un mélange méthylal/méthanol (4/1), séchés à l'étuve à 100 °C, en présence de 5 ml de mélange scintillateur. PPO 5 g; dimethyl POPOP 0,3 g; toluene 1000 ml. Les protéines sont déterminées par la méthode de LOWRY[8].

Résultats. Dans les conditions définies au paragraphe «Matériel et méthodes» et en présence des membranes granulaires, on obtient une synthèse peptidique dont la cinétique est représentée sur la Figure (courbe a). Cette courbe montre que la synthèse peptidique cesse au bout de 20 à 30 min d'incubation.

Si, après avoir réalisé une telle synthèse protéique pendant 30 min en remplaçant les acides aminés marqués par des acides aminés froids, on incube à nouveau pendant 30 min à 37 °C, en présence d'UDP-N-acetylglucosamine ^{14}C, on n'observe qu'un accroissement très faible d'incorporation de N-acetylglucosamine par rapport à un témoin ne comportant pas les acides aminés non marqués; si l'on rapporte la radioactivité mesurée aux protéines de la fraction des membranes granulaires, on trouve une valeur de 40 cpm/mg.

Par contre, si l'UDP-N-acetylglucosamine est introduit dans le milieu en même temps que les acides aminés, on obtient 400 cpm/mg de protéines. Les études cinétiques du transfert de la N-acetylglucosamine sont données sur la Figure (courbe b): en l'absence de synthèse protéique (courbe c): en présence de synthèse protéique; on note une nette dissociation de ces 2 courbes cinétiques. Enfin la puromycine à la concentration 10^{-3} *M* abaisse la synthèse protéique d'un facteur 2,5 et le transfert de N-acetylglucosamine d'un facteur 1,9.

Discussion. Nous avons mis en évidence une activité de transfert de la glucosamine qui semble dépendente de la synthèse protéique; cette N-acetylglucosaminyltransférase présente les particularités suivantes: d'une part elle est inhibée par la puromicyne avec un taux d'inhibition voisin de celui observé pour la synthèse protéique, d'autre part elle est localisée dans les membranes granulaires. La contamination de cette fraction par l'appareil de golgi qui renferme une autre N-acetylglucosaminyltransférase mais non inhibée par la puromycine n'est que de 1% [2–9]. On peut donc la considérer comme responsable du transfert de la première glucosamine établissant la liaison peptide-glucide.

Une synthèse protéique préalable est sans action sur elle; au contraire, si on réalise en même temps une synthèse protéique et le transfert du résidu glucidique, l'activité de cette N-acetylglucosaminyltransférase est nettement augmentée. Il semblerait que ce transfert ne puisse se produire qu'à un moment précis de la synthèse de la chaîne peptidique acceptrice. On sait que cette dernière acquiert tres vite une structure tridimensionnelle[10] alors même qu'elle est toujours liée aux ribosomes, cette dernière évoluant à mesure que la synthése se poursuit. On peut alors supposer qu'au cours de cette évolution, la chaîne peptidique se trouve dans des conditions favorables au fonctionnement de la glucosaminyltransférase voisine, soit par une position spatiale idione, soit en démasquant le triplet-asparagine-x-sérine (ou théorine) – qui constituerait le signal de transfert. Ces conditions seraient fugitives, c'est pourquoi une synthèse protéique à priori augmente la quantité de chaînes peptidiques acceptrices mais pas les capacités réelles de transfert du système.

Summary. The present report is concerned with an N-acetylglucosaminyltransferase which catalyzes the transfer of N-acetylglucosamine from UDP-N-acetylglucosamine to nascent protein chains. This enzyme is localized in a rough microsomes fraction prepared according to the method of DALLNER[3].

J. FROT-COUTAZ et R. GOT

Laboratoire de Biochimie des Membranes, E.R. No. 66, 43, Boulevard du 11 Novembre 1918, F-69621 Villeurbanne (France), 16 juillet 1973.

[8] O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR et R. J. RANDALL, J. biol. Chem. *193*, 265 (1951).
[9] J. FROT-COUTAZ, Thèse de Doctorat es Sciences, No. 131, Lyon (1972).
[10] D. C. PHILLIPS, Scient. Am. *215*, 78 (1966).

Variation in Trehalase Activity in the Fat Body, Intestine and Haemolymph of *Philosamia ricini* (Eri-Silkworm) During Development

Presence of trehalase was first demonstrated by FREREJACQUE[1] in insects that feed on fungi. This enzyme brings about the hydrolysis of trehalose, a non-reducing disaccharide, to glucose whereby trehalose participates in various metabolic activities indirectly in the hydrolyzed form. The occurrence of trehalase in different tissues of various insects with a predominant activity in the salivary gland and intestine has been reported by many investigators[2–16]. While its presence in insect haemolymph or fat body has been demonstrated by HOWDEN and KILBY[17] and others[2, 13, 16], DERR and RANDALL[4] have reported its absence in both these insect tissues. Absence of trehalase in insect haemolymph, as reported by many[2, 14, 16, 18], could be attributed to the presence of inhibitors in the haemolymph[19]. Decline in blood trehalose level accompanied with high trehalase activity has been shown to be associated with a process requiring energy and with another for providing substrate for chitin synthesis[19].

