Cyclic AMP During Development and Ageing of the Insect *Ceratitis capitata*

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Summary. Cyclic AMP was determined during the development of the diptera *Ceratitis capitata*. The concentration of the nucleotide reaches a peak at apolysis with a sharp decline in the pharate adult stage. A gradual increase takes place through the longevity of adult stage reaching a maximum plateau at the end of life.

Cyclic AMP has been suggested to be the second messenger which mediates the action of a variety of hormone and neurohormones in different animal tissues[2]. Its level is regulated by adenyl cyclase which synthetizes it from adenosine triphosphate and cyclic nucleotide phosphodiesterase which degrades it to 5'-adenosine monophosphate. Although the vital role played by cyclic AMP in vertebrates has been studied in detail, its presence and importance in insects has been neglected until quite recently[3].

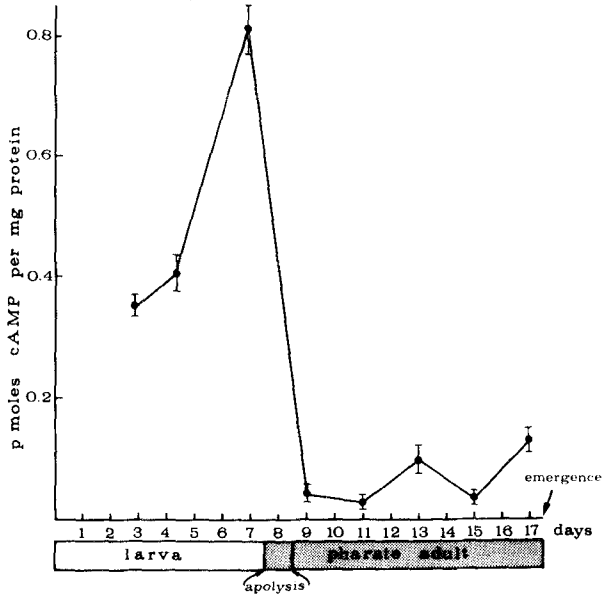

Fig. 1. Cyclic AMP levels of homogenates of the insect *Ceratitis capitata* at different stages of development. Further explanations are given in the text.

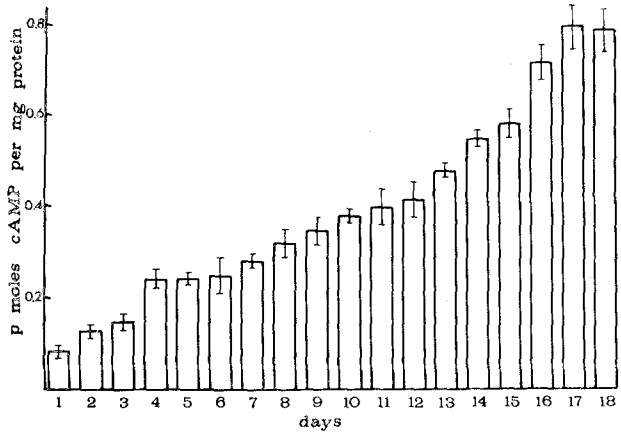

Fig. 2. Cyclic AMP levels of the insect *Ceratitis capitata* during ageing.

The presence of adenyl cyclase in insects, and the fact that the activity of the enzyme in the pharate adult stage is one of the results of the molting hormone action in the diptera *Ceratitis capitata*, has recently been shown[4].

In this paper levels of the cyclic nucleotide during development and ageing of *Ceratitis capitata* are given.

Materials and methods. Culturing of *Ceratitis capitata* (Wiedemann) was carried out as previously described[5]. Frozen tissue was homogenized at 0°C in 0.61 *M* trichloroacetic acid and the protein precipitate again removed by centrifugation. The acid was extracted from the supernatant by 8 washes with 5 volumes of water-saturated ether and the ether removed from the aqueous phase by blowing air on to it. The final pH was 7.5[6].

Cyclic AMP was measured by means of a modification[7] of the competitive binding protein assay of Gilman[8] using a binding protein purified from bovine muscle. Briefly, a standard curve was generated by incubating various concentrations of unlabelled cyclic AMP with ^{3}H-cyclic AMP (The Radio-chemical Centre, Amersham; spec. act. 29 Ci/mmole) and binding protein. Concomitantly, unknowns were incubated with all the preceding components with the exception of unlabelled cyclic AMP. Separation of the protein-bound cyclic AMP from the unbound nucleotide was achieved by adsorption of the free nucleotide on to coated charcoal followed by centrifugation. A sample of the supernatant was removed for liquid scintillation counting. Nucleotide determination was carried out on homogenates of 100 insects by triplicate.

