both by changing the concentrations of lead and substrate[17,19] and by applying this method to unfixed sections[20]. Thus, at present the histochemical validity of the ATPase reaction has been recovered. Therefore our results provide evidence for the presence of ATPase activity in the exposed surface of the vesicular membrane of *C. cellulosae*. This is in keeping with data obtained by Lumsden in the outer surface of tapeworm tegument[21]. Since all postembryonic stages of these parasites lack a digestive tract, the assimilation of material from the environment must take place across the tegument. In this case, those enzyme systems which are concerned with the transport of substances, or with the modification of solutes to which parasites are otherwise impermeable, may be quite important to the survival or specific distribution of the parasite in the host tissues. The demonstration, therefore, of ATPase in *C. cellulosae* may be indicative of the parasite unzymatic dependance for its nutrition and selective tissue dwelling. If it is so, the inhibition of ATPase activity would hamper the survival of the parasite. Recent observation by Sengupta on the presence of a mobility-inhibitory factor which acts as a potent ATPase inhibitor opens the possibility of a search for this factor in various tissue fluids[22]. Theoretically the physiological neutralization or absence of this factor in skeletal muscle and brain tissue fluids would explain such preferential distribution of *C. cellulosae*. Further studies are needed to support this theory.

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Studies on protein composition and synthesis in the ovary of *Rhynchosciara americana* (Diptera, Sciaridae)[1]

M. L. Benozzati and R. Basile

Departamento de Biologia, Instituto de Biociências, Universidade de São Paulo, C. P. 11461, São Paulo (Brazil), 10 January 1977

Summary. A comparative study of electrophoretic protein patterns from ovary and haemolymph was undertaken. The synthetic activity of the ovaries was also studied by measuring protein precursors incorporation. Based on these analysis, the origin of vitellogenic proteins is considered.

Vitellogenic proteins have been extensively studied in many insects with respect to their identification and isolation[2], sites of synthesis[3,4], and hormonal regulation[5]. There seems to be 2 mechanisms involved in yolk formation: the synthesis by the ovary itself and the incorporation of proteins, synthesized in other tissues, from the haemolymph.

A study was undertaken on the synthetic activity of the ovaries of *Rhynchosciara americana*, during pupal and adult stages, when yolk deposition occurs. Ovary follicles of *R. angelae* (*R. americana*)[6] have 1 oocyte and only 1 nurse cell, enveloped by a layer of follicular cells[7]. The ovary grows rapidly from the middle stage of pupa, when typical polytene chromosomes develop in nurse cells.

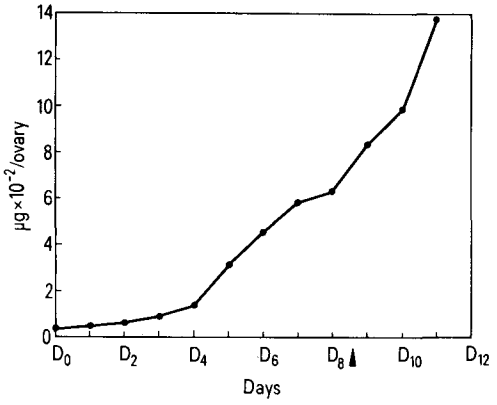

Fig. 1. Determinations of protein content in the ovaries of *R. americana* during pupal and adult stages, using crystalline bovine serum albumin as standard. Each point represents an average of 3 determinations. D_0 corresponds to the newly ecdysed pupae. Arrow indicates adult eclosion.

From this stage up to the adult, a large number of yolk granules can be seen in the oocyte cytoplasm[7]. A fertilized female lays a group of eggs which develop synchronously, producing individuals of the same sex[8].

Quantitative determinations of proteins have been made in order to verify the correlation between protein accumulation and ovarian growth. Following dissection, the ovaries were transferred to 1 ml of a NaOH 1 N solution, at 45 °C for 1 h, and a sample of 0.2 ml from this solution was used for the dosage of soluble proteins, according to Lowry et al.[9]. The spectrophotometric analysis showed an increase of protein concentration at about the 5th day of the pupal stage, which intesifies towards the end of the adult life (figure 1). The ovarian growth observed from mid pupa to adult stage[7] must, therefore, involve a remarkable accumulation of proteins, probably related to vitellogenesis.

The comparative analysis of protein patterns, revealed by polyacrylamide gel electrophoresis[10], showed a re-

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markable correspondence in many protein fractions from fertilized eggs and from ovary and haemolymph of adult females (figure 2). A polymorphic variation involving fractions A and B was detected which may be present or not in different females and groups of eggs. Considering these fractions, groups of fertilized eggs (a total of 14) recently laid by sisters of those flies which had been previously analyzed (60 individual mature ovaries), presented protein patterns probably corresponding to those of the mothers. This indication suggests that fertilized eggs consist, basically, of vitellinic proteins determined by maternal genotype.

