly		3.07	3.40	5.10	4.39	5.17	4.99	5.25	5.16	4.42	6.69
Ala		4.92	5.71	8.46	7.50	5.50	7.48	8.35	9.94	5.06	7.97
Val		7.75	6.48	7.81	5.59	6.84	6.79	7.64	5.24	7.76	9.24
Met		2.82	1.80	1.70	1.96	2.52	2.08	2.66	2.16	2.63	0.00
Ile		4.74	4.56	5.10	4.45	5.68	6.51	7.35	4.30	3.83	2.39
Leu		8.69	8.99	10.24	6.02	7.05	10.94	8.95	8.48	6.12	10.42
Tyr		4.89	4.36	5.06	5.16	3.78	3.60	2.82	2.99	8.87	3.04
Phe		4.49	4.36	2.95	3.47	4.72	4.43	3.49	4.20	6.41	5.03
Lys		7.06	6.16	5.71	7.55	7.75	8.31	7.35	7.47	8.01	9.20
His		4.14	3.15	1.58	3.14	2.90	1.52	3.03	3.00	4.89	4.54
NH <sub>3</sub>		14.54	--	--	15.17	--	--	12.47	--	11.70	11.89
Arg		4.27	6.42	4.05	7.94	5.01	4.99	4.92	5.70	4.67	3.36
Cys/2		0.86	--	1.01	--	--	0.55	--	--	--	--
Try		0.79	--	2.19	0.62	--	1.80	1.13	--	--	--

\* Bg, BgI, BgII are *B. germanica* vitellogenin, serum protein I and II respectively; Gg is *Gallus gallus* lipovitellenin (COOK *et al.*, 1962); Hc is *H. cecropia* vitellogenin; Ld is *Leptinotarsa decemlineata* vitellogenin (DE LOOF and DE WILDE, 1970); Lm is *Leucophaea maderae* vitellogenin (ENGELMAN and FRIEDEL, 1974); Lo is *Locusta migratoria* vitellogenin (CHEN and WYATT, personal communication); Rp is *Rana pipiens* lipovitellin (WALLACE, 1967); Xl is *Xenopus laevis* lipovitellin (FOLLETT *et al.*, 1968).

*Locusta migratoria* (PELED and TIETZ, 1965). Isoelectric points of the two vitellogenins were taken as the pH of zero mobility in electrophoresis (Fig. 5). There is a difference of approximately 0.7 pH unit in isoelectric point which may account for the higher electro-phoretic mobility of *Blattella* vitellogenin seen in Fig. 1. *Blattella* serum protein I has an acid isoelectric point between that of the two vitellogenins while serum protein II has an isoelectric point close to neutrality (Table 1).

The amino acid composition of the vitellogenins and the non-vitellogenins serum proteins is given in mole per cent in Table 2. Compositions of yolk proteins of six other species are included for comparison. A difference function, *SDQ*, of MARCHA-LONIS and WELTMAN (1971) was applied to the composition data of Table 2 to indicate relative differences between the proteins. Table 3 lists the *SDQs* comparing the 10 proteins of Table 2. The vitellins resemble one another as a loose group. The average comparing the seven vitellins is 55 (range 19 to 96). It is noteworthy that the lowest difference in Table 3 is the comparison of the two cockroach vitellogenins.

Both vitellogenins have substantial lipid components, 9 and 15%. The carbohydrate content of the vitellogenins determined by the anthrone reaction was found to be 4.5% mannose equivalents for *Blattella* vitellogenin and 1% mannose equivalents for *Hyalophora* vitellogenin. The larger amount of sugar residues

in *Blattella* vitellogenin was also indicated by the pronounced caramelization of the protein hydro-lysates and by the appearance of a glucosamine/galactosamine peak in the amino acid analysis of the 24

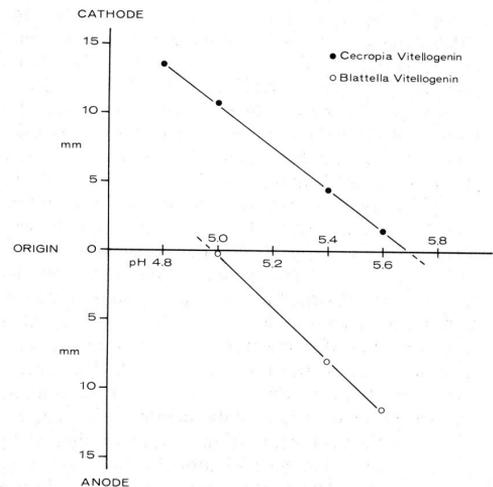


Fig. 5. Isoelectric point determination for vitellogenins. Distance of migration in agarose on microscope slides is measured against the pH of the buffer.

