Tissue specific biochemical alterations during spinning in the eri-silkworm, *Philosamia ricini*

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Summary. Protein, total free amino acid and RNA accumulated in the silk gland of eri-silkworm decrease drastically within 2-4 days of spinning. In contrast, the fatbody presents an augmentation of total protein concentration towards the end of the spinning process. The hemolymph maintains constancy of these molecules with little variation.

Studies on biochemical changes in insects during embryogenesis have been made by many workers, but there is little information during the process of spinning (cocoon formation)[2,3]. Van Der Geest et al.[4] observed increased protein accumulation at the end of last larval instar and its decrease after pupation in *Pieris brassicae.* This accumulated protein in hemolymph at the end of last larval instar is transported to the fatbody during pupation in this insect[5]. Noguchi et al.[6] demonstrated disturbances in the synthesis of silkprotein and fatbody protein caused by gamma radiation resulting into decreased degrading ability of the integument and gut proteins of latest larval instar of *Bombyx mori,* which could partly be the source of silkprotein. Similarly, there occur changes in amino acid spectrum and related components of hemolymph during cocoon formation by the larvae of fly-*Rhynchosciara americana*[7]. The above findings clearly indicate the possible differential changes in various biochemical correlates of the tissues during spinning process. Therefore, the present investigation aims at finding out tissue specific changes in certain biochemical components during spinning by the last larval instar of eri-silkworm, *Philosamia ricini.*

Materials and methods. The larvae of *Philosamia ricini* were reared in the laboratory at 23±1 °C and fed on fresh castor leaves. 5th (last) instar larvae ceased feeding and produced faecal fluid prior to spinning of the cocoon. Spinning continued for about 6 days. The larvae were marked with the date when the spinning started. Daywise tissue specific biochemical analysis were made using these dated larvae. Incomplete cocoons were cut open to take out the larvae for experimental purpose. Hemolymph samples were obtained by making puncture on the dorsal side of the body in the thoracic region and oozing blood was collected with the help of micropipette and immediately mixed with 80% ethanol making 1 ml total volume. After hemolymph collection, the larvae were dissected out in ice-cold water and the silkgland of both sides and sufficient amount of fatbody were taken out and weighed.

Tissue fractionation for amino acid, protein, RNA and DNA was made by conventional procedures. The protein concentration was measured with the help of Folin-Ciocalteau reagent as suggested by Lowry et al.[8], using bovine serum albumin as standard. The quantitative assay of total free amino acids was conducted by Ninhydrin reaction[9], using glycine as the standard amino acid. Yeast RNA-hydrolysate was used as the RNA-standard for the determination of RNA-concentration with the help of orcinol reagent[10]. The determination of DNA-concentration was made following Dische's method[11], and calf-thymus DNA was used as the standard. All the determinations of optical density were made using 'Elico' spectrocolorimeter. The data were expressed as per unit DNA of the tissues. But for hemolymph, it was per unit volume of the body fluid.

Results and discussion. Various metabolites like proteins, amino acids and RNA accumulate to the maximum just prior to spinning but gradually decrease during spinning process, particularly in silkgland. The DNA-content of the

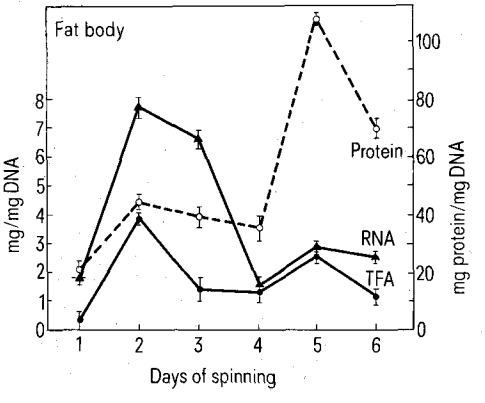

Fig. 2. Biochemical changes in the fatbody of eri-silkworm during the process of spinning. (Mean of 5 samples±SD.)

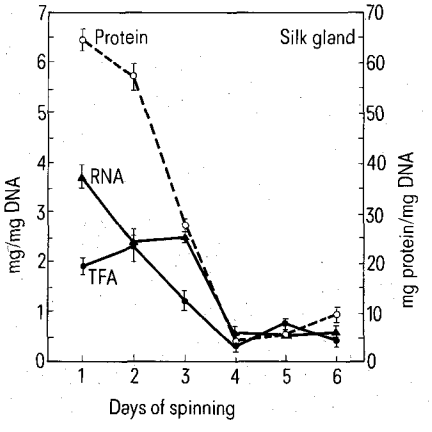

Fig. 1. Biochemical changes in the silkgland of eri-silkworm during the process of spinning. (Mean of 5 samples±SD.)

