Biochemical characterisation of the development of *Musca domestica*

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Abstract. Quantitative changes in DNA, RNA and protein during the entire developmental period—including embryogenesis, larval and pupal stages—in the housefly were determined. The development of housefly from egg to adult takes around 8.5 days at 35±1°C. During the course of embryonic development total RNA and protein decreased. On the other hand, total DNA increased and registered peak value in 4 hr old eggs. The calorific content of the developing eggs showed a decrease from 5649 cal/g dry weight to 5361 cal/g dry weight indicating the utility of energy reserves during embryonic development. Matured larvae registered high calorific content (6260 cal/g dry weight).

The ratios of DNA/DW (dry weight), RNA/DW, Protein/DW, RNA/DNA and DNA/protein were determined from the data obtained to serve as biochemical indices of changes in development. Protein/DW levels showed variations during larval and pupal stages, increased levels being correlated with the synthesis of new cuticle, etc., prior to ecdysis and the histogenesis of adult tissue prior to emergence. RNA/DW levels were highest in the young larvae but declined rapidly during development. Sharp increase was also noticed in pupal stage. DNA/DW levels were very low in the egg but reached highest level in the larvae just emerged and then declined during further development. RNA/DNA ratio was high in the egg and in the larvae between 30-60 hr of development.

Keywords. Housefly; development; embryogenesis; biochemical aspects.

1. Introduction

Housefly is one of the best-known insects from the standpoint of both basic and applied science (Matsumura 1975; Slama et al 1974). The demonstration of microsomal enzymes in this organism, similar to those occurring in mammals, makes it a favourable test animal for toxicological studies (Wilkinson and Brattsten 1972; Srinivasan and Kesavan 1977). The development of housefly a cyclorrhaphan Diptera, involves a series of morphogenetic changes. The development has been characterised biochemically for a number of insects (Chen 1971; Agrell and Lundquist 1973; Ring 1973; Thomson 1975) but such information is available only for certain stages of housefly—mainly embryogenesis and postecdlosion period (Painter and Kilgore 1967; Miller and Collins 1970; Hall et al 1976). The study of protein, RNA and DNA levels would appear to be
a more accurate way of defining different stages of housefly in biochemical terms. These data will provide a baseline for its use in elucidating the mechanism of action of toxicants. Considering this, an attempt has been made to quantitate protein, RNA and DNA throughout the different developmental phases of housefly.

2. Materials and methods

Housefly *Musca domestica* nebulo Fabr. has been maintained in our laboratory at 31 ± 1°C. Techniques used for rearing the flies, collection and incubation of eggs have been the same as described earlier (Srinivasan and Kesavan 1977).

For growth studies, population size and rearing media were maintained at near constancy in all work. To ensure greater uniformity of the experimental larvae, eggs were collected within a period of 30 min. The freshly hatched larvae were transferred to the beaker containing milk-soaked cotton pads by fire brush. Embryogenesis, larval and pupal development were studied at 35 ± 1°C as this temperature supports maximum rate of development (Srinivasan and Kesavan 1978).

Eggs, larvae and pupae were washed with distilled water, dried on filter paper and weighed on an electrobalance for wet weight. For dry weight, the samples were kept in an oven maintained at 100°C for 6–8 hr and then weighed.

2.1. Analytical procedures for DNA, RNA and protein

The sample size taken was around 100 mg wet tissue. It was homogenised in 0.3M perchloric acid (PCA) (1 ml being used for 20 mg of material). The sample was chilled for at least 10 min on ice and the insoluble fraction was collected by centrifugation. The pellet was then extracted with cold 0.3 M PCA, ethanol–ethanol–ether (3:1) and ether. The washed pellet was finally extracted twice with 0.5M PCA for 30 min at 70°C. The combined supernatants from the two perchloric acid extractions (70°C) were used for measurement of DNA and RNA. The sediment was solubilised in 1N sodium hydroxide at 60°C for 1 hr for protein extraction as suggested by Bhargava and Halvorson (1971). DNA was estimated with diphenylamine reagent (Burton 1956) using calf thymus DNA as standard. RNA was estimated with orcinol (Dische 1955) using yeast RNA as standard. Protein determination was carried out by the method of Lowry *et al* (1951) using bovine serum albumin (BSA) as standard. The values indicated in the text figures represent average of three determinations.

2.2. Estimation of calorific content

Calorific content was determined with a PARR 1412 semimicrobomb calorimeter following the procedure given by Pandian (1969).

2.3. Chemicals

Yeast RNA, calf thymus DNA, bovine serum albumin, diphenylamine and orcinol were products of Sigma Co., USA. All other reagents were of analar grade obtained from BDH.