

STRUCTURE AND EMBRYONIC DEGRADATION OF TWO NATIVE VITELLINS IN THE COCKROACH, *PERIPLANETA AMERICANA*

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(Received 27 March 1984; revised 10 August 1984)

Abstract - Multiple vitellogenins (VG) are found in species from all major families of cockroaches. Proof that observed multiple Vgs are actually distinct proteins and not artifacts of differential processing requires careful examination. In *Periplaneta americana* two immunologically and electrophoretically-distinct vitellins (VTs) of similar native size exist in the egg. VT1 is composed of four major peptides of molecular weights 170, 105, 92 and 78 K. VT2 is composed of three major peptides of molecular weights 105, 101 and 60 K. A peptide with molecular weight of about 105 K is found in both VG1 and VG2 and a similar sized peptide is also conserved in the VGs of all other Blattinae subfamily members examined despite the distant immunological relation of these proteins. The 170 K peptide of VT1 is likely to be a processing intermediate of the 92 K and the 78 K peptides. Each VT from a freshly ovulated egg is immunologically identical to and has essentially the same peptide substructure as its serum precursor. The cumulative M_r of the constituent peptides of each VG is ~260K, consistent with 17S native dimeric molecules of approx. 520 K M_r . A specific and limited cleavage of VT major peptides occurs during early embryogenesis (at 30°C, 9 days after ovulation) resulting in a partial loss of immunological determinants. During subsequent yolk utilization the VTs undergo gradual degradation.

Key Word Index: Vitellogenin, vitellin, yolk, embryogenesis, immunoelectrophoresis, electrophoresis, *Periplaneta americana*

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INTRODUCTION

Insect vitellogenesis generally proceeds from the fat body biosynthesis and processing of yolk protein precursors (vitellogenins, VGs), to their secretion into the haemolymph, selective uptake by oocytes, and finally their utilization by the developing embryo (Kunkel and Nordin, 1985). Post-translational modifications of VGs, which occur at all of the above stages (Wojchowski, 1984), are salient features, of vitellogenesis in species which produce group I VGs (Harnish and White, 1982b).

The structure and processing of the group I VG of the American cockroach, *Periplaneta americana*, has received considerable attention due to this species' focal position as a model of insect biochemistry and physiology. In an early study, Bell (1970) used immunological (Oudin single diffusion) and electrophoretic analyses to infer that two VGs and VTs exist in *P. americana*. The claim of multiple Vgs in *P. americana* was independently confirmed using immunoelectrophoresis (Engelmann, 1979). Indeed there is a growing awareness of a multiplicity of VGs in a diversity of animals (Wahli et al., 1979, 1981; Nicolaro and Bradley, 1980; Barnett et al., 1981). Nevertheless, in later work (Sams et al., 1980; Harnish and White, 1982a) and reviews (Bell and Adivodi, 1982) this position was recanted and the previous evidence cited was stated to be artifactual. These authors claim that *P. americana* possesses a single VG composed of three major peptides, and that the second VG is actually an aggregate of native molecules. Because some insect Vts are known to aggregate as a normal

part of vitellogenesis (Dejmal and Brookes, 1972 Oie et al., 1975; Storella and Kunkel, 1979) artifact cannot be dismissed as an explanation of apparent multiple VGs.

The extensive modification and eventual degradation of VG which occurs during vitellogenesis and embryogenesis can make the relationship between involved macromolecules ambiguous. Therefore whenever two VGs or VTs are found it is important to characterize their structural relationship. We present evidence that in *P. americana* two electrophoretically and immunologically distinct VGs and VTs do exist which share no monomer-aggregate relationship. Our analysis of subunit structure indicates that the two native VTs are composed of four analogous peptides, respectively.

Limited and specific VT peptide cleavage early in embryonic development and subsequent slow yolk degradation have been defined as the dominant events of VT processing during embryogenesis (McGregor and Loughton 1974; Storella and Kunkel, 1979). Our findings in *P. americana* concur and suggest that a specific mechanism regulating the timing of VT degradation has been conserved during evolution.

MATERIALS AND METHODS

Animals

Cultures of the American cockroach, *P. americana*, and other Blattinae were maintained at 30°C on Purina Lab Chow and deionized water. The moulting cycles at each instar were synchronized by controlling temperature and food availability (Kunkel, 1966). These cultures provided uniform, last-larval stage, midcycle females for ovariectomy. To produce synchronous batches of eggs, newly extruded oothecae were collected each day from adult females in randomly breeding cultures. The oothecae were incubated at 30°C. In this way oothecae at every age of embryogenesis were obtained. Embryos normally hatched from oothecae 33 days after ovulation.

Isolation of VG and VT

VG was prepared by collecting haemolymph from ovariectomized adults into phosphate buffered saline (0.15M NaCl, 0.01 M Na₂HPO₄, pH7.2). Haemocytes and insoluble or clotted proteins were removed by centrifugation at 28,000 g at 4°C for 15 min. VG was purified by adsorption on to DEAE cellulose in 0.15 M NaCl followed by elution with 0.3 M NaCl. VT was obtained from ovaries of vitellogenic ovaries or newly extruded oothecae. The ovaries or eggs were homogenized in 100mM Tris HCl pH8.0 plus phenyl methyl sulphonyl fluoride (30 µg/ml). The homogenate was centrifuged as above and the supernatant was either used directly or stored for no more than 24hr at 4°C. VT was never frozen as freezing induced aggregation (data not shown).

Sucrose gradient centrifugation

VT from oothecae was centrifuged in 5 to 20% (w/v) sucrose gradients containing 100 mM NaCl and in an SW 41 rotor at 37 K rpm (230,000 g), 4°C for 24hr. Gradients were monitored at 280 nm in a Gilford flow cell and the optical density recorded on a Honeywell linear potentiometer during fractionation.

Antiserum production

Multiple antisera were raised in male New Zealand white rabbits against either VT from eggs or VG from ovariectomized females. Antisera were adsorbed with adult male haemolymph of the corresponding species.

Immuno-electrophoresis

Immuno-electrophoresis (IEP) and quantitative immuno-electrophoresis (QIEP) were performed as described by Laurell (1966). Gels contained 0.90% agarose in a buffer of 50 mM Tris citrate, pH 8.6. Two dimensional (i.e. crossed) QIEP was performed as follows: VG was electrophoresed in the first dimension in gel containing no antibody, and then electrophoresed at right angles into antibody-containing gel. Tris citrate buffer (0.05 M, pH 8.6) was used in both dimensions.

Polyacrylamide gel electrophoresis (PAGE)

Native PAGE was performed in tube or slab gels according to the method of Laemmli (1970) without sodium dodecyl sulphate or B-mercaptoethanol in the buffers (6% acrylamide: 0.2% bis-acrylamide). SDS slab PAGE (15% acrylamide: 0.075% bisacrylamide) was run according to the method of Thomas and Kornberg (1975). Two dimensional PAGE consisted of native 6 mm i.d. tube gels in the first dimension and a 1.5mm thick SDS slab in the second (15% acrylamide). Tube and slab gels were annealed with a thin layer of 1% agarose in SDS stacking gel buffer.

Electroelution of VT

The two electrophoretic forms of VT were separated by native disc PAGE. Separated VTs were electroeluted from gel slices by the method of Green et al. (1982) in buffer containing no SDS. The isolated native VTs were used in QIEP.

RESULTS

Native PAGE of VT and VG

VG from haemolymph and VT from ovaries or eggs were examined by native PAGE (Fig 1). The two main haemolymph protein bands correspond to VGA and B described by Bell (1970). To avoid unwieldy peptide designations we have called them VGI and VG2 respectively. Adult haemolymph also contains two additional major serum proteins, serum protein-I (SP1, related to the arylentins; peptide E of Clore et al., 1978) and serum protein-II (SP2, lipophorin; Duhamel and Kunkel, 1978). Because SPI migrates only slightly behind VG2 it is unresolved here, but separates from VG2 in two dimensional PAGE (see Fig 7). In preparations of ovaries and oothecae VTI and VT2 are visible as the two major proteins.

Quantitative immuno-electrophoresis

Immunological precipitation tests also demonstrated the occurrence of two VGs and VTs. Fused QIEP using antiserum against VG yielded two precipitation lines corresponding to the two forms of the protein (Fig 2). As later demonstrated (Fig 4), the taller peak corresponds to VGI and the shorter to VG2. The fused precipitin lines further demonstrate immunological identity between VG and freshly ovulated egg VT, indicating that endocytosis and storage by the oocyte does not destroy any immunological determinants of VG.