Philosamia ricini fat body[20], as well as the entire larval tissue homogenate[21], have been observed to exhibit elevated glycogen level during larval development and a decline during larval-pupal transformation and early pupal period, followed by a slight increase till ecdysis. The variations in glycogen level could possibly be correlated with the variations in active phosphorylase activity. Coupled with this, a variable pattern obtained for total haemolymph carbohydrate of *Philosamia ricini* (PANT and LACY, unpublished data) induced us to study the variations in trehalase activity of different tissues of *Philosamia ricini*, an insect of economic importance in Indian Silk Industry, during development.

Materials and methods. Rearing of *Philosamia ricini* was carried out as described by PANT and LACY[22]. Tissue homogenates of larvae and pupae were made as described earlier[20]. Larval haemolymph was collected as described by PANT and AGRAWAL[23]. Trehalase activity was assayed as described by DAHLQVIST[24] and DERR and RANDALL[4] by estimating the rate of trehalose hydrolysis. The assay system, consisting of trehalose solution (0.6 ml, 0.14 *M*), phosphate buffer (2.1 ml, 0.1 *M*, PH 5.6) and enzyme solution (0.3 ml) or haemolymph (0.05 ml) was incubated at 30 °C for 30 min, after which the reaction was stopped by heating the tubes in a boiling water bath for 5 min. The glucose released was estimated by employing glucose

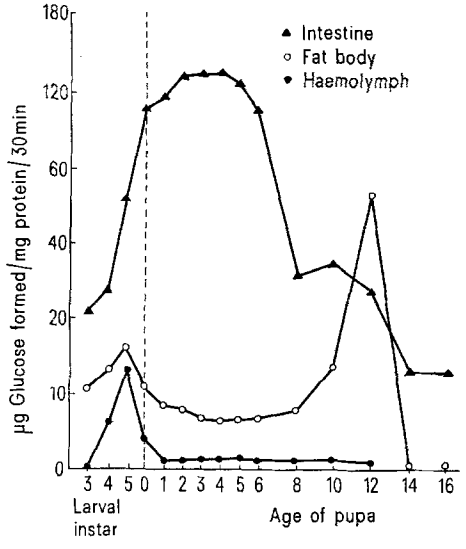
oxidase as described by THOMPSON[25]. Protein was estimated by Folin Ciocalteau's method modified by LOWRY et al.[26] employing bovine serum albumin as the standard. Trehalase activity has been expressed in terms of μg of glucose released/mg protein in 30 min under the assay conditions.

Results and discussion. Sufficient evidence has now accumulated suggesting that the fall in the blood trehalose level during muscular activity or starvation is due to the ready availability of trehalose as an immediate carbohydrate reserve[14, 26] involving UDP-glucose[27]. The rate at which trehalose is utilized has been observed to be almost at the same rate at which it is synthesized and secreted. The enzyme responsible for this has been considered to be trehalase. Predominance of trehalase activity in the larval intestinal tissue of several insects has been demonstrated[13, 14, 16, 28]. Data obtained for *Philosamia ricini* in the present investigation suggest that intestinal trehalase plays an important role in the biosynthesis of glycogen in the fat body as well as in the maintenance of blood sugar level and its absorption.

The intestinal tissues of the larvae of *Philosamia ricini* show an increase in trehalase activity during the 5th instar (Figure). A possible explanation for this high trehalase activity via the intestinal tissue could be ruled out since the enormous consumption of food by the larvae results in the increase of glycogen level of the fat body in addition to its altering the concentration gradient which perhaps brings about the escape of trehalose into the gut.

Trehalase activity has been demonstrated in the fat body of *Celerio euphorbiae*[13], *Bombyx mori*[14], *Samia cynthia ricini*[16] and *Schistocerca gregaria*[17]. Similar data obtained for the fat body of *Philosamia ricini* depicting elevated trehalase activity during the 4th instar followed by a fall in the last 5th instar, suggest that trehalase of the fat body provides a substrate for glycogen synthesis in the fat body.

Presence of trehalase in the haemolymph has been demonstrated in *Celerio euphorbiae*[13], while its absence has been reported in other insects[14, 16, 29]. Data obtained for *Philosamia ricini* support the latter observation, only during the 3rd instar stage. However, larval haemolymph during 4th and 5th instar stages exhibits considerable trehalase activity.



Variation in trehalase activity in the fat body, haemolymph and intestinal tissues of *Philosamia ricini* during larval-pupal development.