Table 3. Relatedness based on mole per cent\* amino acid composition. Departures from identity are measured by the SAQ § statistic of MARCHALONIS and WELTMAN (1971).

k \ j =	Vitellins								Non-vitellins	
	Bg	Lm	Lo	Hc	Ld	Gg	Rp	Xl	Bgl	BglI
1	0	19	82	55	22	39	44	68	76	63
2		0	71	56	42	39	63	72	82	76
3			0	96	80	67	67	76	144	114
4				0	24	64	58	29	107	109
5					0	36	25	65	80	68
6						0	21	40	101	45
7							0	24	121	62
8								0	119	62
9									0	99
10										0

\* For consistency the mole percents of Table 2 were computed ignoring the contribution of NH<sub>3</sub>, Cys/2 and Try to the denominator and these groups were not used in the application of the equation for SAQ.

§  $SAQ_{jk} = \sum (P_{ij} - P_{ik})^2$ , where  $P_{im}$  = mole per cent of amino acid 'i' in protein 'm', 'j' and 'k' index the columns and rows of Table 3. 'i' and 'm' indexes the amino acids and proteins of Table 2.

hr hydrolysates only. No such caramelization nor peak was observed for *Hyalophora* vitellogenin.

To determine if there are detectable antigenic similarities of the two vitellogenins, they were compared immunologically using the Ouchterlony double diffusion test as well as the Oudin single diffusion test. No visible reaction occurred between either protein and the antiserum prepared against the other.

#### Physiological comparison of vitellogenins

Reciprocal experiments were performed to test whether the two vitellogenins could replace each other physiologically. First the two radioactively labeled vitellogenins were tested for their ability to be taken up *in vivo* from the haemolymph into the ovaries of *Blattella*. Four days after molting 60 unfed virgin adult female cockroaches were fed simultaneously at 30°C and allowed to develop for 98 hr, by which time, active yolk deposition was taking place (KUNKEL, 1973). They were then injected with approximately 36 µg of <sup>14</sup>C-*Blattella* vitellogenin (60 dis/min/µg) or <sup>3</sup>H-*Hyalophora* vitellogenin (150 dis/min/µg), or <sup>14</sup>C-*Blattella* serum protein II (105 dis/min/µg) as a control for non-specific uptake. At 2, 4, 8, and 19 hr after injection, five animals were sacrificed for each of the proteins injected. The ovaries were dissected out, rinsed in PBS, and the amount of incorporated radioactivity determined. Figure 6 illustrates the proportion of the injected radioactivity taken up from the haemolymph by the ovaries by different times. The injected *Blattella* vitellogenin was taken up rapidly (initial rate of 0.2 to 0.3 µg/oocyte-te/hr.) while the *Hyalophora* vitellogenin was taken up at a much slower rate similar to that for the con-

trol protein, *Blattella* serum protein II. Therefore, it seems that *Blattella* oocytes do not recognize *Hyalophora* vitellogenin as a vitellogenin protein.

Uptake of the two vitellogenins into the oocytes of *Hyalophora* was tested by incubation of oocytes *in vitro*. Equivalent weights of <sup>14</sup>C-*Blattella* vitellogenin and <sup>3</sup>H-*Hyalophora* vitellogenin were added to 200 µl of medium, making it approximately 1.8% protein. Ovaries were dissected from 18 to 19 day pharate adult moths and individual vitellogenin oocytes with surrounding follicle cells were isolated and graded in size. Medium sized vitellogenin oocytes were placed into the incubation medium. Samples of oocytes were taken from the medium at intervals and rinsed. The diameter of each oocyte was measured before it was dissolved in NCS for counting. The average amount of each protein taken up *per* oocyte as well as the counts per min per unit surface area are given in Fig. 7. In this incubation of *Hyalophora* oocytes, the moth vitellogenin was taken up at a rate of about 0.75 µg/oocyte/hr, while the cockroach vitellogenin was virtually excluded.