Sucrose gradient centrifugation

VT from oothecae was sedimented in sucrose gradients and the gradients monitored at 280 nm during fractionation. Fractions were assayed for relative VT titre by QIEP (Fig 3). Two large u.v. absorbing peaks are apparent. The first peak (non-sedimenting) contains no VT and is primarily small molecules which can be eliminated by prior dialysis. The second peak sedimenting at about 17 S contains both VTI and VT2. As indicated through QIEP, VT2 sediments slightly faster than VTI and it is clear that they do not bear a monomer-aggregate relationship.

Immunological cross reaction

Immunological non-identity is most clearly demonstrated between VTs (Fig 4). Two *P. americana* VTs were separated by native PAGE and isolated by electroelution. Due to electrophoretic trailing, VT1 remains contaminated with VT2. These two forms were tested for immunological cross-reaction by fused-QIEP. This precipitin test identifies the taller peak as VT1 and the shorter one as VT2. The precipitin rockets corresponding to VT1 and VT2 cross without visibly interacting, indicating non-identity. If one VT were a precursor or aggregation product of the other, then they should show at least partial identity. But, as these tests demonstrate, the VTs do not share any determinants. This strongly implies that the two VTs derive from distinctly different precursor VGs.

Peptide composition of VTs and VGs

The peptide composition of total VT was assessed by SDS PAGE (Fig 5) and the molecular weights of seven major bands estimated. At low loadings in SDS PAGE, peptides of 105, 101 and 60K predominate. VT peptides of similar M_r are found in all species examined closely related to *P. americana* (Table 1). An approximate 105 K peptide has been particularly stable within the subfamily Blattinae despite the fact that some of the vitellins in Table I barely cross-react immunologically (Fig 9A).

Two dimensional gel electrophoresis of fresh vitellin clearly shows the different peptide substructures of VT1 and VT2 (Fig 6). Here, the dense 105 K band resolves into two peptides, one derived from each VT. In order to discuss stoichiometry of the parent molecules we assigned letter designations to the peptides of the native VTs: VT1 is composed of peptides A(105 K), B (92 K), C (78 K), D (170 K) and E (40 K). VT2 is composed of peptides X (105 K), Y (101 K), Z (60 K) and W (40 K). We conclude that the D peptide of VT1 is commonly cleaved into peptides B and C because the sum of their molecular weights equals that of peptide D and because the total molecular weight would then be consistent with other group I VGs. We also conclude that peptides E and W are products of degradation within the egg because they do not appear in VG and are found in increasing proportion as the early embryo develops (see Fig 10). Since almost all native group I VGs and VTs are dimers of their high molecular precursors (Table I of Kunkel and Nordin, 1985) we are able to infer the native peptide structures of *P. americana* VG. The native peptide composition of VT1 would thus be (ABC)₂, and rarely ABCAD. The native form of VT2 would be (XYZ)₂.

Purification of VG and VT from related species

TEAE-cellulose chromatography was used to separate VGs from other serum proteins in haemolymph samples from ovariectomized females of four species closely related to *P. americana*. Typically the non-vitellogenic serum proteins eluted or did not adsorb at moderate ionic strengths (0.15 M NaCl) while VGs eluted at high ionic strengths (0.3 M NaCl). These VGs were shown to be free of immunologically detectable serum protein contaminants by QIEP (data not shown). These related VGs were used for the comparative studies below.

Evidence for two VGs throughout the order

Immunological evidence indicates the existence of two native VGs in species from each of the

three major families of cockroaches, Blattidae, Blaberidae and Blattellidae. Figure 8 shows the results of immunoelectrophoresis performed on haemolymph from ovariectomized adult females and nymphal females of these species in the family Blattidae: *Blatta orientalis*, *Periplaneta americana*, *P. australasiae*, *P. fulginosa* and *P. brunnea*. For each species, the antibody was made against VG of that species. While nymphal haemolymph contains no immunoreactive proteins, in all cases adult female haemolymph produced two intersecting precipitin lines. This indicates that the two VGs share no immunological determinants. The separate precipitin lines are especially well illustrated in the case of *P. fulginosa*. The antiserum against *P. australasiae* VT is able to detect two VGs in both *P. australasiae* and *P. fulginosa* VG (Figs 8 and 9A) demonstrating that the two forms are homologous between these two species. However similar amounts of VGs from more distantly related species in the subfamily form much larger and diffuse precipitin rockets, showing that they react with limited components of this VT specific antiserum.

In the Blaberidae, QIEP of adult female haemolymph from *Blaberus discoidalis* also results in two precipitin peaks suggesting two VGs (Fig 9C). This is the type of suggestive evidence of multiple VGs that is available for many species.

We have also found a minor second VG in Blattellid cockroaches. As an example, in sucrose gradients VG from *Blattella germanica* fractionates and sediments bimodally as 10S and 17S particles (not shown). The 17 S VG corresponds to the major VG described elsewhere (Kunkel and Pan, 1976; Storella and Kunkel, 1979; Kunkel, 1981). The 10 S fraction represents a minor VG which can be distinguished as a lower density precipitin rocket in QIEP. Figure 9B shows purified 10 S and 17 S run on either side of yolk samples in QIEP. Measured amounts of each purified VG placed in wells 1 and 6 (Fig 9B) allowed the estimation that the 10 S-VG makes up only 2% of the total yolk vitellin. Notably, both 10 S and 17 S Vgs are taken up rapidly into vitellogenic oocytes (not shown).

These results are in contrast to the situation in *Leucophaea madera*, a Blaberid. This cockroach is unique in that it appears to contain only a single VG (Masler and Ofengand, 1982). Considering that in most species where estimates of relative amounts of the multiple VGs or VTs are available, one dominates, it is easy to see that a minor VG could be overlooked. We tried to find such a minor immunologically distinct VT in *L. maderae*, but failed.

Processing of VT during embryonic development

We followed the modifications of *P. americana* VTs during embryogenesis using SDS-PAGE and QIEP. Oothecae aged one to 33 days were homogenized and their peptides examined with SDS-PAGE (Fig 10). Between 7 and 13 days after ovulation all the original peptides undergo a limited and specific cleavage yielding many smaller peptides, none larger than 40 K in molecular weight. Peptides E and W reach greatest relative concentrations nine days after ovulation indicating that they are normally not peptides of VG but are products of VT degradation in the egg. Some post-11-day vitellin peptides, such as the one with an M_r just smaller than chymotrypsin, survive with little apparent digestion until close to hatching while others progressively disappear from day 13 onward.

At 15 days after ovulation three new peptides appear with M_r s of 93, 85 and 82 K. These are not peptides of VTs but are subunits of haemolymph storage proteins previously described in the closely related species *Blatta orientalis*, SPI and the two subunits of larval specific protein, respectively (Duhamel and Kunkel, 1978, 1983).

To investigate effects on antigenicity of the apparent peptide cleavages which occur between days 7 and 13 inclusive after ovulation, we compared VT from freshly ovulated oothecae to VT from 9-day

oothecae through fused rocket immunoelectrophoresis (Fig 11). At this stage substantial peptide cleavage has already occurred. Both VT1 and VT2 are still detected but each has lost immunological determinants as demonstrated by spurs on the precipitin lines of partial identity between the fresh and 9-day forms of each VT.

DISCUSSION

Insect VGs are complex and diverse due in part to the fact that the vitellogenic process involves sequential modification of the protein at various stages of vitellogenesis. Three groups of insect VGs have been proposed by Harnish and White (1982b) based on the size of the proVG and the degree of processing prior to deposition as VT. In many cases the VGs are modified by peptide cleavage and the addition of carbohydrate, phosphate and lipid (Kunkel and Nordin, 1985). With respect to carbohydrate, microheterogeneity of the attached high-mannose oligosaccharide introduces further diversity (Nordin and Kunkel, 1982; Nordin et al., 1984). Heterogeneity may be artifactually introduced; for example in *Blattella germanica* VG which has been allowed to accumulate in the haemolymph for several days in ovariectomized females (Kunkel and Pan, 1976) exhibits a different peptide substructure from newly synthesized VG (Kunkel et al., 1980). Therefore, when multiple Vgs are found it is important to characterize the relationships among them.

