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Biologia. — *Glycogen in a parasitic crustacean*, Lernaea cyprinacea *L., during development and in adult life.* Nota di BERNARDO FRATELLO, IVAN BENEDETTI, MARIA AGNESE SABATINI e LUCREZIA MOLA, presentata (*) dal Socio A. STEFANELLI.

RIASSUNTO. — È stato condotto uno studio istochimico sulla distribuzione del glicogeno durante l'intero ciclo vitale di *Lernaea cyprinacea* L., crostaceo copepode parassita di pesci di acqua dolce. Da questa indagine è emerso che il glicogeno è una riserva importante sia durante lo sviluppo che nell'animale adulto. Durante la vitellogenesi il glicogeno si distribuisce tra le placchette di tuorlo, alla segmentazione si localizza nei blastomeri e risulta molto ridotto in prossimità delle mute naupliari. I copepoditi, alimentandosi, ristabiliscono rapidamente le riserve che non risultano intaccate dalle successive mute. Negli adulti in accoppiamento il maschio possiede notevoli riserve di glicogeno mentre la femmina ne presenta solo quantità ridotte. Nella femmina trasformata le riserve si ricostituiscono abbondanti. In *L. cyprinacea* sedi preferenziali di accumulo del glicogeno sono i muscoli, le cellule della parete del corpo e le cellule che sospendono il tubo digerente: in particolare queste sono un riferimento costante per l'accumulo dai primi stadi larvali all'adulto. Pertanto è da ritenere che in questi animali le cellule che sospendono il tubo digerente svolgano la funzione di accumulo di glicogeno che nei crostacei malacostraci è propria dell'epatopancreas.

INTRODUCTION

Lernaea cyprinacea L. is a cyclopoid parasitic copepod of freshwater fish. Its life cycle after hatching is characterized by three free-living naupliar stages followed by a period where the animals remain on the surface of a host (copepodid I-V stages and cyclopoid stage) (Grabda, 1963). Sexual differentiation occurs at the copepodid V stage and copulation takes place at the cyclopoid stage (Shields, 1978). The inseminated female then begins its transformation (Thornton Bird, 1968). It burrows into the host body, remaining partially protruding from the site of penetration, and completely looses the ability to move.

Essential for the study of this " metamorphic " process in the adult is the knowledge of the morphological, physiological and biochemical changes these animals encounter throughout the entire life cycle. The literature reports only a few data on this subject. Indeed, the morphology of *L. cyprinacea* has been described only by Wilson (1917). His observations are very detailed for sexually mature animals and transformed females but are scarce for the developmental stages. More recent studies have examined the effect of temperature on larval

(*) Nella seduta del 15 dicembre 1984.

development and tranformed females (Nakai and Kokai, 1931; Lahav and Sarig, 1964; Shields and Tidd, 1968), the karyology (Fratello and Sabatini, 1972), the effect of salinity on osmotic relationships throughout development (Shields and Sperber, 1974), and the proteolytic enzyme system of transformed females (Juhasz *et al.*, 1980).

It seemed of interest to begin our research with the study of localization and profile of the metabolic sources of energy in relation to specific phases of the life cycle. Here the results on the distribution of glycogen during the life cycle are reported.

Materials and methods

The source of *L. cyprinacea* was a laboratory stock which had been experimentally maintained upon *Gambusia affinis*. Egg sacs were removed from the parasite and hatched in Petri's dishes with spring water at a temperature of 26° C. After hatching, the larvae developed until the first copepodid stage without the addition of any nutrients. Within 24 hours after moulting the copepodids I must be added to an experimental host in an aquarium and maintained at a temperature of 26° C. The development of the larvae proceeds on the fish, reaching the adult stages and egg production.

This study examined:

- embryos taken out at the start of segmentation and at 6 hour intervals until hatching;

- all naupliar and copepodid stages, immediately after moulting, far from moulting and immediately prior to the subsequent moulting;

- male and female cyclopoids, copulating animals, females at 12, 24, 48, 72, and 96 hours after insemination, females at the time the first egg sacs are produced (about 110 hours after insemination) and after this, every 6 hours until the next egg sacs production.

The various larval stages were identified following the observations of Grabda (1963).

For histochemical evidentiation of glycogen, at least 10 specimens for each stage were cold fixed in Duboscq and Brasil fluid. All the material, embedded in celloidin-paraffin, was cut in transversal or longitudinal sections 5 μm thick. The histological specimens were oxidized for two hours with 1% periodic acid dissolved in 70% alcohol (Palladini and Reitano, 1973). The specimens were then treated with Schiff's reagent, according to the usual techniques (Lison, 1960). A control slide, incubated with 1% diastase for one hour at 37° C, was prepared for each stage.

Results

Glycogen can be detected among the yolk platelets of *L. cyprinacea* oocytes (Tav. I, fig. 6). Its content increases progressively and is very abundant by

the time the eggs enter the egg sacs. During superficial segmentation, glycogen is confined to the blastomeres (Tav. I, fig. 1), and during subsequent development it gradually becomes depleted in some areas. At hatching, a lot of glycogen granules are present in muscles and both inside and outside the cells nearest the yolk.