#### DISCUSSION

The mechanism by which oocytes recognize vitellogenin is of interest as an example of how a cell communicates with its environment through its cell surface. The oocyte surface comes in contact with many serum proteins during vitellogenesis but only vitellogenin is recognized and singled out for rapid pinocytotic uptake. A previous result (SCHJEIDE *et al.*, 1963) has suggested that the method of recognition of vitellogenin by an oocyte might not be highly specific.

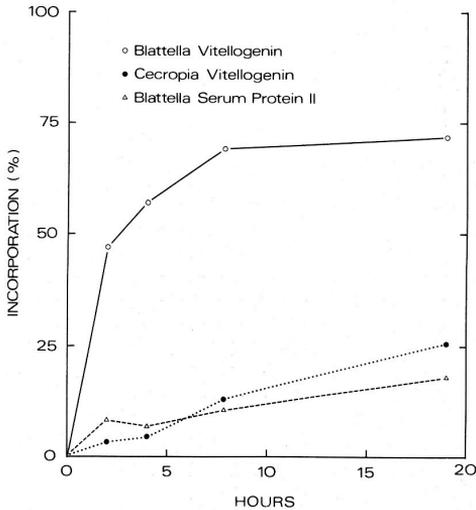


Fig. 6. Uptake of serum proteins into *Blattella* oocytes *in vivo*. At zero time approximately 36  $\mu$ g of radioactively labelled protein was injected into groups of females which had been fed for 98 hr. The proportion of the injected protein taken up is plotted against time since injection.

In testing this hypothesis we have taken two insect vitellogenins and compared their physical, chemical and physiological properties.

Vitelins and their precursors, vitellogenins tend to be large macromolecules (8.9 to 28s; COOKE *et al.*, 1962; WALLACE *et al.*, 1967; BROOKES and DEJMAL, 1968; PAN and WALLACE, 1974): the cockroach and silkmoth vitellogenins studied above conform to this generality, both sedimenting close to a rate of 16s with diameters between 140 and 150 $\text{\AA}$ . The partial specific volumes ( $V$ ) computed from composition data are all higher than those usually assumed for simple proteins even for *Blattella* serum protein I, which lacks carbohydrate and lipid. The partial specific volume computed for silkmoth vitellogenin agrees well with a previous determination by pycnometry (PAN and WALLACE, 1974). The mol. wt computed from the Stokes' radii, sedimentation coefficients, and partial specific volumes show that all the serum proteins studied are large, 476,000 to 659,000 mol. wt. The computed frictional ratios, *ffo*, show them all to be close to spherical. This large spherical size of the vitellogenins may be an adaptation for serving as an efficient transport and storage protein. A more general reason that all the major serum proteins of *Blattella* may be of large diameter is to avoid filtration by the pericardial cells, which have specialised intracellular filtration junctions to prevent large molecules from reaching the pinocytotic surface (CROSSLEY, 1972; LIBERTOFF and KUNKEL, in prep.).

Both vitellogenins are heteropolymers with two apparently different size classes of subunits. However, since the vitellogenins contain a substantial amount of

carbohydrate the determinations of mol. wt may not be accurate (WEBER *et al.*, 1972). *Blattella* serum protein I which lacks substantial carbohydrate has a single subunit of about 80,000 mol. wt. This suggests that the native protein has six subunits. *Blattella* serum protein II although homogeneous on sucrose gradients, disc electrophoresis pH 7.5, and immuno-electrophoresis pH 6.5, gives multiple bands on SDS gel electrophoresis. This is similar to a serum lipo-protein from *Locusta* which also shares an extremely similar amino acid composition (PELED and TIETZ, 1975).

The isoelectric points of vitellogenins in this study were low, 5.0 and 5.7, so that at physiological pH they would have a pronounced negative charge. This could not be a sufficient criterion to allow for vitello-genic recognition since the non-vitellogenic serum proteins of *Blattella* would also be negatively charged. Negative charge would also be an unusual general criterion for uptake since in other model pinocytotic systems most proteins will only stimulate pinocytosis below their isoelectric points i.e. when they are positively charged (GIESE, 1973).