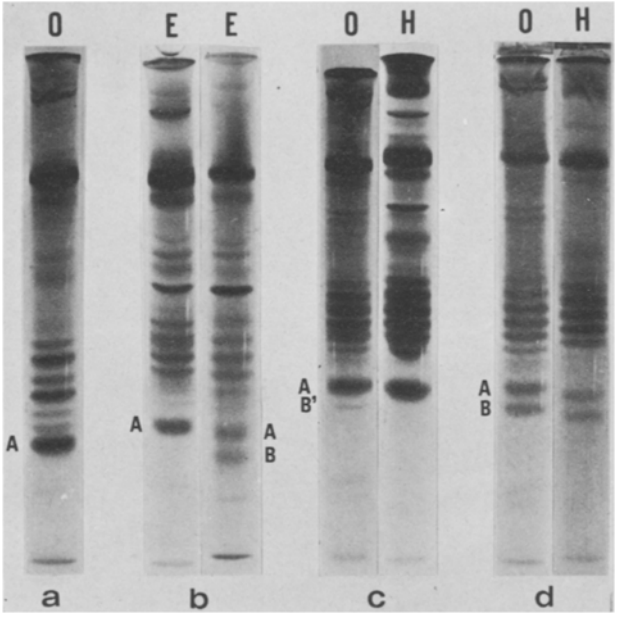

Fig. 2. Gel electrophoresis of fertilized eggs (E), ovary (O), and haemolymph (H) from: a) adult female presenting only fraction A; b) 2 different groups of eggs, one of them having only fraction A, and the other, fractions A and B; c) newly ecdysed, untanned adult, presenting fraction A in both ovary and haemolymph, and fraction B′ in the ovary; d) newly ecdysed, untanned adult, having fractions A and B in both ovary and haemolymph.

A great correspondence have also been found between protein fractions from ovary and haemolymph of newly ecdysed, untanned adults (figure 2, c–d). Females presenting fractions A and B in the haemolymph, also presented both fractions in the ovary. Females, of that specific stage, having only fraction A in the haemolymph, presented also an ovarian protein fraction, B′, with the same mobility of fraction B. Fraction B′ should not be taken by fraction B, since it has not been detected in ovaries of older flies, as well as in fertilized eggs having only fraction A (figure 2, a–b). This fraction (B′) would probably be obscured by fraction B, in those samples showing AB pattern.

The occurrence, in fertilized eggs, of proteins detected in mature ovaries and in the haemolymph of *R. americana* could suggest the incorporation of vitellogenic proteins from the haemolymph. This kind of mechanism has been described in other insects, in which vitellogenesis greatly depends upon proteins synthesized outside the follicles and incorporated from the haemolymph[11,12].

Protein synthesis has been quantitatively determined by in vitro incorporation of H^3-amino acids, in order to verify the synthetic activity of the ovary itself during vitellogenesis. Individual pairs of ovaries were incubated at room temperature in 200 µl of 0.02 M phosphate buffer (pH 7.0) with 4.5% sucrose, containing the isotopes. The amino acid incorporation was determined as described by Clever et al.[13].

The results have shown a remarkable synthetic activity in adult females. The maximum level of amino acids incorporation was detected 4 days after the adult emergence (figure 3). During this period, therefore, there is a considerable contribution from the follicles themselves for the production of proteins probably involved in vitellogenesis.

In preliminary experiments, an intense amino acid incorporation during adult eclosion had been detected. This incorporation was not observed in the experiment presented in figure 3. Therefore, a more detailed analysis was done during that period; it was divided in shorter intervals and protein synthesis was measured in each one

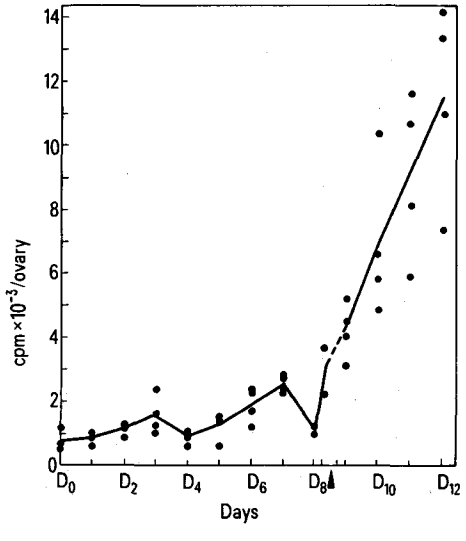

Fig. 3. Incorporation of amino acids at pupal and adult stages. Ovaries were incubated for 75 min in tritiated L-amino acid mixture [NEN] (50 µCi/ml). Each point represents an average of 2 ovaries from different females. D_0 corresponds to the newly ecdysed pupae. Arrow indicates adult eclosion.