We have examined VG and VT in *P. americana*. These molecules fall into group I of the classification of Harnish and White (1982b). Group I VGs are large (>400K M_r) native proteins composed of numerous peptides with M_rs between 50 and 200 K. In this group, large putative primary translation products (approx, 250 K M_r) are cleaved to yield the peptides found in mature VGs (Koepe and Ofengand, 1976; Chen, 1980). VG is taken up into the oocyte by specific adsorptive endocytosis and subsequently is termed VT (Pan et al., 1969). *P. americana* VG is immunologically unchanged by this process and its major peptide subunits remain, at least temporarily, intact. Therefore our assertions which are demonstrated most clearly for newly ovulated egg VT hold equally well for VG.

The existence of two VG and VT molecules in *P. americana* is indicated by the presence of two precipitation lines in immunological tests and by two protein bands in native PAGE. Although the two VTs elute from TEAE anion exchange resins at the same salt concentration and sediment at only slightly different rates, their analytical separation can be accomplished by native gel electrophoresis followed by electroelution. This results in purified VT2 but only partially purified VT1. Separation of two vitellogenins of *B. discoidalis* has also been successfully accomplished using NaBr gradients (Wojchowski, 1984).

The separated *P. americana* VT molecules were distinct in two ways. First the two molecular forms of VT showed no immunological cross-reaction, indicating their non-identity. Second, in 2D-PAGE VT1 and VT2 showed substantially different peptide substructures. These two significant differences are the necessary proofs that *P. americana* produces two distinct VGs for storage in its eggs.

We examined the peptide substructure of *Periplaneta* VT and found seven major peptides, four belonging to VT1 and three belonging to VT2. The peptides of VT1 are: 170, 105, 92 and 78K. Those found in VT2 are: 105, 101 and 60 K. We propose that two VG precursors of molecular weight about 260 K are processed as follows (Fig 12): the VG1 precursor is cleaved to give the 170 K peptide and the 105 K peptide. Most often the 170 K peptide is cleaved into the 92 K peptide and the 78 K peptide. The VG2 precursor is also cleaved into three peptides yielding the 105K, the 101 K and the 60K peptides. As we have discussed, there is precedence for this cleavage scheme in other group I VGs of insects. Considering that most group I VGs are composed of peptides of 50 and 100 K M_r size classes,

this processing scheme may have been highly conserved during evolution.

The existence of two rather than one VG in *P. americana* has not been firmly established until now. Previously, evidence had been presented asserting both possibilities. While assuming a single VG, workers proceeded to determine the peptide structure of the molecule (Table 2). We believe that the structural properties these investigators have deduced correspond to VG2, the more abundant of the two VGs. It has a generally denser-staining precipitin pattern with rocket heights and precipitin arcs, consistent with higher concentration, and it has a more densely-staining native PAGE pattern. Only VG2 and its peptides stain on lightly loaded SDS-PAGE gels. A comparison of our determinations of peptide sizes for two VTs with those previously published for one VT show that the latter correspond most closely in pattern and relative peptide molecular weights to VG2.

The exception is the 189 K "beta" peptide of Clore *et al.* (1978), which in our interpretation corresponds to our 170K D peptide of VT1. It should be noted that these workers also recognized an "alpha" peptide, found only in haemolymph early in the first reproductive cycle, which we interpret as a precursor to our VT2 peptides Y and Z. This implies a common cleavage pattern in both VT1 and VT2 (i.e. $D \rightarrow B + C$ and "alpha" $\rightarrow B + C$ and "alpha" $\rightarrow Y + Z$; Fig 12). The Clore *et al.* model of VT, (A₂D) does not recognize multiple VTs and suffers from the lack of resolution of the 101-105 K peptides.

Our examination of yolk peptides after ovulation and during embryogenesis reveals that VTs undergo a limited specific cleavage 9 to 11 days after ovulation and that further complete degradation proceeds after this time point. Some of the peptides resulting from that limited cleavage appear to survive at least 20 days in the developing egg until close to hatching. It is possible that those peptides are being reserved for late embryonic or early larval nutrition. It would be interesting to examine the generality of this mode of processing of group I VG in the holometabolous insects.

This study illustrates that the existence of multiple forms of native VG is more widespread than previously believed. Indeed, it is more the rule than the exception among those cases closely studied. We have demonstrated multiple antigenic forms in members of all three major cockroach families including *Periplaneta spp.*, *Blatta orientalis*, *Blaberus discoidalis* and *Blattella germanica*. In addition, previous work has identified multiple antigenic forms in other species possessing group I VGs including another Blaberid cockroach, *Byrsotria fumigata* (Barth and Bell, 1970), the termite *Zootermopsis angusticollis* (Greenberg *et al.*, 1978) and the cricket *Acheta domesticus* (Nicolaro and Bradley, 1980). In two other well studied systems, *Locusta migratoria* and *Leucophaea maderae*, only one immunologically discrete native protein has been described. Nevertheless, considering the often low titre of the secondary VGs, a closer electrophoretic and immunological examination of the native VGs of the later species is warranted.

The problem of multiple VGs must finally be solved by genetic analysis. Multiple non-allelic genes for VG have already been demonstrated in *Drosophila melanogaster* (Barnett *et al.*, 1981) and in *Xenopus* (Wahli *et al.* 1979, 1981). The multiple VG genes of *Xenopus* may correspond to multiple processed forms of VG, lipovitellin, phosvitin and phosvettes which have been reported (Wiley and Wallace, 1981). Clearly, an understanding of the number and the relationship of primary protein products to genes will simplify the complex phenomenology of vitellogenic systems.

Why should multiple forms of vitellogenin exist in any animal? First, the multiple forms may reflect multiple genes encoding them. Two genes may be necessary to provide large amounts of VG protein over a short VG deposition time. Second, different forms may have different functions. If so we would expect the two forms to be treated differently. Two such instances have been reported: the multiple antigenic forms of *Acheta domestica* VG are differentially distributed along the length of the

cricket oocyte (Nicolaro and Bradley, 1980). Differential utilization of the multiple VTs of *Blatta orientalis* has been reported (Kunkel and Nordin, 1984). This later hypothesis assumes that the embryo has different nutritional requirements at different stages of development. In this respect it might be expected that in insect species with heavily sclerotized larval cuticle a later used VT might reflect cuticular composition and tanning requirements. It is thus possible that general and specific physiological roles for the multiple VGs will be delineated.

Acknowledgements — The authors thank Dick Poccia and Ray Green for their generous technical help and advice. JRS and DMW were supported as trainees by the Program in Molecular and Cellular Biology, NIH GM07473. Research was supported in part by NSF PCM790653 and PCM820653 to JGK and JHN.

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TABLES

Table I. Estimated peptide molecular weights of vitellogenins of the subfamily Blattinae of cockroaches

Genus species	Peptide molecular weights (x 10 ⁻³)				Sum of peptides in predominant
	Peptide weight classes				
	I	II	III	IV	VG
<i>Blatta orientalis</i>	104.0	98.4	55.9		258
<i>Periplaneta americana</i>	105.1	100.9	60.2		266
<i>P. brunnea</i>	104.9	91.5	57.5		254
<i>P. australasiae</i>	104.1	96.0	59.2		259
<i>P. fuliginosa</i>	104.7	97.4	60.5	63.2	262 (265)
Mean class wgt. (x 10 ⁻³)	104.6	96.8	58.7		
Coefficient of variation	0.4	3.5	3.1		

Determined by SDS tube PAGE with multiple internal molecular weight standards run within each gel. The molecular weights are quoted to hundreds of daltons which represents the accuracy of the measured relative mobilities achieved. It is recognized that the high precision in differentiating relative mobilities of two peptides using this technique may not be reflected in an accurate molecular weight difference. This is due to unexplained systematic departures of the log molecular weight of standards from their predicted mobilities of molecular weight were the only important factor.

TABLE 2

Table 2. Peptide molecular weights for *P. americana* VG/VTs

Storella et al. (1984)		Harnish and White (1982)	Sams et al. (1980)	Clore et al. (1978)	
VT1: (ABC), (ABCAD)	VT2: (XYZ)	VT	VG: (ABC) ₂	VG	VT: (A ₂ D)
				205 K	[alpha]
D 170 K				189 K	[beta]
A 105 K	X 105 K	140 K [1]	123 K [A]	112 K	[A]
	Y 101 K	135 K [21]	118 K [B]		
B 92 K					
C 78 K					
	Z 60 K	62 K [3]	57 K [C]	63 K	[D]
		59 K [4]			

Separate molecular weight standardizations were used by each group and thus reported peptide weights are not exactly comparable but the relative values allow homologies to be established. Since VT1 is a minor component it was ignored by the earlier workers with the exception of Clore et al. (1978). Prior peptide designations are given in brackets. Models of VT proposed by each group are included within parentheses and discussed in the text.