In the naupliar stages glycogen distribution is the same as that observed at hatching. However, glycogen content varies: in particular, marked decreases in content are found before the moulting processes (Tav. I, fig. 2). The copepodid I not yet on the host has few granules and these only in the peculiar cells joining and suspending the gut to the body wall. We indicate these cells ar suspending cells. The feeding copepodid I and successive stages show a progressive increase of glycogen, at times quite marked, in the muscles, in some gut cells, always in the suspending cells and in the eye. In the nervous system glycogen persists until the copepodid IV stage, and is localized in cell body areas.

Sexual differentiation occurs in the copepodid V stage. Glycogen is very abundant in the muscles and suspending cells of both males and females (Tav. I, fig. 3). Copulating animals show different glycogen content: in males it is enhanced with respect to copepodid V, whereas in females it is diminished (Tav. I, fig. 4). In the transformed female, glycogen granules can be seen in the body wall cells and in the suspending cells (Tav. I, fig. 5). In particular, body wall cells contain a variable quantity of glycogen: those near the genital pore have more granules. The suspending cells are remarkable for their constant and abundant glycogen content (Tav. I, figs. 3-4-5-6).

DISCUSSION

Histochemical evidence of glycogen throughout the entire life cycle of a crustacean is new in literature.

Our data show that in *L. cyprinacea* glycogen is an important metabolite during development and in adults.

During vitellogenesis glycogen is stored among the yolk platelets and at the initial stages of development is confined to the blastomeres where it is progressively used.

Variations in glycogen amount described during naupliar development are due to the utilization of glycogen already present in the cells and of the glycogen produced from yolk demolition. Glycogen production from the yolk is confirmed by the appearance of granules between cells and the yolk.

In this way, the increase of glycogen observed by Hoshi (1953) in a daphnid embryo may also be explained.

Yolk demolition in *L. cyprinacea* leads to greater amount of glycogen in nauplius II prior to moulting. The same trend can be observed in nauplius III, which moults to copepodid I.

A relationship between glycogen content and moulting cycle has been observed only in adult Malacostraca (Hohnke and Scheer, 1970, for review).

Copepodid I, upon feeding, shows rapid storage of glycogen, which continues into the successive larval stages. The copepodids do not show remarkable fluctuations in glycogen levels during the moulting cycle. This may be due to the large amount of glycogen stored and to the short moulting periods.

Variations in glycogen levels in gut cells and muscles can be attributed to the functional state of each cell.

In copepodids V, the distribution and level of glycogen do not differ in the two sexes. In copulating adults, instead, notable differences can be found; females contain much less glycogen than males and this may be related to the use of glycogen to prepare for transformation.

The inseminated female undergoes a process of elongation and widening and transforms into the so-called " anchor-worm ". Now the most evident structures of *L. cyprinacea* are the body wall cells, gut cells, and, above all, the gonads. Glycogen is abundant in the body wall cells, probably related to the functional need to lengthen and widen the cuticle, and in the suspending cells. It is remarkable that the suspending cells are the only structure to maintain a permanent glycogen storage function throughout all changes undergone by the animal. Thus, in *L. cyprinacea*, these cells may have the glycogen storage function which in malacostracan Crustacea belongs to the hepatopancreas.

References

Fratello B. and Sabatini M.A. (1972) - *Cariologia e sistematica di* Lernaea cyprinacea *L. (Crustacea, Copepoda)*. « Rend. Acc. Naz. Lincei », ser. VIII, *53*, 209.

Grabda J. (1963) - *Life cycle and morphogenesis of* Lernaea cyprinacea *L.*. « Acta parasitologica Polonica », *11*, 169.

Hohnke L. and Scheer B.T. (1970) - *Carbohydrate metabolism in Crustaceans*. In: « Chemical Zoology », vol. V (M. Florkin and B.T. Scheer Eds.), Academic Press, New York.

Hoshi T. (1953) - *Studies on physiology and ecology of plankton. IX. Changes in the glycogen content during development of the daphnid,* Simocephalus vetulus *under aerobic and anaerobic conditions*. « Sci. Rep. Tohoku Univ. », *20*, 6.

Juhasz S., Galfi P. and Molnar K. (1980) - *Data on the proteolytic enzyme system of* Lernaea cyprinacea. « Acta Veterinaria Academiae Scientiarum Hungaricae », *28*, 57.

Lahav M. and Sarig S. (1964) - *Observations on the biology of* Lernaea cyprinacea *L. in fish ponds in Israel*. « Bamidgeh. », *16*, 77.

Lison L. (1960) - *Histochimie et cytochimie animales*. Gauthier-Villars, Paris.

Nakai N. and Kokai E. (1931) - *On the biological study of parasitic copepod* Lernaea elegans *Leigh-Sharpe, infesting japanese fresh-water fishes*. « J. Imper. Fish Exp. Stat. », *2*, 123.

Palladini G. and Reitano M. (1973) - *Une nouvelle méthode pour l'identification des groupes aminiques*. « Micr. Acta », *75*, 32.