A very curious variation pattern (Figure) has been exhibited by the intestinal tissue of *Philosamia ricini* during metamorphosis period. It shows a sharp increased trehalase activity at the onset of metamorphosis that continues till day 4, a period during which histolysis takes place more rapidly than histogenesis. After this the activity starts first gradually and then sharply decreasing till day 8 and again after day 10 till ecdysis. Fat body, on the other hand, shows an increase in trehalase activity on day 0 which is followed by a decrease till day 6. On day 8 it shows a slight increase which is followed by a sharp rise on day 10, then falls again on day 12. However, no activity was observed on days 14 and 16. Haemolymph, on the other hand, shows a fall in the 0 day pupa which is followed by a decline till day 12 evincing only traces of trehalase activity. However, on days 14 and 16, before the moth emerges, no activity could be detected in the haemolymph.

Thus the present investigation suggests that the intestinal tissue trehalase probably plays an important role in regulating the blood sugar level and also in increasing glycogen synthesis in the fat body during feeding period.

Zusammenfassung. Es wurden die Veränderungen des Trehalasegehaltes, der Trehalaseaktivität im Darmgewebe, Fettkörper und Hämolymphe des Seidenspinners *Philosamia ricini* während der Metamorphose und deren Rolle in der Regulation des Blutzuckerspiegels und der Glykogensynthese im Fettkörper untersucht.

RADHA PANT and INDIRA D. MORRIS

Department of Biochemistry, Allahabad University, Allahabad-2 (India), 19 July 1972.

[1] M. FREREJACQUE, C. r. Séane. Soc. Biol., Paris *213*, 88 (1941).
[2] E. C. ZEBE and W. H. MCSHAN, J. Cell comp. Physiol. *53*, 21 (1959).
[3] P. EHRHARDT and G. VOSS, J. Insect Physiol. *8*, 165 (1962).
[4] R. F. DERR and D. D. RANDALL, J. Insect Physiol. *12*, 1105 (1966).
[5] W. A. L. EVANS and D. W. PAYNE, J. Insect Physiol. *10*, 657 (1964).
[6] F. DUSPIVA, Verh, dt. zool. Ges. *63*, 440 (1954).
[7] P. EHRHARDT, Z. vergl. Physiol. *46*, 169 (1962).
[8] F. MUKAIYAMA, J. seric. Sci., Tokyo *30*, 1 (1961).
[9] T. YUSHIMA and S. ISHII, Oyo-Kontyu *8*, 51 (1952).
[10] L. K. ALLMANN and F. DUSPIVA, Experientia *22*, 231 (1966).
[11] H. LAUFER, Y. NAKASE and J. VANDENBERG, Devl. Biol. *9*, 367 (1964).
[12] W. A. L. EVANS and J. MARSDEN, Nature, Lond. *177*, 478 (1956).
[13] C. PETRYSZYN and L. SZARKOWSKA, Bull. Acad. pol. Sci. Cl. II, Sér. Sci. biol. *7*, 491 (1959).
[14] S. SAITO, J. Biochem., Tokyo *48*, 101 (1960).
[15] G. DUCHATEAU-BOSSON, CH. JEUNIAUX and M. FLORKIN, Archs int. Physiol. Biochim. *71*, 566 (1963).
[16] G. K. CHANG, F. LIU and H. FENG, Acta ent. sin. *13*, 494 (1964).
[17] C. F. HOWDEN and B. A. KILBY, Chemy. Ind., *1956*, 1453.
[18] S. FRIEDMAN, Arch. Biochem. Biophys. *93*, 550 (1961).
[19] M. FLORKIN and CH. JEUNIAUX, Bull. Acad. r. Belg. Cl. Sci. *51*, 541 (1965).
[20] R. PANT and I. D. MORRIS, J. Biochem., Tokyo *1*, 1 (1972).
[21] R. PANT and I. D. MORRIS, J. Biochem., Tokyo *66*, 29 (1969).
[22] R. PANT and P. S. LACY, Indian J. exp. Biol. *3*, 274 (1965).
[23] R. PANT and H. C. AGRAWAL, Biochem. J. *96*, 824 (1965).
[24] A. DAHLQVIST, Acta chem. scand. *14*, 9 (1960).
[25] R. H. THOMPSON, Clin. chim. Acta *13*, 133 (1966).
[26] O. LOWRY, N. ROSEBROUGH, A. FARR and R. RANDALL, J. biol. Chem. *193*, 265 (1951).
[27] J. S. CLEGG and D. R. EVANS, J. exp. Biol. *38*, 771 (1961).
[28] B. A. KILBY, *Aspects of Insect Biochemistry:* Biochem. Soc. Symposium No. 25 (Ed. T. W. GOODWIN; Academic Press, London 1965), p. 39.
[29] F. LIU and H. FENG, Acta ent. sin. *14*, 432 (1965).