The relative chemical composition of vitellogenins might give some clue to specificity. The amino acid composition of the eight vitellogenins show some superficial similarities such as high aspartate and glutamate and low methionine which are characteristics of proteins in general (KING and JUKES, 1969). In the absence of amino acid sequence data it is difficult

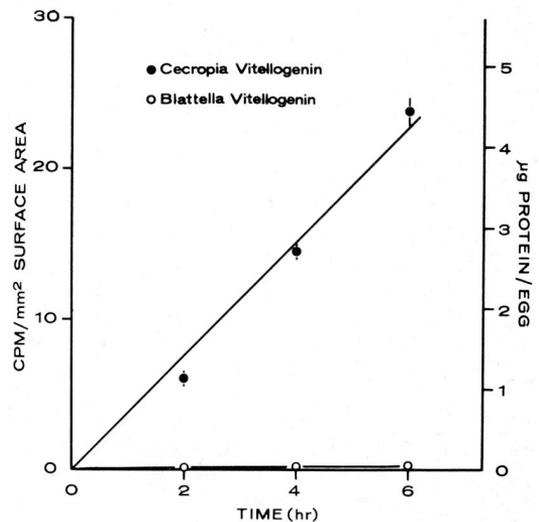


Fig. 7. Uptake of vitellogenins into silkmoth oocytes *in vitro*. Medium-sized vitellogenic oocytes were placed into 200  $\mu$ l of a culture medium (PAN *et al.*, 1967) containing approximately 9 mg/ml each of C-14-*Blattella* vitellogenin and H-3-*Hyalophora* vitellogenin. The uptake over time with associated standard error (vertical bars) was calculated in counts per min per square mm of oocyte surface and is also displayed in  $\mu$ g of protein entering the average sized egg.

to make judgements of the homology of the proteins being compared. We applied the difference function of MARCHALONIS and WELTMAN (1971) to the amino acid composition data. This function,  $SAQ$ , measures departures from identical mole percent amino acid composition on a scale from zero to 20,000. Identical proteins analysed in different laboratories are found to have  $SAQ$ s of up to 4. The distribution of  $SAQ$  for large numbers of comparisons of related and unrelated proteins has been published (MARCHALONIS and WELTMAN, 1971), providing a background for comparison of the tabulated analyses of vitellogenins. The vitellogenins form a group in terms of amino acid composition. None of the 28 comparisons of vitellogenins result in  $SAQ$ s over 100, while fewer than 2 percent of the 820 cross comparisons of 41 unrelated proteins have  $SAQ$ s lower than 100 (MARCHALONIS and WELTMAN, 1971). The uniformly low  $SAQ$ s demonstrate a conservatism in amino acid composition of vitellogenins. However, immunological cross-reaction, a measure of amino acid sequence difference (PRAGER and WILSON, 1971), suggests that vitellogenins are not conservative in primary structure. As reported above, the cockroach and silk moth vitellogenins do not show any immunological crossreaction, and subsequent work has shown that insect vitellogenins do not crossreact far outside the genus level of relationship (KUNKEL, JOHNSON, HAGGERTY and SARGENT, in prep.). This suggests marked divergence in amino acid sequence among vitellogenins, despite similarity in mole percent composition.

The vitellogenins also vary in the amount of conjugated carbohydrate and lipid that they contain. *Blattella* vitellogenin contains about five percent mannose equivalents compared to one percent in *Hyalophora* vitellogenin and none in *Rana pipiens* lipovitellin. While the gross physical properties of vitellogenins do not seem sufficient to account for specificity of uptake, the differences in primary structure and carbohydrate content may form the basis of that specificity.

#### *Physiological properties of the serum proteins*

The physiological tests showed that the oocytes of the cockroach and silk moth could not recognize each others vitellogenins for selective uptake. The uptake by *Hyalophora* oocytes *in vitro* demonstrates a high specificity for their particular vitellogenin: very little cockroach vitellogenin entered the oocytes in the six hours of incubation. *Blattella* oocytes *in vivo* over a twenty hr period demonstrate uptake of *Hyalophora* vitellogenin at a low rate which did not exceed that of a control non-vitellogenic cockroach serum protein under the same conditions. A previous study (SCHJEIDE *et al.*, 1963), claiming specific uptake of alligator yolk protein into chicken oocytes, did not control for non-specific uptake, and thus fails to provide evidence for lack of specificity of the uptake

process. Non-specific uptake at a low rate may occur as an accompanying leakage during rapid pinocytotic uptake of vitellogenin. Alternately or in addition, non-specific uptake could be occurring by way of an independent set of pinocytotic vesicles as suggested by ANDERSON (1970).

