

The image obtained from the video camera was displayed on a TV monitor. Upon the surface of the monitor a photocell array of four cells was placed linearly to cover the entire field from infundibulum to pelviureteric orifice.

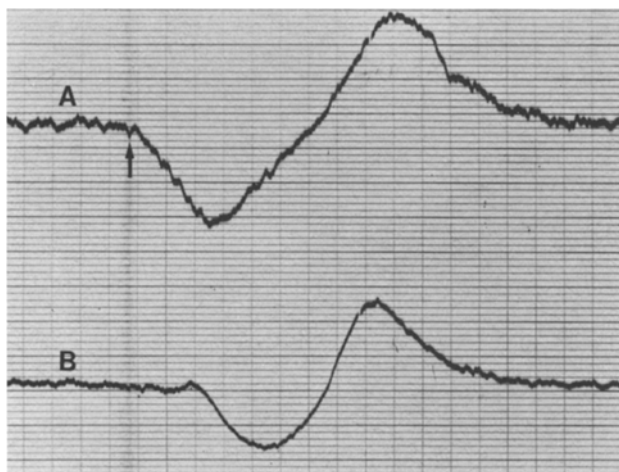


Fig. 2. Record showing the change in optical density of the pelvis as a contracting wave transverses its surface. Trace A demonstrates the photocells located at the calyceal terminus while trace B, the mid pelvis. Note that the contraction is first initiated at the A calyx, shown by the arrow. The mid pelvis responds 2.1 sec later. Note also that the total time taken to transverse the 2 top photocells is 6.8 sec while the photocells at the mid pelvis, 4.6 sec. As the 2 photocell detectors are equidistant this time difference demonstrates that the velocity of the wave accelerates within the renal pelvis.

Each pair of photocells, wired in a bridge configuration, were connected to a recorder.

Results. Observation of the TV monitor shows that the origin of renal pelvic contractions is located at the extreme periphery of the renal pelvis. Changes in the reflective properties of the surface of this structure due to the propagating contraction can readily be detected by the photocells. Figure 2 demonstrates a typical recording thus obtained. The first pair of photocells records the incidence and the speed of propagation of renal pelvic contractions transversing the screen at the 2 points. The incidence of pyeloureteral contraction can be computed from each biphasic ensemble obtained. The speed of propagation is derived by measuring the time taken for the peak of contraction to transverse across the 2 photocells (figure 2, A). Similarly, the same parameters are measured at the mid pelvis with the remaining 2 photocells (figure 2, B). The velocity change by the contractile wave transversing across the cells located at the calyx to mid pelvis can thus be measured. The results show the existence of a velocity gradient of 2.1 ± 0.3 cm/sec/cm along the renal pelvis.

Discussion. The use of an optical system for the measurement of the contraction frequency and velocity gradient of the pyeloureteral system has the distinct advantage of enabling the observer to record the properties of the system without interfering with the movement of the tissue. In addition, such a system can be used to record the variation between different areas of the multicalyceal system simultaneously.

- 1 This work was supported by an NIH, grant No. AM 19366.
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Tissue specific biochemical alterations prior to spinning in the eri-silkworm, *Philosamia ricini*

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Summary. Silkgland, fatbody, hemolymph and midgut of eri-silkworm present tissue specific turnover in their biochemical correlates in preparation for spinning of the cocoon which is followed by larval-pupal transformation.

Information about most of the biochemical alterations involved during development of holometabolous insects is based on studies made on the homogenates of whole insects^{2,3}. However, recent investigations have stressed the need of tissue specific studies to explain the metabolic processes during insect development⁴⁻⁶.

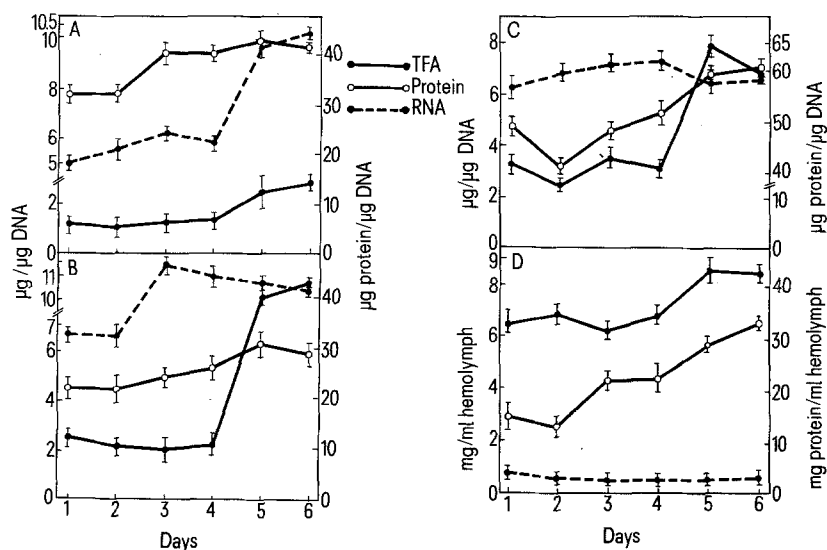
The functional role played by individual tissue seems to be of vital physiological significance, particularly in relation to cocoon formation⁷⁻⁹. Accordingly, the present investigation was planned to find out the changes in certain biochemical correlates in different tissues of 5th instar larvae of eri-silkworm, *Philosamia ricini*, in order to understand the possible metabolic processes involved in the preparation for the spinning of the cocoon.