FIGURES

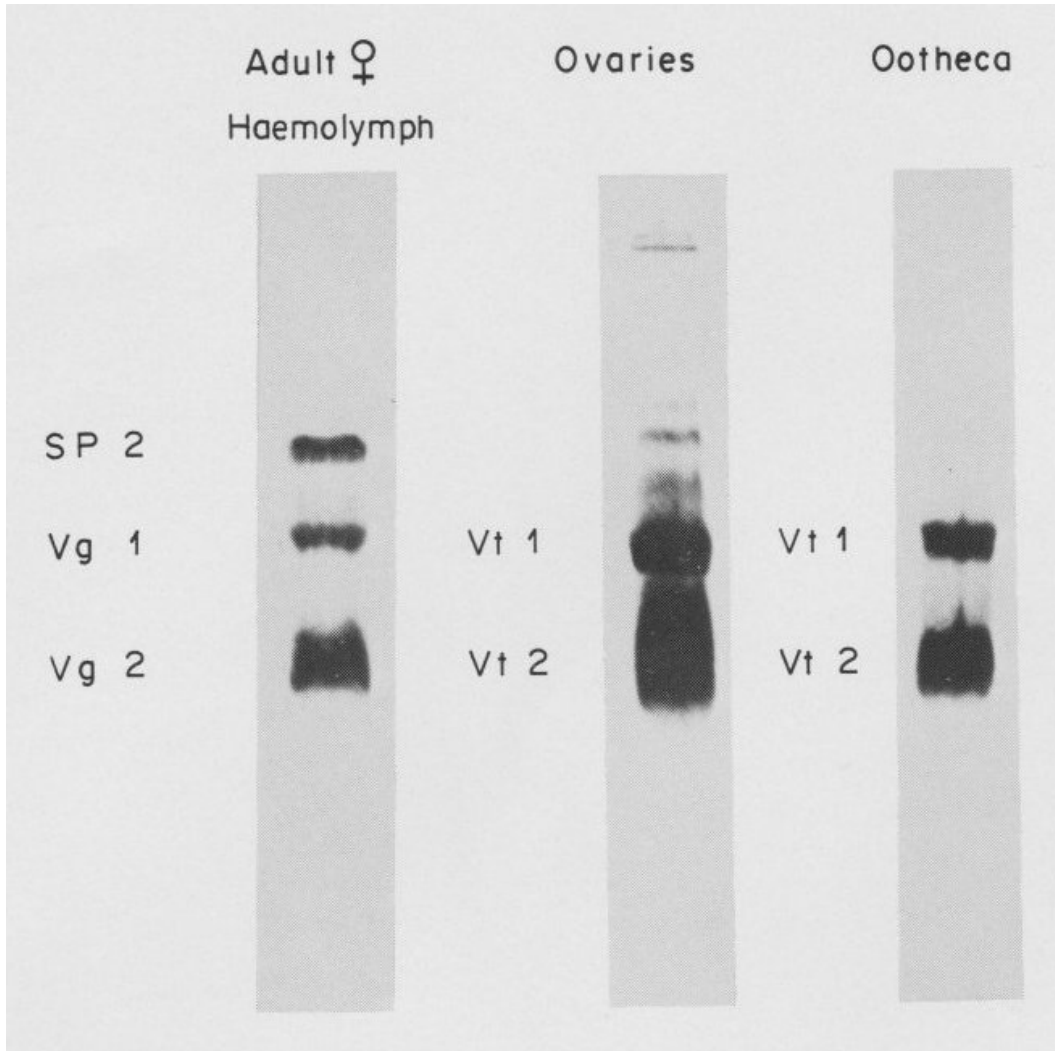


Fig. 1. Native gel electrophoresis of protein from ovariectomized adult female *P. americana* hemolymph, vitellogenic ovaries and freshly extruded oothecae. The identified proteins are: vitellogenin (Vg), vitellin (Vt) and serum protein two (SP2).

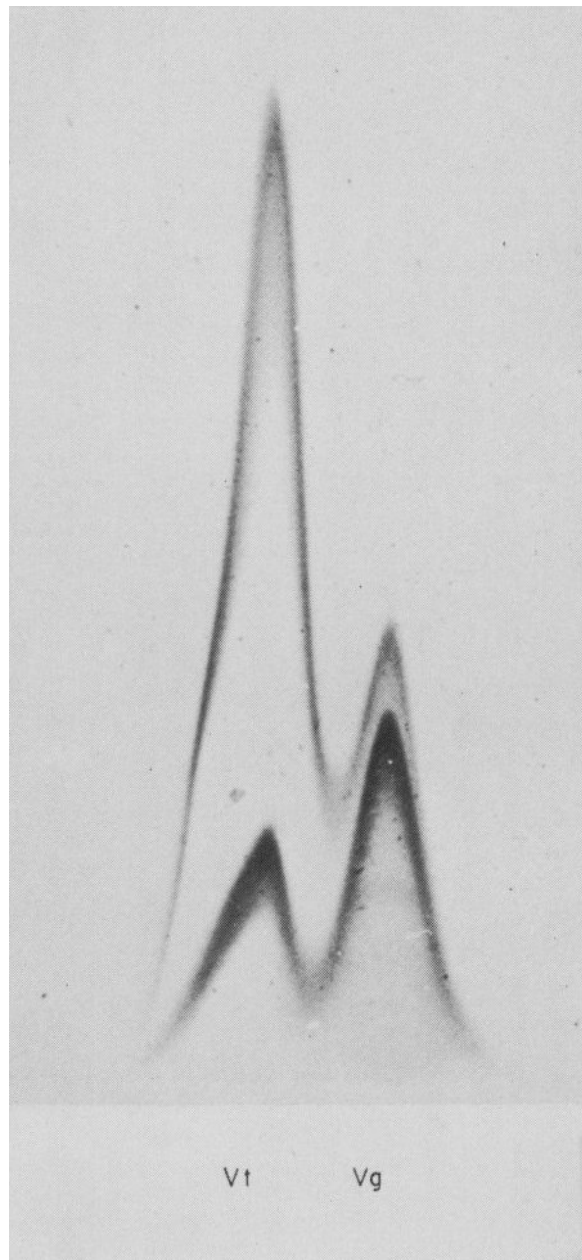


Fig. 2. Fused-QIEP of purified *Periplaneta americana* vitellin (Vt) and vitellogenin (Vg). Proteins were allowed to diffuse in antiserum free agarose 2 hr prior to electrophoresis into agarose gel containing anti-*P. americana*-Vg.