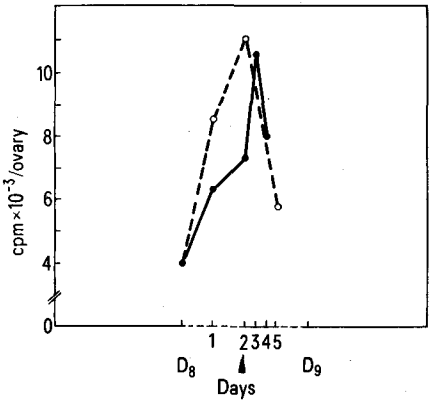

Fig. 4. Incorporation of amino acids during adult moult. Ovaries were incubated in tritiated L-amino acid mixture [NEN] (50 µCi/ml), for 75 min. ○---○ and ●—● represent experiments using 2 different groups of pupae. Each point is an average of 2 ovaries from different females. D_8 corresponds to pharate adults (9 days after larval-pupal ecdysis); *1* pharate adults with the movements of eclosion; *2* newly ecdysed, untanned adults; *3*, *4* and *5* adults respectively 2, 4 and 6 h after eclosion.

of them (figure 4). Ovaries of pharate adults which could be characterized by the movements of eclosion, and adults during the 1st 2 h after eclosion, exhibited an intense incorporation of protein precursors. This outstanding synthetic activity does not seem to be related to any visible cytological alteration in the ovaries. Although we have no data on protein synthesis in other tissues, the synthetic activity observed at this stage should not be specifically related to vitellogenesis itself. It could, however, be part of a more generalized metabolic process, at that specific developmental stage of the organism, as discussed by Pan[14]. Considering these observations, yolk deposition in *Rhynchosciara* seems to involve 2 kinds of mechanism: the incorporation of proteins from haemolymph, as suggested by the corresponding electrophoretic patterns detected for fertilized eggs, ovary and haemolymph, and the synthesis by the ovary itself, mainly during the adult stage, as indicated by its intense incorporation of protein precursors.

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Effect of low density lipoprotein on proteoglycan synthesis by aorta cells in culture[1]

K. Ehrlich and M. Murray

Department of Cytology and Applied Biochemistry, Gulf South Research Institute, P. O. Box 26518, New Orleans (Louisiana 70186, USA), 8 July 1977

Summary. Increasing the content of human serum low density lipoprotein in the growth medium led to greater incorporation of ^{35}S-sulfate into proteoglycan (mostly into dermatan sulfate) by primary aorta cells but did not affect similar incorporation by fibroblast cells. These results suggest a mechanism which can explain the increased deposition of lipid in aorta due to hyperlipidemia.

Several recent studies suggest that lipid deposition in atherosclerotic lesions may be due, in part, to formation of insoluble complexes of serum lipoproteins with dermatan sulfate proteoglycans secreted by aorta smooth muscle cells[2–4]. It has been shown that of all glycosaminoglycans dermatan sulfate has the greatest affinity for low density lipoprotein (LDL)[5] at physiological pH and ionic strength and is present in high concentration in arterial fatty lesions[6]. Bovine aorta contains a dermatan sulfate-chondroitin sulfate hybrid proteoglycan molecule as the major proteoglycan in the tissue[7,8]. Changes in the proteoglycan composition of aorta tissue can be correlated with the extent of atherosclerotic involvement of the tissue[9,10]. Although aorta smooth muscle cells in tissue and organ culture grow more rapidly in the presence of hyperlipemic serum[11], such increased growth alone cannot explain the lipid accumulation which leads to atherosclerosis. We present evidence that serum lipoproteins can stimulate proteoglycan synthesis by aorta cells in culture and hypothesize that such increased secretion by aorta cells in vivo leads to the increase in lipid deposition observed in the formation of fatty streaks and fibrous lesions in the aorta.

Materials and methods. LDL was prepared fresh for each experiment from pooled normal human serum obtained from fasting people. Separation from other serum components was achieved by the method Ewing et al.[12]. In this method, 2 volumes of serum overlaid with 1 volume of a 1.006 g/ml NaCl solution are centrifuged for 20 h at 40,000 rpm in a Spinco Ti60 rotor at 16–20 °C to remove very low density lipoprotein (VLDL). The bottom layers containing LDL are transferred to a clean centrifuge tube (2 volumes) and overlaid with 1 volume of a 1.182 g/ml NaCl-NaBr solution. After centrifugation at 40,000 rpm in the above rotor for 24 h, the floating LDL is removed. The material from the bottom half of the tube is collected and saved as serum minus VLDL and LDL. The various fractions were dialyzed against 0.15 M NaCl with 0.001 M EDTA, sterile filtered under pressure through a 0.22 μm membrane, and stored for less than 2 weeks in plastic tubes at 8 °C before use.

The human foreskin fibroblast (passage 11) cells were obtained from Dr C. Sanders at the LSU Medical School, New Orleans. Rabbit and monkey aorta cells were obtained by primary explant from aortas of 6 2-week-old rabbits and an adult male rhesus monkey. The aortas

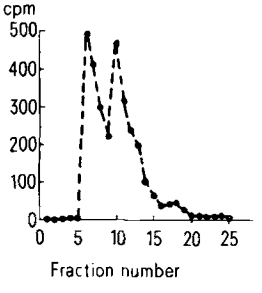

Fig. 1. Bio-Gel P-10 column chromatography of ^{35}S-sulfate-labeled proteoglycans from rabbit aorta cell culture medium (extracellular proteoglycans) after hyaluronidase digestion. The sample was from the experiment in which medium contained 3 times the normal amount of LDL. The sample, 0.2 ml, was applied to a 1.0 × 27 cm column and eluted at a flow rate of 3.3 ml/h.

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