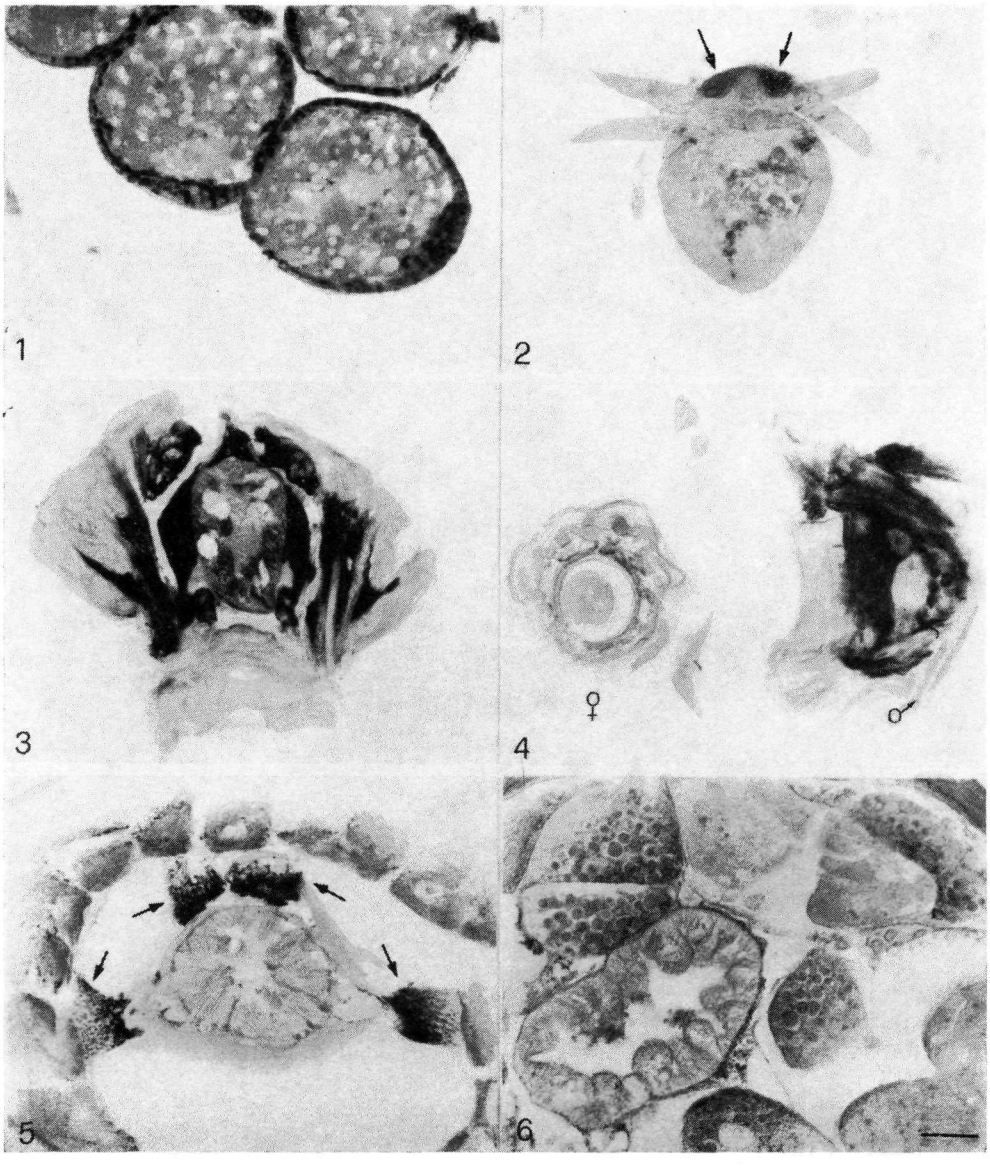
Shields R.J. (1978) - *Procedures for the laboratory rearing of* Lernaea cyprinacea *(Copepoda)*. « Crustaceana », *35*, 259.

Shields R.J. and Sperber R.G. (1974) - *Osmotic relationship of* Lernaea cyprinacea *L. (Copepda)*. « Crustaceana », *26*, 157.

SHIELDS R.J. and TIDD W.M. (1968) – *Effect of temperature on the development of larval and transformed females of* Lernaea cyprinacea *L.* (*Lernaeidae*). « Crustaceana », Suppl. *1*, 87.

THORNTON BIRD N. (1968) – *Effects of mating on subsequent development of a parasitic copepod.* « J. Parasit. », *54*, 1194

WILSON C.B. (1917) – *The economic relations, anatomy and life history of the genus* Lernaea. « Bull. Bur. Fish. », *35*, 165.



Glycogen in 12 hour old embryos of *Lernaea cyprinacea* L. (fig. 1); in nauplius II prior to the moult (arrows indicate labral glands: PAS positive diastase resistent) (fig. 2); in copepodid V female (fig. 3); in copulating animals (fig. 4); in transformed female showing snspending cells (arrows) between gut and the body wall cells (fig. 5); in transformed female showing same oocytes at various stages of vitellogenesis (fig. 6).