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BIOCHEMICAL ASPECTS OF *MUSCA DOMESTICA* VITELLOGENESIS

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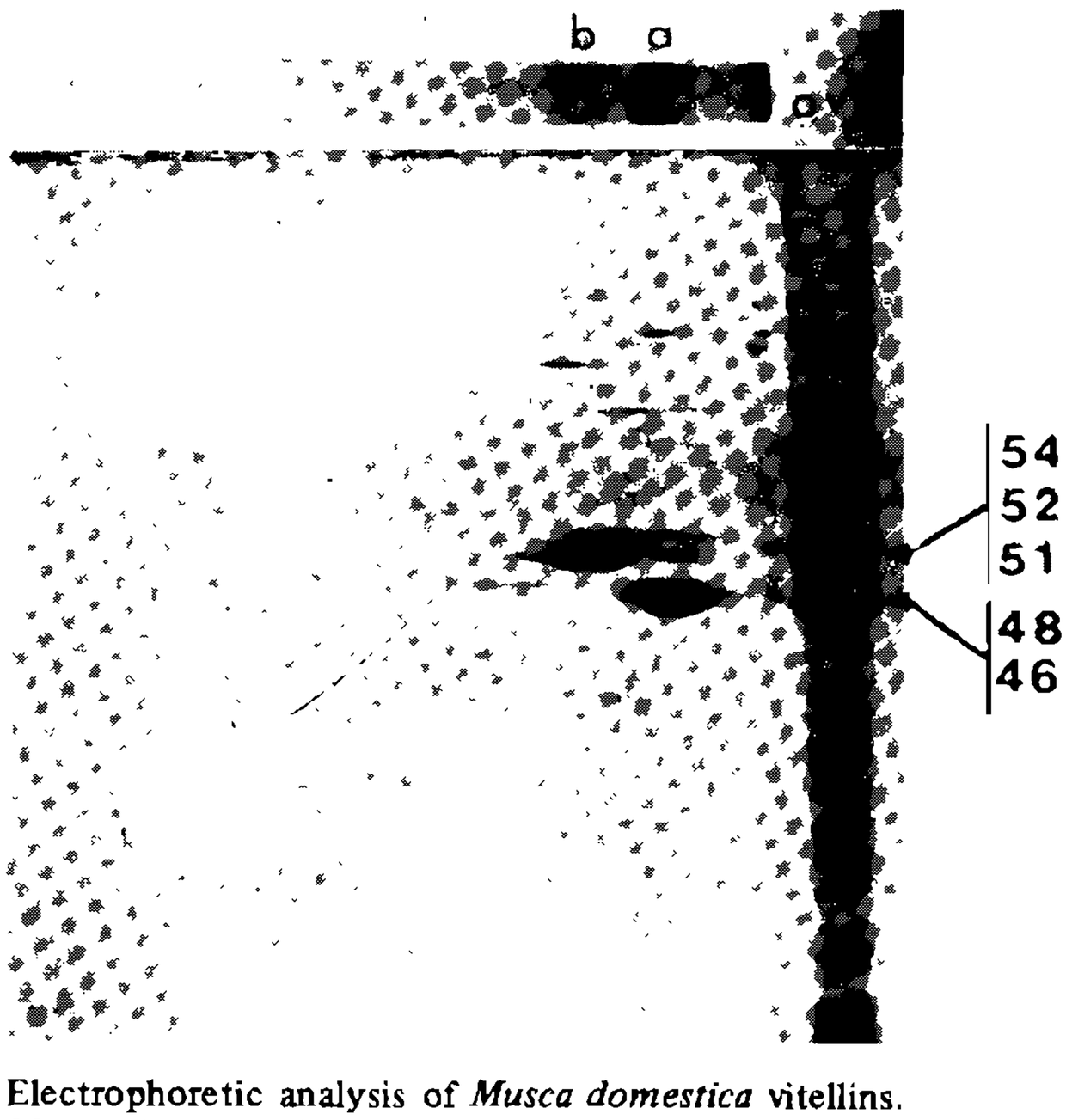
Vitellogenin, the precursor of yolk proteins, is only synthesized by the fat bodies in the majority of insect species studied (Hagedorn & Kunkel, 1979; Engelmann, 1979). However, vitellogenin synthesis by ovaries and fat bodies was described to occur in *Drosophila melanogaster* (Bownes, 1980), *Sarcophaga bullata* (Huybrechts et al., 1983), *Coccinela septempuctata* (Zhai et al., 1984), *Musca domestica* (Bianchi et al., 1985) and *Leptinotarsa decemlineata* (Peferoen & De Loof, 1986).

Some biochemical aspects of *Musca domestica* vitellogenesis were recently studied by Adams & Filipi (1983), Bianchi et al. (1985), Adams et al. (1985), Agui et al. (1985a, b) and Bianchi & Pereira (1987). Some precursor works were made by Bodnaryk & Morrison (1966, 1968), Peltzelt & Bier (1970) and Hall et al., (1976).