The possibility of an adapter or recognition molecule produced by the follicle cells (ANDERSON and TELFER, 1969; BELL and SAMS, 1974) although intrinsically interesting does not alter the necessity for a recognition site on the vitellogenin. We propose that this site depends on subtle differences in the structure of the vitellogenin which allow it to be picked out from a mixture of grossly similar serum proteins.

*Acknowledgements*--We are indebted to G. R. WYATT for coordination, for advice and encouragement, and for the use of his laboratory at Yale. We thank J. S. FRUTON for the use of the amino acid analyser, and S.S. HUSAIN for instruction in its use. This research was supported by grants from the National Institutes of Health (HD-02176), and the Whitehall Foundation to G. R. WYATT, and by a University of Massachusetts Faculty Research Grant, and a grant from the National Institutes of Health (AI-11269) to J.G.K. We are grateful for the fine technical help of R. MCCARTHY.

## REFERENCES

- ACKERS G. K. (1964) Molecular exclusion and restricted diffusion processes in molecular sieve chromatography. *Biochemistry* 3, 723-730.
- ACKERS G. K. (1967) A new calibration procedure for gel filtration columns. *J. biol. Chem.* 242, 3237-3238.
- ANDERSON E. (1970) Two types of coated vesicles in oocyte development. *de Microscope* 8, 721-738.
- ANDERSON L. M. and TELFER W. H. (1969) A follicle cell contribution to the yolk spheres of moth oocytes. *Tissue Cell* 1, 633-644.
- BARZEV A., WAJIC E., COHEN E., SAPIR L., APPLEBAUM S. W., and EMMERICH H. (1975) Vitellogenin accumulation in the fat body and haemolymph of *Locusta migratoria* in relation to egg maturation. *J. Insect Physiol.* 21, 1257-1263.
- BECKER E. L., MUNOZ J., LAPRESLE C., and LEBEAU L. J. (1951) Antigen-antibody reactions in agar II. Elementary theory and determination of diffusion coefficients of antigens. *J. Immunol.* 67, 501-511.
- BELL W. J. (1972) Yolk formation by transplanted oocytes. *J. exp. Zool.* 181, 41-48.
- BELL W. J. and SAMS G. R. (1974) Factors promoting vitellogenin competence and yolk deposition in the cockroach ovary: the post ecdysis female. *J. Insect Physiol.* 21, 173-180.
- BRAY G. A. (1960) A simple efficient liquid scintillator for counting aqueous solutions in a scintillation counter. *Analyt. Biochem.* 1, 279-285.
- BROOKES V. J. and DEJMAL R. K. (1968) Yolk protein: structural changes during vitellogenesis in the cockroach *Leucophaea maderae*. *Science, Wash.* 160, 999-1001.
- BUSH E. T. (1964) Liquid scintillation counting of doubly-labeled samples. *Analyt. Biochem.* 36, 1082-1089.
- COOK W. H., BURLEY R. W., MARTIN W. G., and HOPKINS J. W. (1962) Amino acid composition of the egg-yolk lipoproteins and a statistical comparison of their amino acid ratios. *Biochem. Biophys. Acta* 60, 98-103.