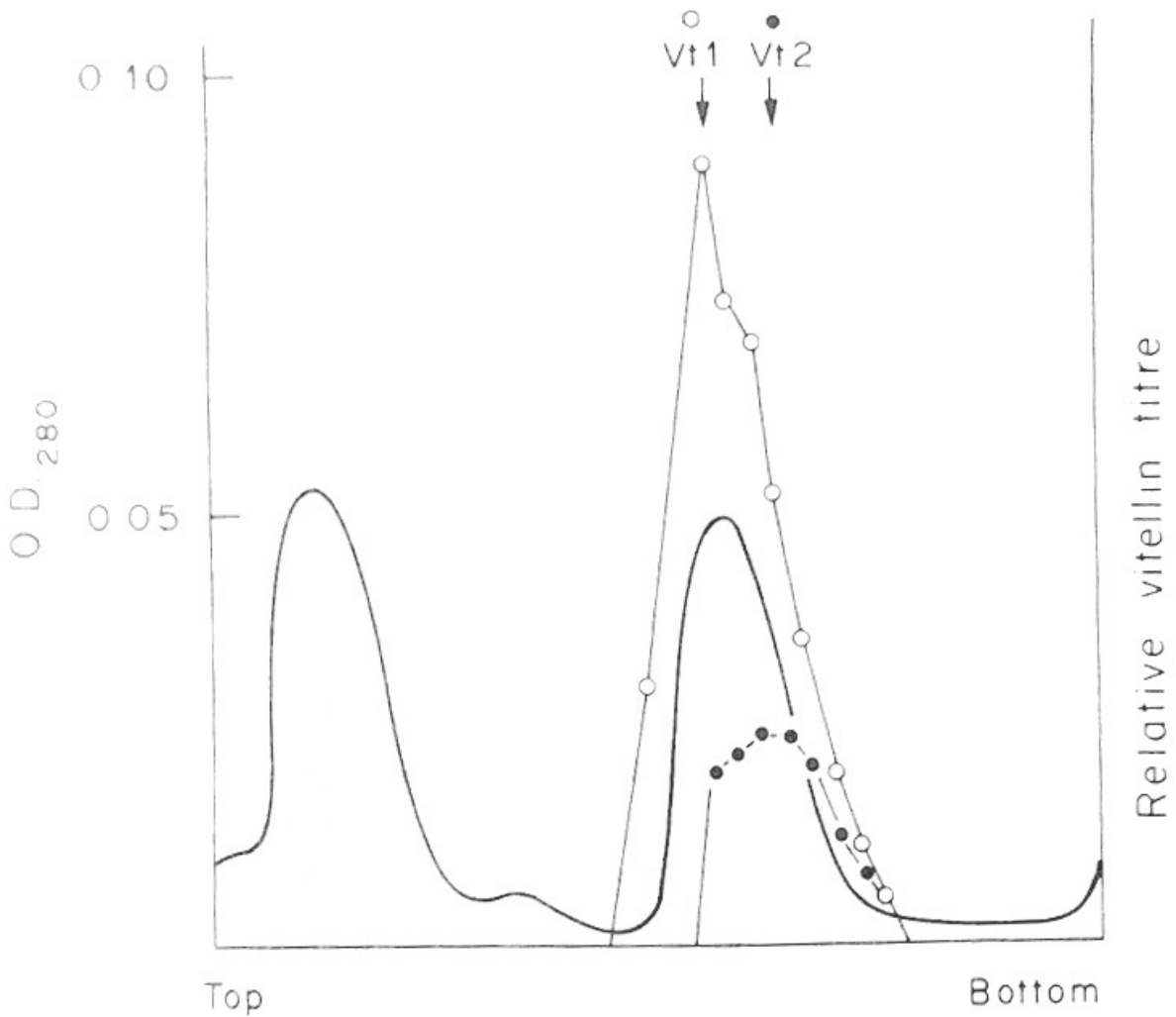
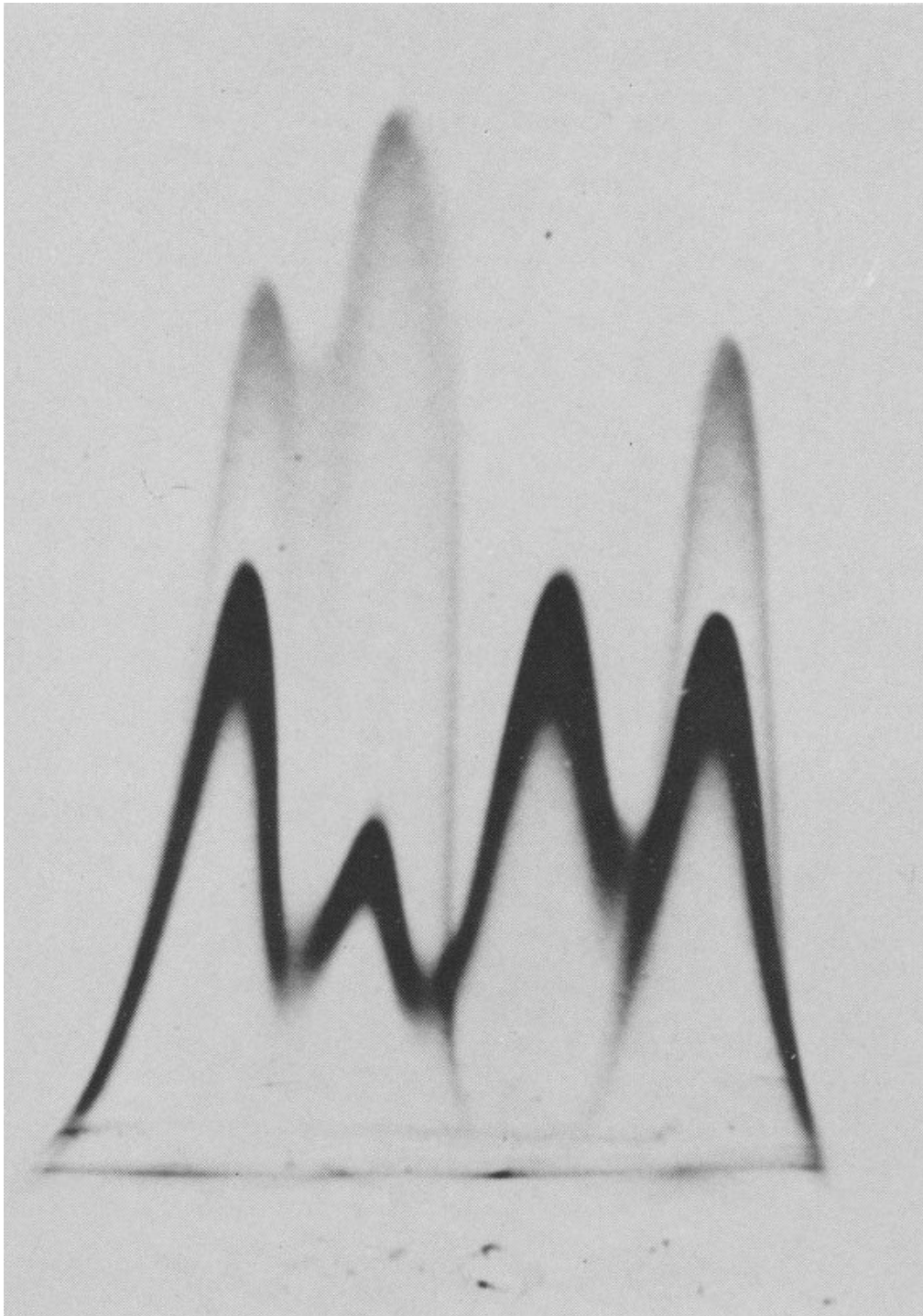


Figure 3. *Periplaneta americana* vitellin freshly extracted in 0.3 N NaCl PBS layered on a (5-20%) sucrose gradient and centrifuged in an SW 41 rotor at 37K rpm for 24 hr. The gradient was scanned at 280 nm (solid line) and fractionated. Each fraction was tested for relative titre of vitellin I (open circles) and vitellin 2 (solid circles) by QIEP.



Vt fresh Vt 1>2 Vt2 Vt fresh

Fig. 4. Fused-QIEP of VT freshly extracted from *Periplaneta americana* eggs (Vt fresh) and enriched VT1 (Vt 1>2) and purified VT2 (Vt2) electroeluted from native polyacrylamide gels.

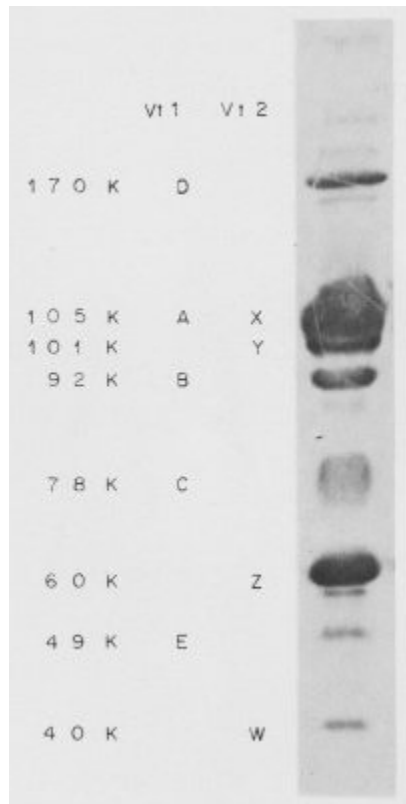


Fig. 5. SDS-PAGE of fresh *Periplaneta americana* oothecal extract. Standards allowed estimation of molecular weights of constituent peptides. The assignments of peptides V21 or Vt2 based on two dimensional PAGE as seen in Fig. 6.