The analysis of egg extract and vitellogenic female hemolymph led Adams & Filipi (1983) to conclude that *Musca domestica* vitellin and vitellogenin are composed by three types of subunits with molecular weights of 48,45 and 40K. Agui et al. (1985a) reported that in the flies studied by them the vitellin (and vitellogenin) subunits present molecular weights of 51, 43 and 42 K. Our results (Bianchi et al., 1985) show that *Musca domestica* vitellin and vitellogenin are composed by five types of subunits that may be distributed in a group of higher molecular weight (54, 52 and 51K) and another group of lower molecular weight polypeptides (48 and 46K) (Fig.). The comparison among the results of Adams & Filipi (1983), Agui et al. (1985a) and Biachi et al. (1985) suggests that probably different polypeptides are being considered as vitellin subunits by the different authors. Thus for Adams & Filipi (1983) the subunits with closer molecular weights (48 and 45K) are probably related to our group of higher molecular weight subunits while the reported results of Agui et al. (1985a) suggest the two subunits with closer molecular weights (43 and 42K) are probably related to our group of lower molecular weight subunits. We do not know if these discrepancies are due to difficulties of analyzing the obtained electrophoretic patterns or is due to differences among the strains of *Musca domestica* used by the different investigators.

Adams & Filipi (1983) suggested the presence of two native vitellins (and vitellogenins) in *Musca domestica* through the data obtained by immunological technique. Meanwhile only one vitellin and one vitellogenin could be resolved by pore limiting gradient gel technique. These proteins showed apparent molecular weights of 281 and 283K. These authors do



Electrophoretic analysis of *Musca domestica* vitellins. 10 ovaries were homogeneized in 0.5 ml of 43 mM Tris, 46 mM glycine buffer, pH 8.9 containing 2 mM phenylmethylsuphonyl fluoride. After centrifugation at 10000 xg, 20 μl of the supernatant was submitted to native electrophoresis in a 7% polyacrylamide gel (Reisfeld & Small, 1966). The polyacrylamide cylinder (top of the figure) was laid on the top of a 10% polyacrylamide slab and submitted to a transversal electrophoresis in the presence of SDS (Laemmli, 1970). In the same slab, a sample of ovary homogenate was analysed (OV). The proteins were stainned with Coomassie blue R. The numbers in the right side indicate the two groups of vitellin(s) polypeptides and their molecular weights.

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not report the relationship between the native proteins and their subunits.

Results recently obtained (Fig.) suggest that one of the *M. domestica* vitellins is composed by the subunits of 54, 52 and 51K and another native vitellin is composed by the subunits with 48 and 46K. Several other evidences obtained by $(NH_4)_2SO_4$ fractionation, gel filtration chromatography, hydroxyapatite chromatography and ion exchange chromatography indicate the occurrence of more than one native vitellin (and vitellogenin) in *M. domestica* (Pereira et al., unpublished results).

Vitellogenin is first detected in the hemolymph at the previtellogenic phase (Bianchi et al., 1985; Agui et al., 1985a) and the induction of its synthesis is not dependent on the intake of a proteic food by the flies. However the vitellogenic phase, that leads to egg maturation, is dependent on consumption of a proteic food by the flies (Bianchi et al., 1983; Bianchi & Pereira, 1987).

The pattern of vitellogenin synthesis, in flies maintained on a proteic diet, showed that fat bodies are more active during the initial phase of the gonotrophic cycle. The vitellogenin synthesis by fat bodies attains its maximum at stagium 6 (S_6) declining to low levels at S_7. However, the ovaries are more active on vitellogenin synthesis during the second half of gonotrophic cycle attaining maximum values at $S_7 - S_9$ (Bianchi & Pereira, 1987). The massive accumulation of vitellin by the developing oocytes begins at S_7 and is completed at S_{10}. The comparison between these data about time of vitellogenin synthesis and accumulation by the ovaries, makes possible the conclusion that in *M. domestica* the ovaries contribute with the major part of egg vitellin (Bianchi & Pereira, 1987).

The *M. domestica* vitellin is totally used during the embryogenesis, showing a pattern of utilization very similar to that described for *Drosophila melanogaster* by Bownes (1982) (Pereira et al., unpublished results).

Besides vitellogenins, others hemolymphatic proteins were analyzed in relation to *M. domestica* vitellogenesis. The adult *M. domestica* hemolymph shows a female protein, distinct from the vitellogenins. This protein was first described by Bodnaryk & Morrison (1966, 1968) as fraction 4. The molecular weight of this monomeric protein subunit was determined as 75K and it was quantified in the animals during the first gonotrophic cyle. The results suggest that its synthesis induction is dependent on the intake of proteic food by the flies, showing therefore a different control of synthesis induction from that described for vitellogenin. Despite the high concentration of this protein in the hemolymph, during the vitellogenesis, it is not taken up by the growing oocytes (Pereira et al., unpublished results). The relationship between this hemolymphatic protein and the ovarian development will be investigated.

Another *M. domestica* protein studied was the major hemolymphatic lipoprotein, lipophorin. The involvement of lipophorin in the transport of lipids to the oocytes and/or as a stored nutrient in the eggs of some insects have been subject of debates (Kunkel & Nordin, 1985). The *M. domestica* lipophorin was purified, characterized and quantified during the life cycle (Bianchi et al., 1987). During the adult life of the insect we do not observe any major variation of the quantity of lipophorin in the animals, even during the vitellogenesis, but some modifications in the density of the protein are verified to occur during the gonotrophic cycle. The variation in the density of lipophorin could be related to a lipid mobilization for egg formation (Capurro et al., unpublished results). In contrast to data obtained with other insect species, we were not able to detect lipophorin into the eggs of *M. domestica.* Whether this result indicate a distinct mechanism of lipid accumulation in the housefly oocytes, in relation to other insects, is not yet known.

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