Detection of Juvenile-Hormone-Induced Gene Activity in the Colleterial Gland Nuclei of *Periplaneta* by ^{3}H-Actinomycin-D 'Staining' Technique

Based on the observation that ecdysone induced puff formation in the salivary gland chromosomes of *Chironomus* larvae CLEVER and KARLSON[1] proposed that ecdysone acts directly on the genome so as to induce the synthesis of messenger RNA (mRNA). KROEGER[2] has questioned the validity of the above assumption since puffs can be induced by changes in electrolytes. He has further suggested that ecdysone and Juvenile hormone (JH) do not act directly on the genome but indirectly through changes in the permeability of the nuclear membrane[3]. By employing isolated salivary gland chromosomes of *Chironomus tentans* LEZZI and GILBERT[4] provided further evidence in support of the hypothesis that insect hormones act on the genome via changes in the ionic composition of the nucleoplasm.

The above mentioned studies employed the dipteran salivary glands which possess polytene chromosomes. These giant chromosomes provide an excellent assay system for determining differential gene activation during metamorphosis. The techniques employed were either cytological and/or autoradiographic techniques after incorporation of labelled precursors of RNA into specific regions of the chromosomes. The interphase nuclei of other tissues which do not possess polytene chromosomes are not suitable for similar studies. Hence the present study was undertaken with a view to finding out whether it was possible to detect enhanced genetic activity, if any, after JH treatment in the colleterial gland nuclei of *Periplaneta americana*, which do not possess polytene chromosomes, by using the cytochemical technique 'staining 'by ^{3}-H-actinomycin-D followed by autoradiography[5].

The colleterial glands of cockroaches are involved in the production of ootheca[6]. Studies involving decapitation, surgical extirpation and implantation of corpus allatum (CA) have established that the secretory activity of the left colleterial gland which secretes the structural protein and glucoside is regulated by the corpus allatum (juvenile) hormone[7]. Although ZALOKAR[8] showed that corpus allatum stimulated RNA and protein synthesis in the colleterial glands of *Blatta germanica* it is not established that this stimulation involves prior activation of the genes in the colleterial gland nuclei.

Female cockroaches (*Periplaneta americana*) carrying oothecae were collected from the colony and allatectomised as described by BODENSTEIN[9]. The oothecae were removed and the insects were maintained on dog biscuits and water at 25°C. If no ootheca developed within 3 weeks after the operation, allatectomy was considered as successful. Those that produced oothecae were discarded. One group of allatectomised cockroaches were topically applied with 1 µg of a JH analogue, *trans, trans*-N, N-diethyl-3, 7, 11-trimethyl-10, 11-epoxydodeca-2, 6-dienamide in 1 µl of acetone. Another group of allatectomized cockroaches received 1 µl of acetone alone. These served as controls. 24 h after the treatment the left colleterial gland was dissected out from each of the control and JH-treated insects and fixed by freeze substitution, embedded in paraffin and sectioned at 5 µm thickness. After deparaffinizing and hydration these sections were placed in a solution of ^{3}H-actinomycin-D (5µCi/ml, Sp. act. 8.4 Ci/mM, Schwarz) for 1 h. They were then transferred to a solution of non-radioactive actinomycin-D (20 µg/ml) for 1 h and later in running tap water for 24 h. Autoradiography was carried out with NTB-2 nuclear emulsion (Eastman Kodak). They were developed after 4 days of exposure in the dark. Using a squared graticule the number of silver grains per 15 µm^2 of nuclear area was determined from 100 nuclei for each group. The means of both the groups were compared using 't'-test for significance.

The results obtained show that the number of silver grains per 15 µm^2 of nuclear area in the JH-treated insects is 9 ± 0.26, whereas in the controls it is 4 ± 0.17. The difference between the two is statistically significant ($P < 0.001$). There is sufficient evidence to indicate that the binding of actinomycin-D to chromatin is related to the degree of gene activity. BRACHET and HULIN[10] showed that during spermatogenesis the binding of actinomycin-D to chromatin was progressively reduced and in the fully differentiated sperm there was very little of binding by actinomycin-D. These results on spermatogenesis as well as those on embryonic cells indicate that once the cell has reached its final phase of differentiation the chromatin is fully repressed in which state the capacity for actinomycin-D binding is considerably reduced as a result of masking of the free sites by chromosomal proteins. Since the application of JH increases the number of free sites on the DNA molecule, as is evidenced by the increase in the number of silver grains on the nuclei, it appears that the hormone increases the activity at the transcriptional level. Whether this increase in activity is due to direct action of the hormone on the genome[1] or via ionic changes in the nucleoplasm[2] or both[11] remains to be established.

Zusammenfassung. Es wird die genetische Aktivität der Zellkerne in den akzessorischen Drüsen von *Periplaneta americana* durch Juvenilhormon gesteigert, ein Nachweis, der mittels Autoradiographie nach «Anfärbung» mit ^{3}H-Actinomyzin-D erbracht werden konnte.

K. K. NAIR and MAYA MENON

Pestology Centre, Department of Biological Sciences, Simon Fraser University, Burnaby (B.C., Canada), 25 October 1971.

[1] U. CLEVER and P. KARLSON, Expl Cell Res. *20*, 623 (1960).
[2] H. KROEGER, Nature, Lond. *200*, 1235 (1963).
[3] H. KROEGER, in *Metamorphosis* (Eds. W. ETKIN and L. I. GILBERT; Appleton-Century-Crofts., New York 1968), p. 185.
[4] M. LEZZI and L. I. GILBERT, Proc. natn. Acad. Sci., USA *64*, 498 (1969); J. Cell Sci. *6*, 615 (1970).
[5] J. BRACHET and A. FICQ, Expl Cell. Res. *38*, 153 (1965).
[6] M. G. M. PRYOR, Proc. R. Soc. B *128*, 378 (1940).
[7] D. BODENSTEIN and I. B. SPRAGUE, J. Exp. Zool. *142*, 177 (1959). – J. H. WILLIS and P. C. J. BRUNET, J. exp. Biol. *44*, 363 (1965). – D. BODENSTEIN and E. SHAAVA, Proc. natn. Acad. Sci., USA *59*, 1223 (1968). – E. SHAAYA and D. BODENSTEIN, J. exp. Zool. *170*, 281 (1969).
[8] M. ZALOKAR, J. Insect Physiol. *14*, 117 (1968).
[9] D. BODENSTEIN, J. exp. Zool. *123*, 413 (1953).
[10] J. BRACHET and N. HULIN, Nature, Lond. *222*, 481 (1969).
[11] L. F. CONGOTE, C. E. SEKERIS and P. KARLSON, Expl. Cell Res. *56*, 336 (1969).
[12] The research was supported by National Research Council of Canada operating grant No. A4669 awarded to Dr. K. K. NAIR. We thank Dr. J. B. SIDDALL of Zoecon for the generous gift of the juvenile hormone analogue.