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Short Contribution

The contents of proteins, carbohydrates, lipids and DNA during the embryogenesis of *Drosophila*

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ABSTRACT The concentration of proteins, sugars, lipids and DNA has been determined during the embryogenesis of *Drosophila*. The protein content decreases after fertilization, being in the late embryo only 60%the value of the oocyte. The total sugar increases about 2.5-fold, from 3.5 h on until the end of embryogenesis. The lipids increase with a sharp peak at about 4 h and decrease during the rest of embryogenesis. DNA increases exponentially from the beginning of embryogenesis.

KEY WORDS: *Drosophila embryogenesis, proteins, carbohydrates, lipids, DNA*

Changes in mass and more specifically in the amounts of protein, lipids, carbohydrates and nucleic acids are in general associated with changes in growth and differentiation. During *Drosophila* development, the changes in macromolecules that take place from the hatching of the embryo to the adult have been studied (Robertson, 1977). During this period, large increases in mass are observed, whereas no significant change in mass is observed during embryogenesis. The embryo develops in a closed system, with the only exception of gas exchange with the outside (Bownes, 1982). To our knowledge, no information is available on the concentration of macromolecules during this period. We here present data on the changes that macromolecules experience during the embryogenesis of *Drosophila*.

Variations in the protein content during Drosophila *embryogenesis*

Mature oocytes and embryos of different times of development were homogenized and the protein content determined. As shown in Fig. 1, the protein concentration started to decrease upon fertilization and dropped in late embryos to 60% of the value of the oocyte. The main decrease in protein may correspond to yolk granule degradation, which occurs during embryogenesis (Medina *et al.*, 1988; Medina and Vallejo, 1989). The yolk protein is utilized in part to synthesize proteins in other subcellular compartments. This can be inferred from the observation that yolk protein represents 80% of the total protein (Medina *et al.*, 1988) and yolk granules are completely broken down during embryogenesis (Medina and Vallejo, 1989). It is interesting to note the comparatively small change in protein contents that occurs during embryogenesis in comparison with the many-fold increase observed during larval growth (Robertson, 1978). The larva feeds itself from the outside while the embryo develops in a closed system.

Variation in sugar content during Drosophila *embryogenesis*

Total sugar content was observed to increase from 3.5 h on until the end of embryogenesis (Fig. 2). We have not found any report on the sugar concentration during the larval atage. It is conceivable that the increase in sugar is synthesized at the expense of the decrease in protein (Fig. 1).

Variation in lipid content during Drosophila *embryogenesis*

The total lipid (Fig. 3) concentration of the embryo increased after fertilization until peaking at 4 h, when it started to decrease continuously until hatching (Robertson, 1978). After hatching, the lipid concentration rose again and peaked at the end of the larval period.

Variation in DNA content during Drosophila *embryogenesis*

The DNA content of the embryo increased exponentially from the time of fertilization. As is well known, nuclei divide rapidly and synchronously during the first 3 h of development. Then, the cellular membranes are synthesized and, until the end of embryogenesis, the

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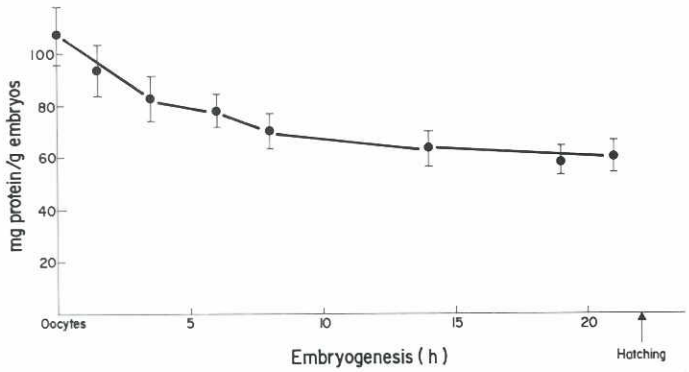

Fig. 1. Decrease in protein during *Drosophila* embryogenesis. *Mature oocytes and embryos of different times of development were obtained, homogenized and the protein determined as explained in Experimental Procedures. 1g contains 10^5 animals. The data represent the average 5-10 experiments.*

cells divide asynchronously and much more slowly (Campos-Ortega and Hartenstein, 1985). The mitDNA is synthesized with a different timing, replication does not commence until after the first 10 h of development and ceases by 18 h (Rubenstein, 1977). The amount of mit DNA in relation with total DNA has been determined during the embryogenesis of *Drosophila* and found to be 50% in mature oocytes, less than 10% at 10 h and 1% at the end of embryogenesis (Spradling and Rubin, 1981).

The data on macromolecule content presented here and related to the time immediately before hatching fits well with the data for the period shortly after hatching reported previously (Robertson, 1978). Some of the variations in the concentration of macromolecules herein described may appear to be unimportant, particularly in comparison with the changes described during the larval period. But this is certainly not the case of DNA, especially considering that the embryo is not nourished from the outside. The changes observed during embryogensis are produced at the expense of materials present in the fertilized oocyte. This report completes therefore the pictures of the changes in macromolecules that take place during the development of *Drosophila*.