Materials and methods. The larvae of *Philosamia ricini* were reared at $27 \pm 1^\circ\text{C}$. The removal of the tissues and their fractionation for total free amino acids, protein, RNA and DNA and their quantitative measurements were made according to the procedures already described by Singh and Singh¹⁰. All the determinations of OD were made on an 'Elico' spectro-colorimeter, and the data were expressed as per unit DNA of the tissues. But for hemolymph it was per unit volume of the bodyfluid.

Results and discussion. Metamorphosis in holometabolous insects is accompanied with complex physiological and biochemical processes responsible for morphogenetic activity in different tissues. Moreover, such metabolic processes are further significantly affected prior to and during the process of moulting when the 2 concurrent processes of histogenesis and histolysis proceed together. The changes observed in the level of total free amino acid, protein content, RNA and DNA in fatbody, silkgland, midgut and hemolymph of *Philosamia ricini*, prior to spinning, have been presented in the figure. Generally all the tissues mentioned above exhibit a tendency towards continuous enhancement in the levels of various biochemical components throughout the growth and development of 5th instar larvae and reach the maximum at the time when spinning of the cocoon starts. Thus there is an increased level of biosynthetic activity, presenting close interrelation between the different tissues.

Figure A presents such changes in biochemical parameters in fatbody of last instar larvae. While protein content from an initial level of $30 \mu\text{g}/\text{DNA}$ rises to about $40 \mu\text{g}/\text{DNA}$ on day 3 after, which an approximately similar level is maintained till the end of 5th instar; the RNA content of the

A Biochemical changes in fatbody, B silk-gland, C midgut and D hemolymph of eri-silkworm prior to spinning. (Mean of 5 samples \pm SD.)



tissue steadily rises from the level of 5 $\mu\text{g}/\text{DNA}$ to 6 $\mu\text{g}/\text{DNA}$ till day 4 followed by a sharp jump to the level of 9.5–10.5 $\mu\text{g}/\text{DNA}$ on day 5 and 6. This is in contrast to the findings of Takahashi⁷ who reported an initial high level of RNA:DNA ratio in early days of 5th instar. Thus present observation is in conformity with the enhanced protein biosynthetic activity in fatbody prior to spinning³. On the other hand, total free amino acid of fatbody is maintained at a steady state till day 4, followed by a little increase on day 5 and 6.

As evident from figure B, total protein content of silk-gland (per unit DNA) consistently rises from the level of 22.0 to 32.0 till day 5 which is maintained approximately at the same level on day 6 but gets further increased on 1st day of spinning¹⁰. RNA:DNA ratio in this tissue takes a sharp rise on day 3 from an initial level of 6.6 to 11.3, which is probably in response to the increased RNA polymerase activity reported by Sridhar et al.⁸ in *B. mori*. From day 4 onwards, this ratio gradually declines towards the end of 5th instar but is still maintained at a higher level than that in early days. Total free amino acid concentration of the tissue is maintained at a steady state till day 4, after which there is a remarkable increase from about 2 $\mu\text{g}/\text{DNA}$ to 10 $\mu\text{g}/\text{DNA}$ on day 5 and 6. This surprising high level of total free amino acids in silk-gland just prior to spinning is indisputable and would certainly be in accordance with the demand of free amino acids for their incorporation into fibroin synthesis. Remarkable enhancement in the level of total free amino acids and RNA in silk-gland towards late days of 5th instar may also be correlated with high demand of structural proteins needed for the massive growth of the silk-gland tissues itself. However, such high level of biosynthetic activities do not appreciably affect the total protein concentration of silk-gland, because the same has been expressed in the present work as per unit DNA which also significantly increases.

Midgut presents an almost similar nature of accumulating the various biochemical components towards the late days of 5th instar (figure C). Protein content of the tissue decreases from a level of about 50 $\mu\text{g}/\text{DNA}$ on day 1 to about 40 $\mu\text{g}/\text{DNA}$ on day 2, after which there is consistent increase in the level of this parameter, reaching the maximum (60 $\mu\text{g}/\text{DNA}$) on day 6. Total free amino acid concentration in midgut fluctuates within the range of 2.5–3.5 $\mu\text{g}/\text{DNA}$ till day 4 followed by almost 2-fold rise in this parameter on day 5, which shows a little decline on day 6. This increased level of amino acid pool size in midgut at the end of 5th instar is probably because the larvae at this

stage are maximally engaged in active feeding. RNA:DNA ratio of this part of alimentary tract presents gradual increase till day 4 followed by little decrease on day 5 and 6, but these fluctuations are within limited range of 6.0–7.0 $\mu\text{g}/\text{DNA}$. Thus almost the same level of RNA:DNA ratio in midgut tissue indicates a consistent protein biosynthetic rate throughout the 5th instar stage.

Figure D exhibits the overall status of these biochemical parameters in hemolymph of 5th instar larvae. Protein concentration of the bodyfluid presents a gradual increase from an initial level of 15 mg/ml in early days to about 32.0 mg/ml bodyfluid in late days of 5th instar. This high level of blood protein towards late days of 5th instar is apparently because of remarkable sharp increase in RNA:DNA ratio of fatbody during the same days, indicating an active biosynthesis of proteins which get simultaneously secreted into hemolymph. Probably this is the reason why the fatbody itself does not show appreciable enhancement in the protein level during these days. A high titre of free amino acid pool size is maintained within the bodyfluid, which further increases on day 5 and 6. This indicates a continuous influx of amino acids after digestion of proteins (ingested food) from midgut, which also shows similar increase in total free amino acid pool size during the same days. In contrast, RNA content in the hemolymph is at a very low level and presents almost no change throughout 5th instar larval stage.

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