- CROSSLEY A. C. (1972) The ultrastructure and function of pericardial cell and other nephrocytes in an insect *Calliphora erythrocephala*. *Tissue Cell* 4, 524-560.
- DELOOF A. and DEWILDE J. (1970) The relation between haemolymph proteins and vitellogenesis in the Colorado Beetle, *Leptinotarsa decernlineata*. *J. Insect Physiol.* 16, 157-169.
- ENGELMAN F. and FRIEDEL T. (1974) Insect yolk protein precursor, a JH induced phosphoprotein. *Life Sciences* 14, 587-594.
- GIESE A. C. (1973) *Cell Physiology*, 4th Edition, W. B. Saunders, Philadelphia, 741 pp.
- HIRS C. H. W. (1967) Determination of cystine as cysteic acid. *Methods in Enz.* XI, 59-62.
- KING T. H. and JUICES J. L. (1969) Non-Darwinian Evolution. *Science, Wash.* 164, 788-798.
- KUNKEL J. G. (1966) Development and the availability of food in the German Cockroach, *Blattella germanica* (L.) *J. Insect Physiol.* 12, 227-235.
- KUNKEL, J. G. (1973) Gonadotrophic effect of Juvenile hormone in *Blattella germanica*: a rapid simple quantitative bioassay. *J. Insect Physiol.* 19, 1285-1297.
- MARCHALONIS J. J. and WELTMAN J. K. (1971) Relatedness among proteins. *Comp. Biochem. Physiol.* 38B, 609-625.
- MARTIN R. G. and AMES B. N. (1961) A method for determining the sedimentation behavior of enzymes: Application to protein mixtures. *J. Biol. Chem.* 236, 1372-1385.
- MUNN E. A., FEINSTEIN A., and GREVILLE G. D. (1971) The isolation and properties of the protein calliphorin. *Biochem. J.* 124, 367-374.
- PAN M. L. and WALLACE R. A. (1974) Cecropia Vitellogenin: Isolation and Characterization. *Amer. Zool.* 14, 1239-1242.
- PAN M. L. and WYATT G. R. (1971) Juvenile hormone induces vitellogenin synthesis in the monarch butterfly. *Science, Wash.* 174, 503-505.
- PELED Y. and TIETZ A. (1975) Isolation and properties of a lipoprotein from the haemolymph of *Locusta migratoria*. *Insect Biochem.* 5, 61-72.
- PRAGER E. M. and WILSON A. C. (1971) The dependence of immunological cross-reactivity upon sequence resemblance among lysozymes II. Comparison of precipitin and microcomplement fixation results. *J. Biol. Chem.* 246, 7010-7017.
- RAO C. R. (1965) *Linear Statistical Inference and its Applications*, Wiley, New York 533p.
- SCHACHMAN H. K. (1957) Ultracentrifugation, diffusion and viscometry. *Meth. Enz.* 4, 32-103.
- SCHJEIDE O. A., WILKINS M., MCCANDLESS R. G., MUNN R., PETERSON M., and CARLSEN, E. (1963) Liver synthesis, plasma transport and structural alterations accompanying passage of yolk proteins. *Am. Zool.* 3, 167-184.
- SCHNEIDERMAN H. A. and WILLIAMS C. M. (1954) The physiology of insect diapause. IX The cytochrome oxidase system in relation to the diapause and development of the cecropia silkworm. *Biol. Bull. Woods Hole*, 106, 238-252.
- SIEGEL L. M. and MONTY K. J. (1966) Determination of molecular weights and frictional ratios of proteins in impure systems by use of gel filtration and density gradient centrifugation. *Biol. Biophys. Acta* 112, 346-362.
- SPACKMAN D. H., STEIN W. H., and MOORE S. (1958) Automatic recording apparatus for use in chromatography of amino acids. *Anal. Chem.* 30, 1190-1206
- SPACKMAN D. H. (1967) Accelerated amino analysis. *Meth. in Enz.* XI, 3-15.
- SPANDE T. F. and WITIKOP B. (1967) Determination of the Tryptophan content of proteins with N-bromo succinimide. *Meth. in Enz.* XI, 498-506.
- SPIRO R. G. (1966) Analysis of sugars found in glycoproteins. *Meth. in Enz.* 8, 3-25.
- TELFER W. H. (1954) Immunochemical studies of insect metamorphosis. II The role of sex limited blood protein in egg formation. *J. Gen. Physiol.* 37, 539-558.
- TELFER W. H. (1960) The selective accumulation of blood proteins by the oocytes of saturniid moths. *Biol. Bull. Woods Hole*, 118, 338-351.
- TELFER W. H. and SMITH D. S. (1970) Aspects of egg formation. *Roy. ent. Soc. Sympos.* 5, 117-134.
- WALLACE R. A. (1963) Studies in Amphibian Yolk IV. An analysis of the main-body component of yolk platelets. *Biochem. Biophys. Acta* 74, 505-518.
- WALLACE R. A. (1964) Studies on amphibian yolk. VI. A protein kinase from the ovary of *Rana pipiens*. *Biochem. Biophys. Acta* 86, 286-294.
- WALLACE R. A. and DUMONT J. N. (1968) The induced synthesis and transport of yolk proteins and their accumulation in the oocytes in *Xenopus laevis*. *J. Cell Physiol.* 72, suppl., 72-102.
- WEBER K. PRINGLE J. R., and OSBORN M (1972) Measurement of Molecular Weights by Electrophoresis on SDS-Acrylamide Gel. *Meth. in Enz.* 26, 3-27.
- YAMASAKI K. (1973) Characterization and partial purification of a mannin-like polysaccharide in the eggs of *Locusts migratoria*. *Insect Biochem.* 3, 79-90.