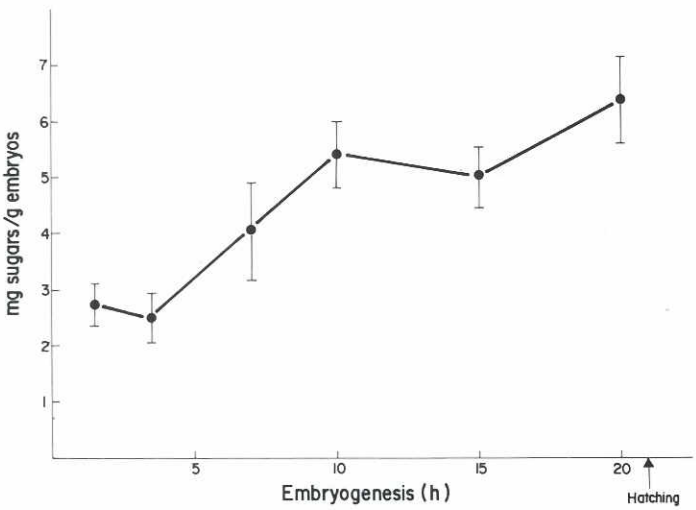

Fig. 2. Increase in sugars during *Drosophila* embryogenesis. *The embryos of different times of development were obtained, homogenized and the sugar determined as explained in Experimental Procedures. 1g contains 10^5 animals. The data represent the average of at least 3 experiments.*

Experimental Procedures

Drosophila melanogaster (Oregon-R strain) was cultured at 25°C as usual (Elgin and Miller, 1977). Oocytes were obtained as indicated (Petri *et al.*, 1977). Embryos were collected routinely for 2 h, with the embryos considered on average to be 1 hour old. The collected embryos were then allowed to develop the additional time required (for example, 5 h to obtain 6 hour old embryos).

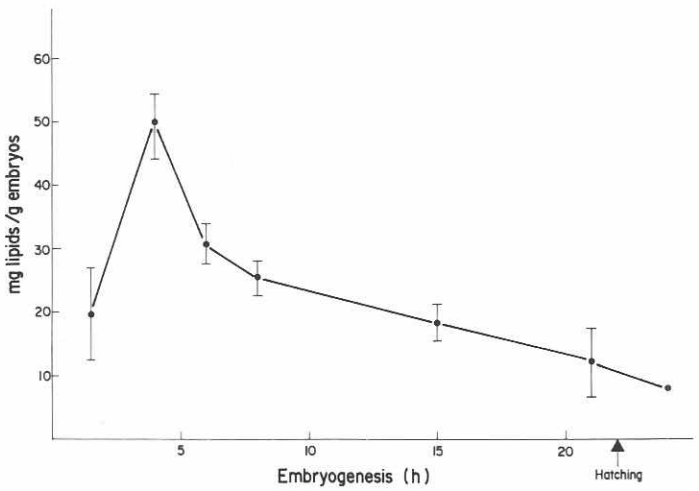

Fig. 3. Variation in lipids during *Drosophila* embryogenesis. *The embryos of different times of development were obtained, homogenized and the total lipid determined as explained in Experimental Procedures. 1g contains 10^5 animals. The data represent the average of at least 3 experiments.*

Determination of weight variation during embryogenesis

A certain quantity of embryos (1 hour old), collected as described, was divided into two parts. One part was counted, filtered and weighed immediately. The other portion was counted, allowed to develop for an additional 19 h and weighed in the same conditions. No significant differences were encountered between the group of early embryos and that of late embryos when the ratio weight/number of em-

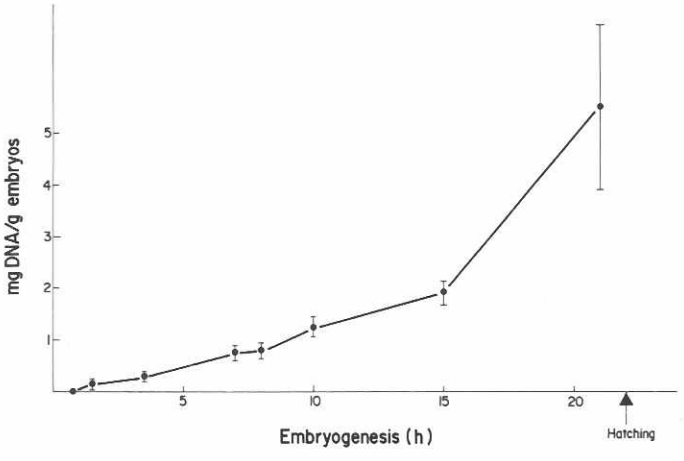

Fig. 4. Increase in DNA during *Drosophila* embryogenesis. *The embryos of different times of development were obtained, homogenized and the DNA determined as explained in Experimental Procedures. 1g contains 10^5 animals. The data represent the average of at least 3 experiments.*

bryos was calculated. The value was close to 10µg per embryo. We find therefore 10^5 embryos per gram.

Protein determination

Embryos and oocytes were dechorionated prior to hand homogenization in 2-4 vols of a medium (Vallejo *et al.*, 1981) composed of 0.3 M sucrose, 15% Ficoll 400, 25 mM Hepes buffer, 60 mM NaCl, 5 mM $MgCl_2$, 0.5 mM $CaCl_2$, 1 mM sodium borate, adjusted to pH 7.5. Protein was determined in the homogenates by a modification of Bradford's method (Read and Northcote, 1981).

Sugar determination

Total sugars were determined in the homogenates obtained by homogenating the embryos in 5 vols of 0.2M buffer potassium phosphate pH 7.3 supplemented with 100 µg/ml soybean trypsin inhibitor and the sugar content was determined by the anthrone method (Herbert *et al.*, 1971). Trehalose was used as standard.

Lipid determination

Embryos were homogenized in 20 vols of chloroform/methanol (2:1) and the total lipids were determined by a gravimetric method as described (Radin, 1969).

DNA determination

Embryos were homogenized in 4 vols of cold 0.2 N perchloric acid and the method of Pestaña *et al.* (1978) was followed to isolate and determine fluorometrically the DNA content. Salmon sperm DNA was used as standard.

Ackowledgments

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