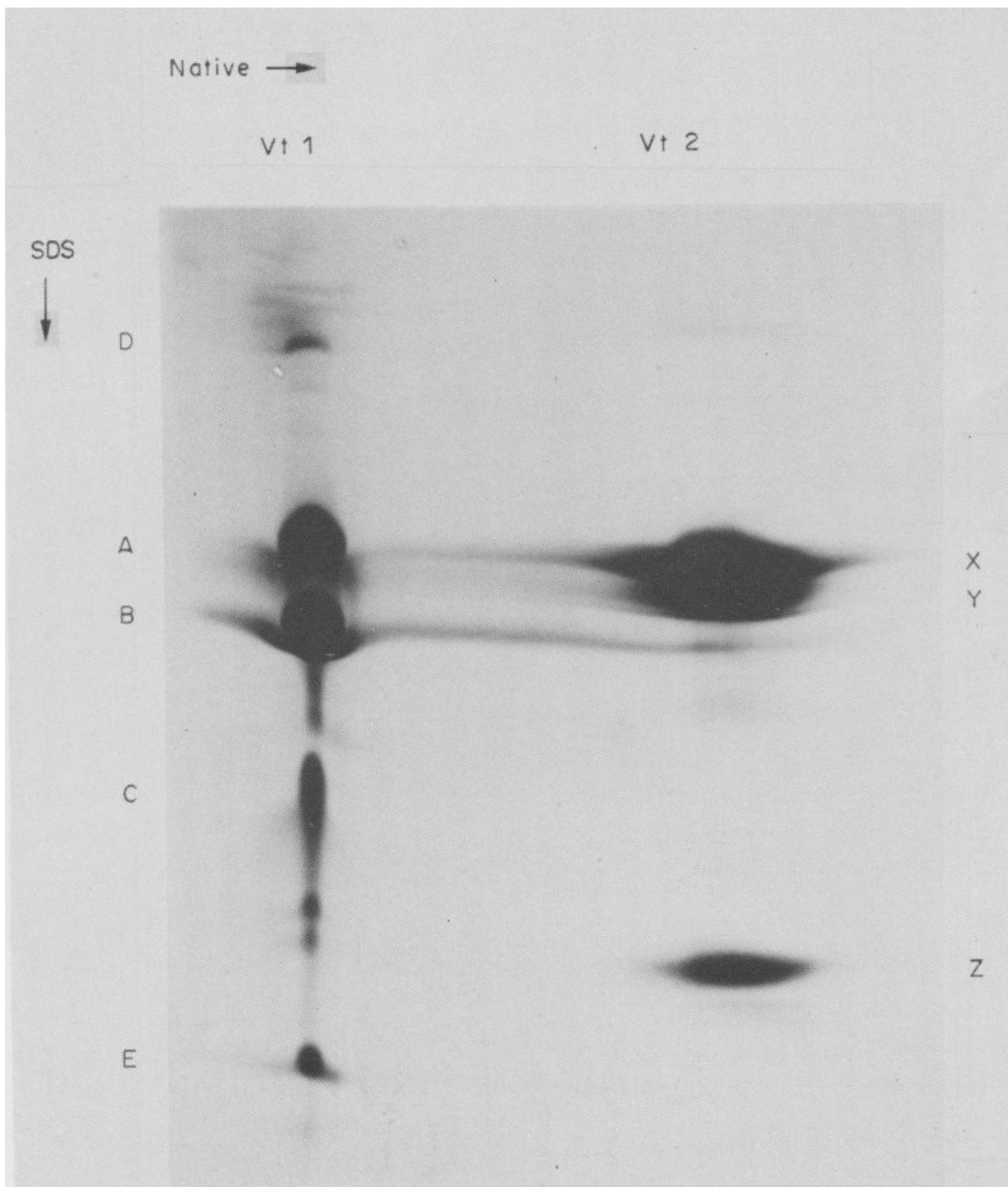


Fig. 6. Two dimensional PAGE of fresh *Periplaneta americana* VT. Proteins were separate by native PAGE in the first dimension and then SDS-PAGE in the second dimension.

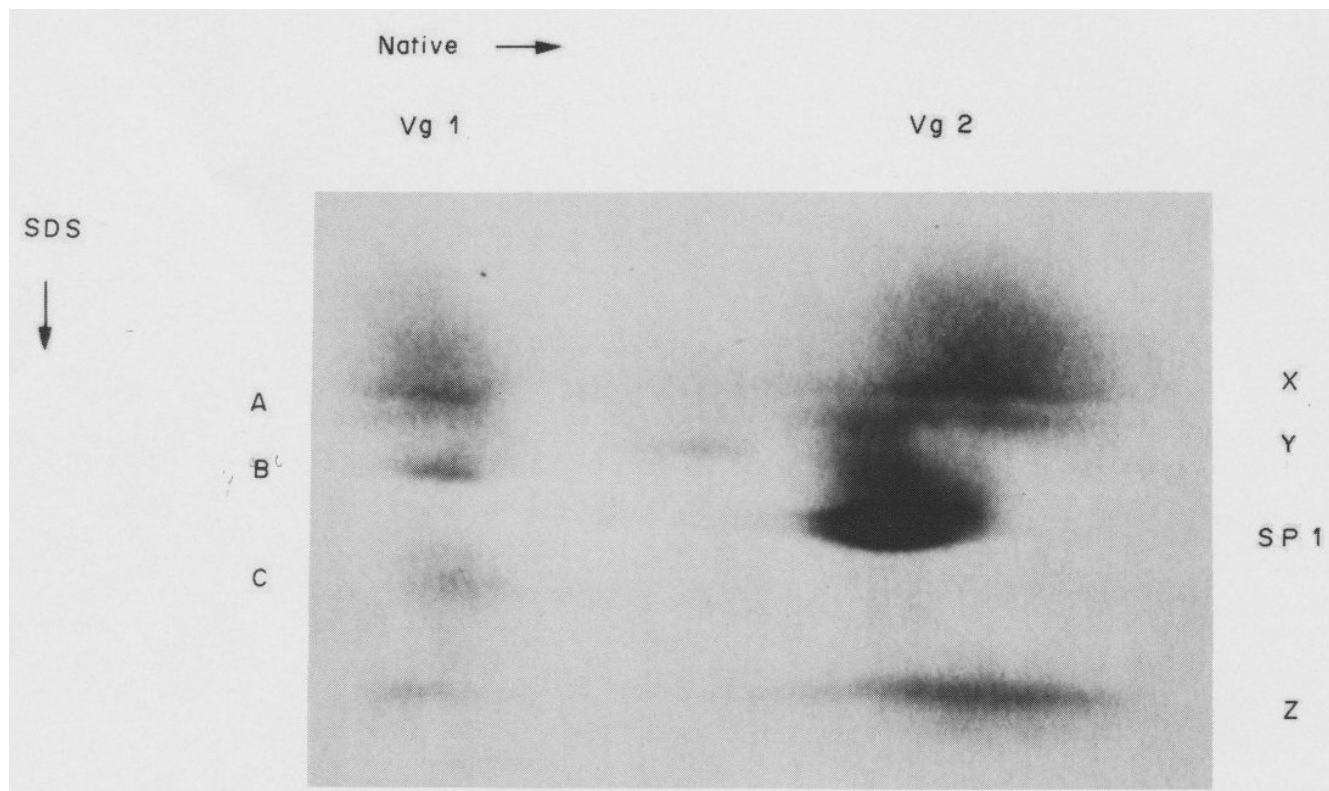


Fig. 7. Two dimensional PAGE of serum proteins from ovariectomized adult *Periplaneta americana* females. Proteins were separated by native PAGE in the first dimension and by SDS-PAGE in the second dimension.