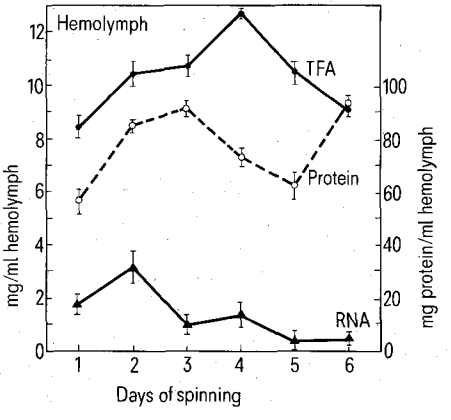

Fig. 3. Biochemical changes in the hemolymph of eri-silkworm during the process of spinning. (Mean of 5 samples±SD.)

whole silkgland along with its wet wt continues to decrease till complete loss of the gland prior to pupation.

Figure 1 demonstrates that total free amino acid, protein and RNA-concentrations in silkgland continue to decrease drastically till day 4. The level of all the 3 components is minimum and at constant level from day 4 to day 6, beyond which the whole silkgland dwindles away. This sequencial loss of these biochemical components can be well anticipated on successive days of spinning, when the proteins accumulated in the silkgland are utilized for the formation of silk fibres. It clearly indicates that whatever silk protein is needed for spinning, is already stored prior to spinning and thus continued synthesis of silkprotein during spinning process cannot be anticipated in this silkworm. Noguchi et al.[6] suggested integument and gut proteins as the source of precursors for silkprotein in *Bombyx mori*, and it is known that the integument and gut proteins start hydrolyzing prior to ecdysis.

In contrast to silkgland, fatbody presents an increased accumulation of protein towards the end of spinning accompanied with constant level of amino acid pool size, except for slight increase on day 2 (figure 2). Such protein enhancement might be in relation to preparation for organogenesis in the next instar. Similar protein accumulation in fatbody of *Pieris brassicae* has been reported by Chippendale et al.[5] at the prepupal stage. RNA, however, initially increases on day 2 and 3, after which it is maintained approximately at the same level throughout.

Although hemolymph also demonstrates little variation in the concentration of amino acids and RNA (figure 3), the variations are within limits, and there is tendency for the maintenance of constancy of these metabolites which might help in strict regulation of osmotic balance in the body-fluid. However, the proteins tend to increase in hemolymph at the end of spinning, as also reported by Van Der Geest et al.[4] in *Pieris brassicae* at the beginning of pupal stage. It is possible that hemolymph may partly help to provide proteins as such to silkgland during spinning, due to which towards end of spinning there occurs an increase in protein concentration of hemolymph.

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Inhibition de l'arthrite à adjuvant par l'oxonate: influence de l'uricémie[1]

Inhibition of adjuvant arthritis by oxonate: Influence of uricemia[1]

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Summary. Adjuvant arthritis in rat is inhibited by an oxonate diet, which increases uricemia. The inhibition is proportional to blood uric acid level and not to oxonate concentration in the diet. Oxonate alone does not exert an inhibitory effect.

Nous avons étudié expérimentalement l'exclusion mutuelle de la goutte et de la polyarthrite rhumatoïde en combinant l'arthrite à adjuvant du rat et l'hyperuricémie provoquée par une alimentation enrichie en oxonate de potassium et en acide urique. Nos expériences ont montré que ce régime à l'oxonate inhibe l'arthrite à adjuvant et que cette inhibition ne semble pas impliquer le métabolisme des pyrimidines[2-4]. Le but du présent travail est de déterminer si cette inhibition est causée directement par l'oxonate ou si elle est liée à un mécanisme plus complexe associé à l'uricémie.

Matériel et méthodes. Des rats Wistar mâles, pesant environ 270 g, ont été répartis au hasard en 12 groupes de 6 rats. Ces 12 groupes comprennent 6 groupes de rats «témoins» et 6 groupes de rats «injectés» correspondant à 6 régimes alimentaires différents. Les pourcentages (en poids) d'oxonate et d'acide urique qui sont ajoutés à l'alimentation habituelle (Purina Laboratory Chow) sont indiqués au tableau 1.

15 jours après l'introduction de ces différents régimes alimentaires, les 6 groupes «injectés» ont reçu une injection

Tableau 1. Influence du régime alimentaire sur l'arthrite à adjuvant

Groupes injectés	Alimentation Oxonate (%)	Acide urique (%)	Uricémie mg %	(± DS)	Valeurs relatives* Score arthritique	Œdème de la patte non-injectée	Œdème de la patte injectée
1	0	0	2,75	(0,34)	100	100	100
2	0	1	2,82	(0,63)	103,0	122,5	105,4
3	2,5	0	1,60	(0,32)	112,4	121,2	98,9
4	2,5	1	4,57	(0,34)	74,6	64,6	90,3
5	5	0	1,92	(0,37)	102,3	122,7	103,3
6	5	1	5,65	(1,56)	78,0	44,5	78,9

* Surfaces relatives des différentes courbes entre le jour 9 et le jour 50.