The dose-response curve was linearized and the final results calculated with a computer program utilizing a Hewlett-Packard computer 2116 B according to the logit-log method of Rodbard et al.[9]. The validity of the assay procedure was confirmed by the linearity of sample dilution and by phosphodiesterase destruction of measured cyclic AMP. Recovery studies ranged between 95–100%. The intraassay and interassay coefficient of variation was less than 7%.

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Extreme values of the 3 analytical values are represented in Figures 1 and 2. Protein was determined by the method of LOWRY et al.[10]. For the study of longevity, adult flies were maintaind at 25 °C. The flies were kept in small plastic cages with approximately 200 flies of each sex per cage and food was removed every other day. Flies were removed every 24 h and immediately processed.

Results and discussion. Figure 1 shows the results obtained during development of the insect. As it can be seen, the concentration of the nucleotide reaches a peak practically just at apolysis with a sharp decline in the pharate adult stage. As has been reported previously[4], the amount of adenyl cyclase increases during this stage; therefore it is quite possible to imagine that the much lower cyclic AMP level could indicate the presence of potent cyclic AMP phosphodiesterase activity in splitting the cyclic AMP. With this regard, we must underline that it is generally considered that phosphodiesterase governs the tissue level of cyclic AMP[11]. The possibility that this enzyme may be the rate limiting step for maintaining steady state levels of cyclc AMP in the insect must be determined. Levels of cyclic AMP are close to those described by KUO et al.[12] in silkmoth fat body. We must point out also that the fact that *Ceratitis capitata* produces cyclic AMP is not surprizing, since it has been shown to exhibit cyclic AMP dependent protein kinase[13].

Due to the ubiquity of cyclic AMP in nature and the tissues of different organisms, and because of the different nature of processes influenced by this nucleotide, it would play a very important role in the ageing phenomena. We have determined the levels of cyclic AMP during longevity, as is shown in Figure 2. A gradual increase takes place through the longevity reaching a maximum plateau at the end of life. Although it is very hard to explain such a pattern during ageing, one could think in a gradual maturation[14] of biochemical systems. Such phenomena in some enzymes in insects has been demonstrated and the increase of activity after several days of emergence has been related to the initiation of the flying activity[15,16].

The significance of the presence of cyclic AMP in *Ceratitis capitata* can only be speculated, but it can be imagined that the insect contains a primitive control system with several features in common with the intercellular metabolic system found in vertebrates.

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Glycoprotein Biosynthesis in Splenic Cells. Purification of a Microsomal Galactosyl-Transferase[1]

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Summary. One part of the microsomal galactosyl-transferase activity of splenic cells of rats can by solubilized by the action of Triton X-100 and Tween 20. Its purification on a Sephadex G-200 column and by electrophoresis on a polyacrylamide gel leads to a solution of high specific enzymic activity.

Previous work[2,3] has shown that diverse glycosyl-transferases activities exist in splenic cells. From this, we have been able to locate a mannosyl-transferase, a N-acetyl-glucosaminyl-transferase, a sialyl-transferase, a fucosyl-transferase and a galactosyl-transferase.

A more refined subcellular fractionation shows that these enzymes are distributed in the microsomal fractions sedimenting in the different zones of a sucrose gradient[2] or in the mitochondria[3]. Amongst the diverse galactosyl-transferases prepared from different tissues, some have microsomal localization[4,5], others are in a soluble state in the cell sap[6], and yet others are integrated in the membranal structures, but can be dissolved by the careful action of some detergents[7]. The microsomal galactosyl-transferase of splenic cells belongs to this last group. Conditions for its purification are the subject of this paper.

Material and methods. The preparation of microsomal fractions and the control of their quality are extensively

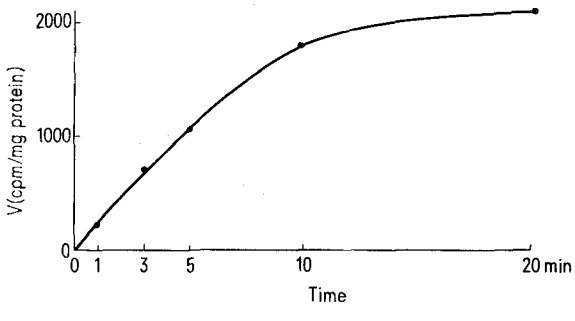

Fig. 1. Kinetic study of galactosyl-transferase (*Tris*-HCl buffer, 0.05 *M*, pH 6.8).

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