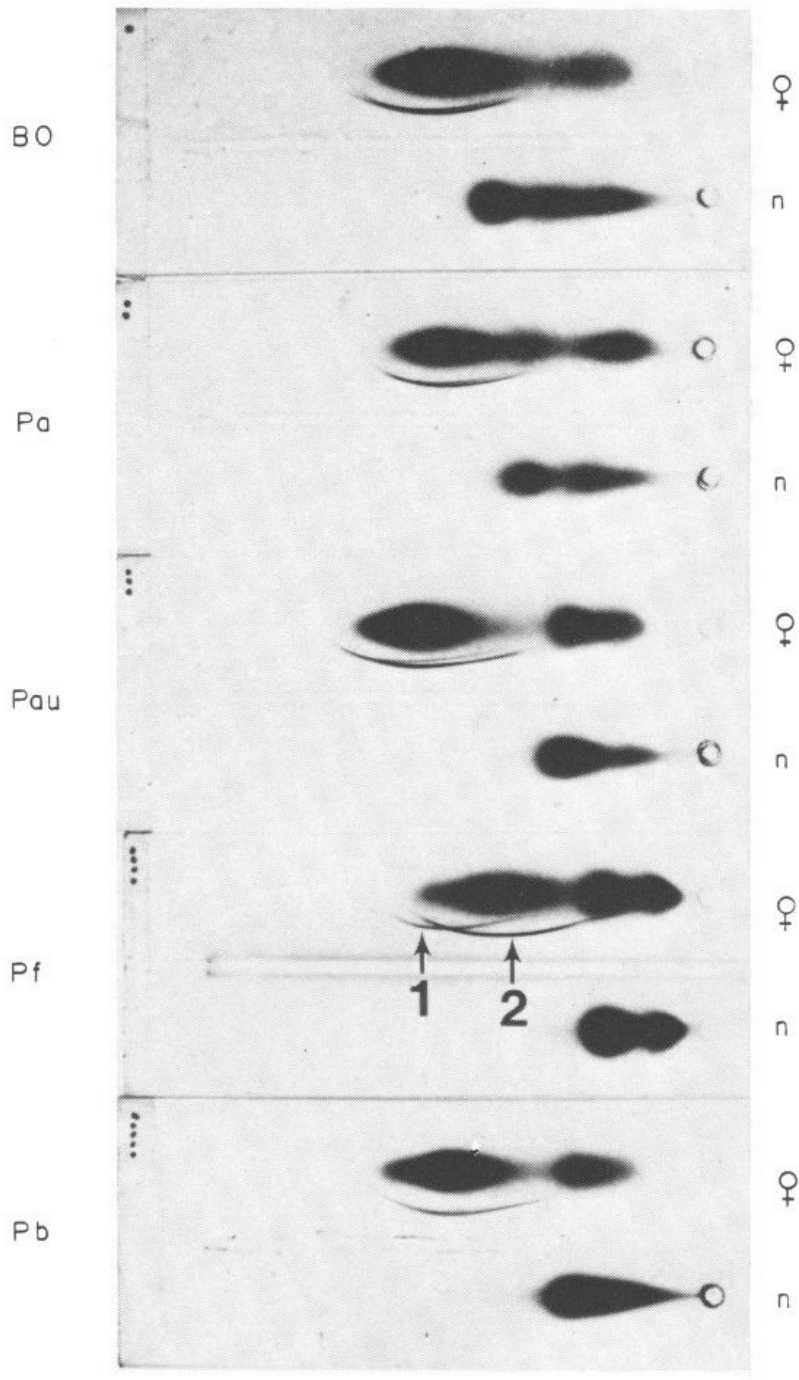


Fig. 8. Immunoelectrophoresis of serum from nymphs and ovarietomized adult females of the following species: *Blatta orientalis* (Bo), *Periplaneta americana* (Pa), *Periplaneta australasia* (Pau), *Periplaneta fulginosa* (Pf), and *Periplaneta brunnea* (Pb). Aliquots of adult female (f) and nymphal (n) serum were placed in the sample wells. After electrophoresis of duplicate slides, one was stained to visualize separated proteins and one had troughs cut for immunodiffusion. Antibody specific against each species' vitellogenin was added to the respective troughs. The two VGs of *P. fulginosa* are indicated by "1" and "2" as the clearest immunoelectrophoretic examples of multiple VGs

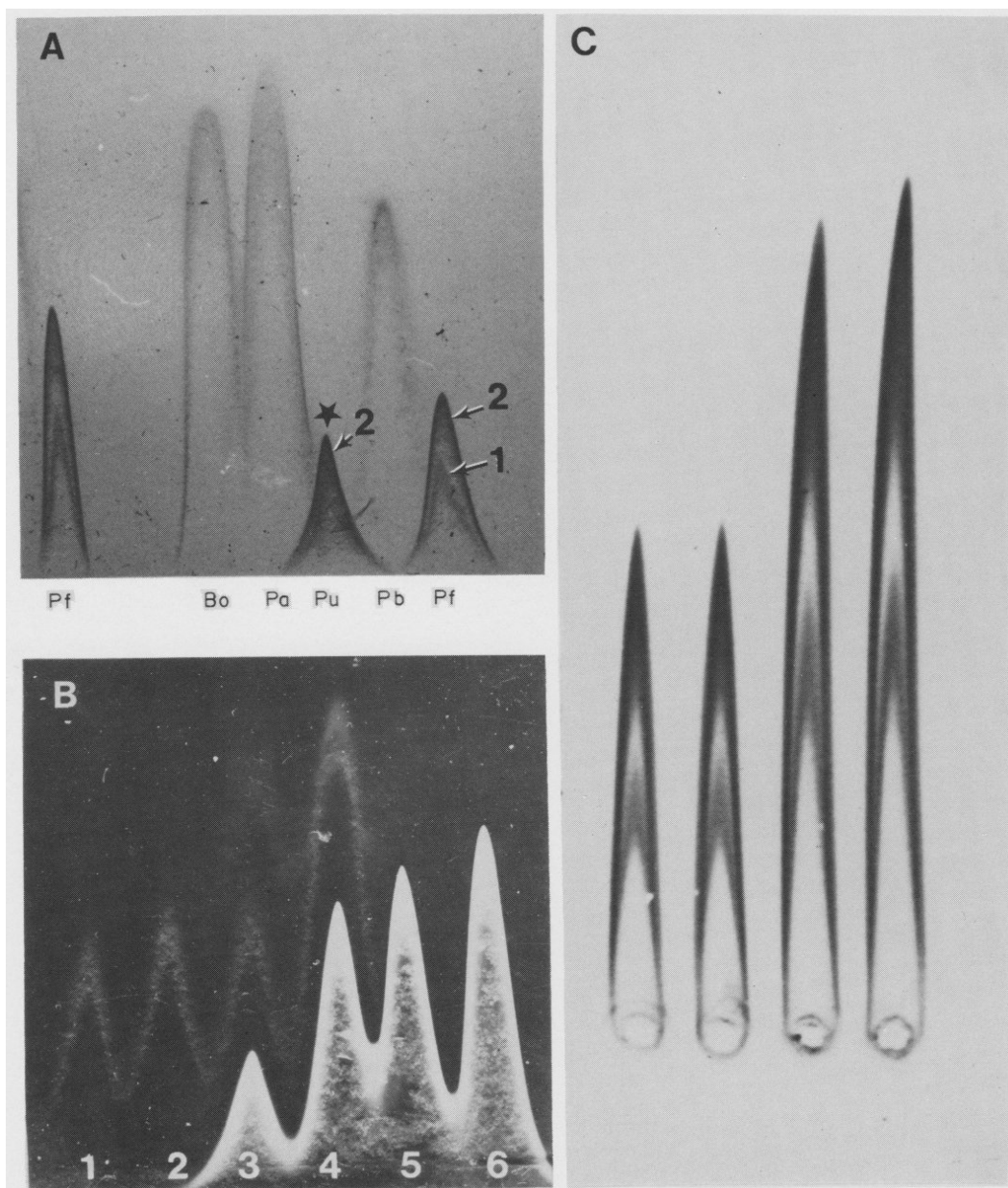


Fig. 9. Examples of multiple VGs in other species demonstrated by quantitative immunoelectrophoresis (QIEP). (A) Fused-QIEP of cross-reacting vitellogenins of members of the Blattinae. *Blatta orientalis* (Bo), *Periplaneta americana* (Pa), *P. australasiae* (Pu), *P. brunnea* (Pb) and *P. fuliginosa* (Pf). Antiserum (3%) against *P. australasiae* vitellin is in the agarose phase; 3.2 milli-O.D. units of purified Vg of each species was placed in the wells and allowed to diffuse for 2 h prior to adding the antibody agar to the slide. In the leftmost well Pf Vg was added just prior to electrophoresis to indicate the effects of diffusion. The homologous antigen (star) gives the smallest and densest rockets, actually a second rocket is visible but obscured by the dense staining of the VG2 rocket. In the closest related species within the genus, Pf, there are clearly two precipitin rockets which correspond to the two VGs seen in immunoelectrophoresis in Fig. 8. (B) Fused QIEP of *Blattella germanica* VGs. A minor VG immunologically distinct from the major VG is found in both haemolymph and yolk. Purified 10S-VG (0.26 μ g) in wells 1 and 2. Purified 16S VG (5.2 μ g) in wells 5 and 6. Yolk extract (2.5 and 5 μ g respectively) in wells 3 and 4. Calculations of absolute amounts of 10S and 18S VT, based on heights of the rockets, suggest that 10S-VT makes up about 2% of the total VT concentration. (C) QIEP of *Blaberus discoidalis* VG showing two distinct precipitin rockets.

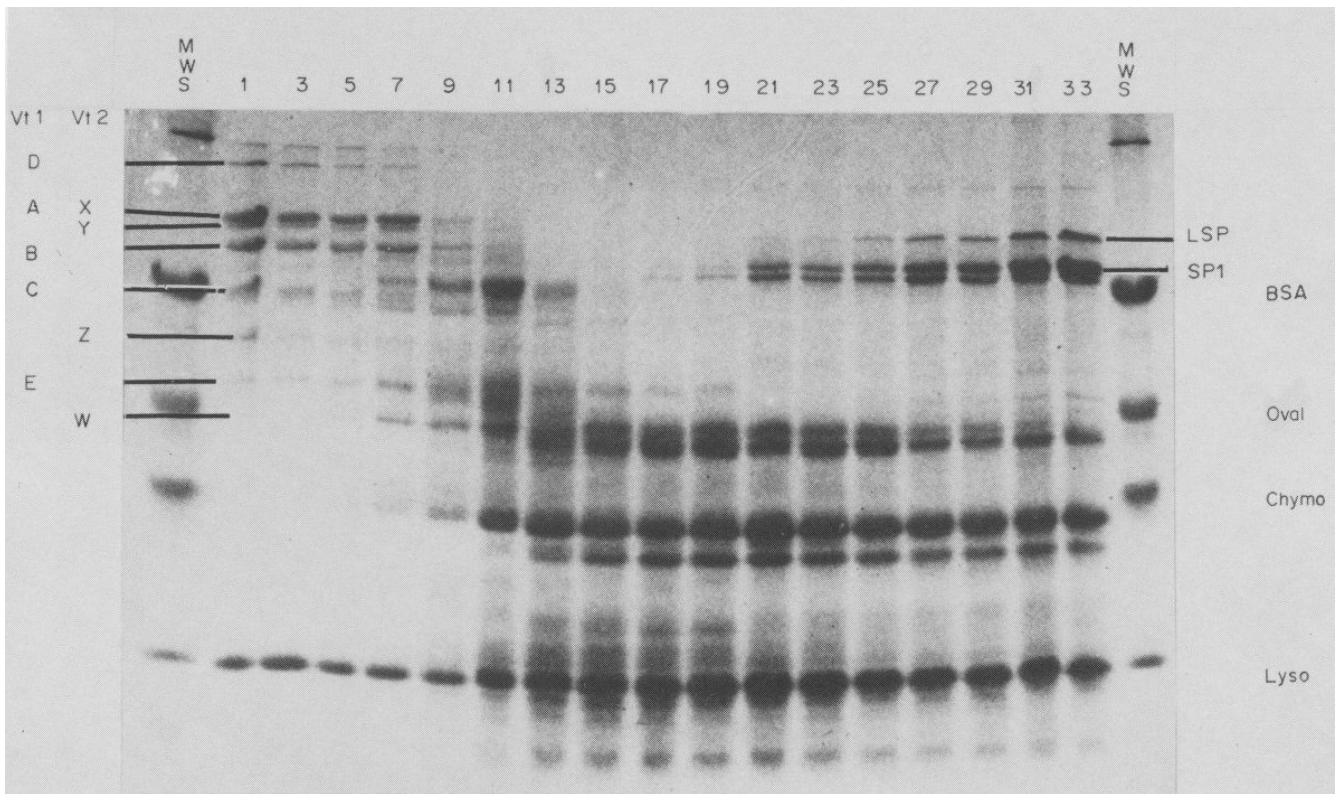


Fig. 10. SDS-PAGE of extracts from *Periplaneta americana* oothecae on days 1 to 33 after ovulation. Molecular weight markers are: bovine serum albumin (BSA), 68 K; ovalbumin (Oval), 43 K; chymotrypsinogen (Chymo), 25 K; lysozyme (Lyso), 17 K. On the left border we designate our interpretation of the native VT source of the peptides.

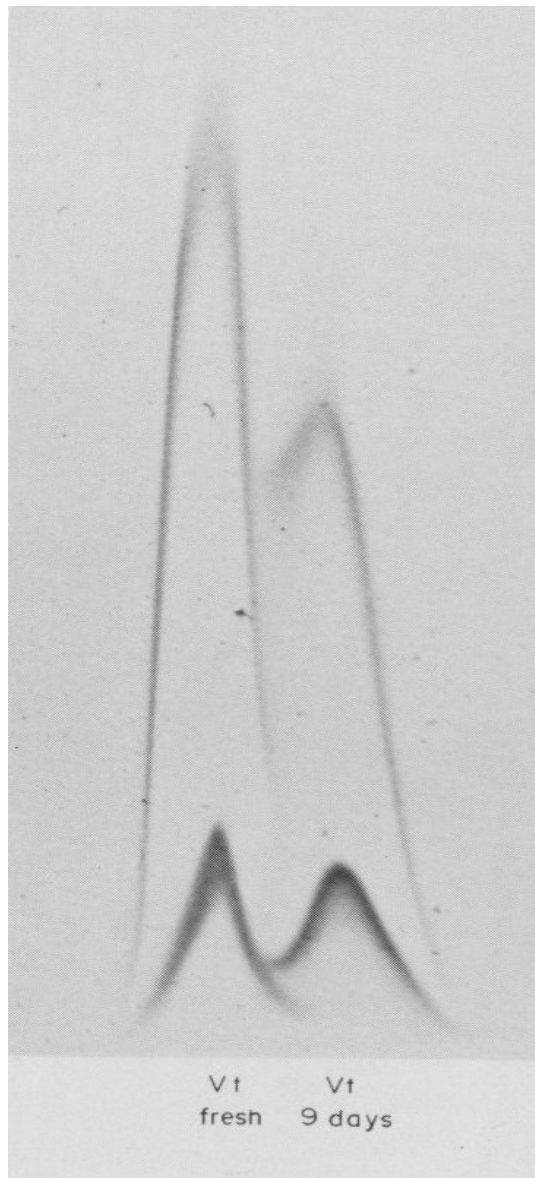


Fig. 11. A processing step of *Periplaneta americana* VT in ovulated eggs. Fused-QIEP is used to compare VT of newly ovulated eggs and 9-day VT. Both VT1 and VT2 display a spur of partial identity indicating loss of immunological determinants in both VTs between days 1 and 9 of embryogenesis, corresponding to the cleavage of peptides seen in Fig. 10.

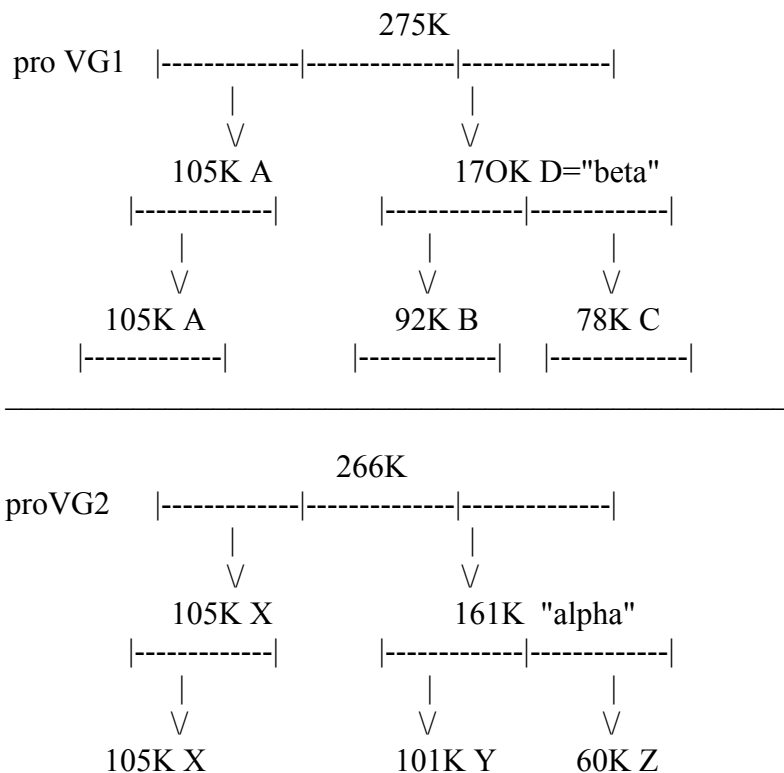


Fig 12. Model of ontogeny of *Periplaneta americana* vitellogenin/vitellin peptide structure based on Table 2 peptides. Pro-vitellogenin (proVg) is synthesized in the fat body, processed and secreted into the haemolymph in an already cleaved state. It is not clear whether the 170K peptide is normally cleaved in the haemolymph or whether this is an artifact of accumulation after ovariectomy. The "alpha" peptide of Clore et al. (1978) is hypothesized to belong to VG2. The mature peptide pattern is seen in freshly ovulated eggs, however some 170K peptide survives over the first seven days of embryogenesis, Fig 10. The size of the 105 K peptide is highly conserved despite the fact that two vitellins have been presumably separated at least since the origin of the *Periplaneta* genus and immunological cross-reaction has totally disappeared.