CHANGE IN PATTERNS OF INHIBITION BY ACTINOMYCIN D OF URIDINE-H^3 INCORPORATION INTO SALIVARY GLAND RNA OF <u>RHYNCHOSCIARA</u> AT DIFFERENT LARVAL AGES

R. Meneghini, H.A. Armelin and F.J.S. Lara
Laboratório de Biologia Molecular, Deptº de Botânica-Faculdade de Filosofia, Ciências e Letras - Universidade de São Paulo - C.P. 8105 - S.Paulo,Brazil

Received August 1, 1968

Recent work from this laboratory has shown striking variations in the salivary gland RNA metabolism during the larval development (Lara and Hollander, 1967; Armelin, Meneghini and Lara, unpublished results). These results are substantiated by the data of the present study on the kinetics of uridine-H^3 incorporation into that RNA at two distinct periods of larval life - before and during the appearance of the giant puffs. It was also observed that, at both these stages, inhibition by actinomycin revealed the presence of unstable RNA classes. However, before the appearance of the giant puffs, the inhibition by the antibiotic is followed by a rather unexpected recovery of incorporation capacity. This effect is possibly due to an active DNA multiplication at this particular stage.

<u>Experimental</u>

Larvae were cultured as previously described (Lara, Tamaki and Pavan, 1965). Determinations were made at two typical stages: a) before the appearance of the large puffs, at the time corresponding to the 3rd period of Guaraciaba and Toledo (1967) and b) at the beginning of the 5th period of Guaraciaba and Toledo (1967) when puff B-2 reachs maximum size.

Uridine and actinomycin were administrated by injections into the body cavity of larvae previously anesthetized by ether. Incorporation was

stopped by placing the larvae in crushed ice. The salivary glands were dissected into a solution with the same Na^+/K^+ ratio and osmolarity as R.angelae hemolymph (38mM-KCl and 76mM-NaCl). When a sufficient number had accumulated this solution was withdrawn and the glands were immediately placed in ice cold 0.05N NaOH and homogenized during 15 seconds: Half a volume of 0.6M $HClO_4$ was then added. After this, RNA extraction was made as described by Fleck and Munro (1962). The final hydrolysate was made 0.1M in relation to $HClO_4$. One aliquot was taken for spectrophotometric RNA assay at 260 mu. Another was pippeted into Whatman GF/B glass-fiber filter and dried at 60°C for one hour. Radioactivity measurement was then carried out as described by Scherrer et al. (1966).

Actinomycin D-H^3 was used to determine the levels of this antibiotic in the larval tissues at various times after its administration. For determination of the level in the hemolymph, 5λ samples were placed on glass-fiber filters and the radioactivity was measured as indicated above. The level of antibiotic in the glands was determined after they had been washed with non-radioactive actinomycin. The glands were then placed on glass filters and homogenized in a plastic scintilation vial with 2.0 ml of 0.05N NaOH. After drying overnight at 60°C, 10 ml of scintillation liquid was added and radioactivity measurements were carried out as indicated above.

Results and Discussion

Figure 1 shows the kinetics of uridine-H^3 incorporation into salivary glands of 3rd period larvae and the effect of actinomycin D. The incorporation in the control experiment is linear, and independent determinations have shown that it proceeds in this way for at least 24 hrs.

Actinomycin causes a large decrease in the specific activity, but unexpectedly and reproducibly there is a recovery of the incorporation capacity which now proceeds with a different kinetics from that observed in the absence of the antibiotic.

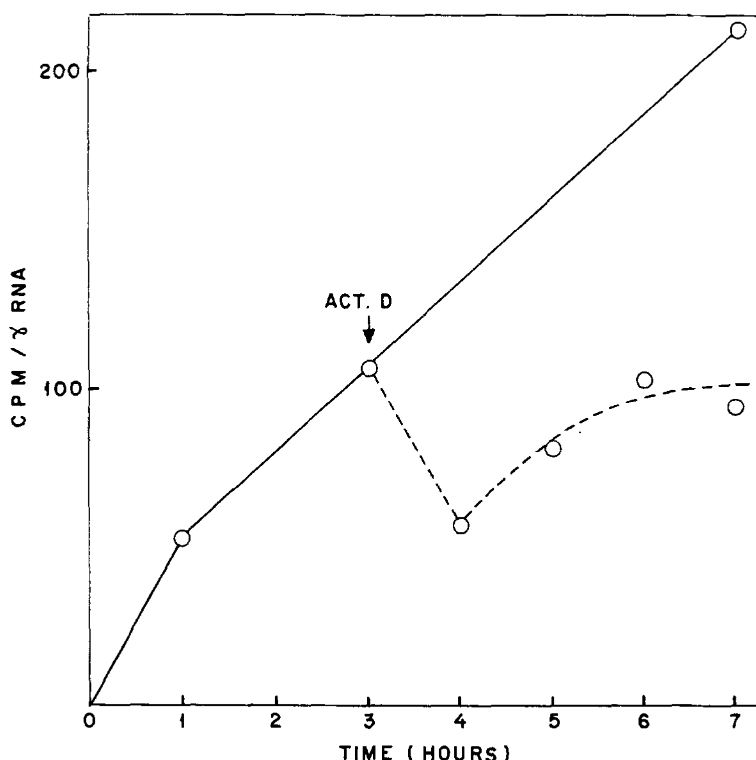

Figure 1. Kinetics of uridine-H^3 incorporation into RNA, and inhibition by actinomycin D in 3rd period larvae. Each larva was injected with 2λ of uridine-H^3 (7.2 C/mM, 1mC/ml). Three hours after the isotope injection, part of the larvae was injected with 2λ of a 2.2mg/ml actinomycin D solution. Incorporation was stopped at indicated times (for lots of ten larvae) and the specific activity of the total salivary gland RNA was measured as described in the text.

0 ——— 0 uridine-H^3 at zero time

0 - - - - 0 uridine-H^3 at zero time + actinomycin at three hours

---

The drop in incorporation level observed within the first hour after actinomycin administration can be understood by assuming the existence of classes of unstable RNA, as in the case of other cells (Scherrer, Latham and Darnell, 1963).

The subsequent recovery cannot be explained by a drop of actinomycin concentration in the hemolymph to low enough levels which would permit the synthesis of classes of RNA less sensitive to the antibiotic, similar as in the case described by Perry (1963). As indicated by the results shown

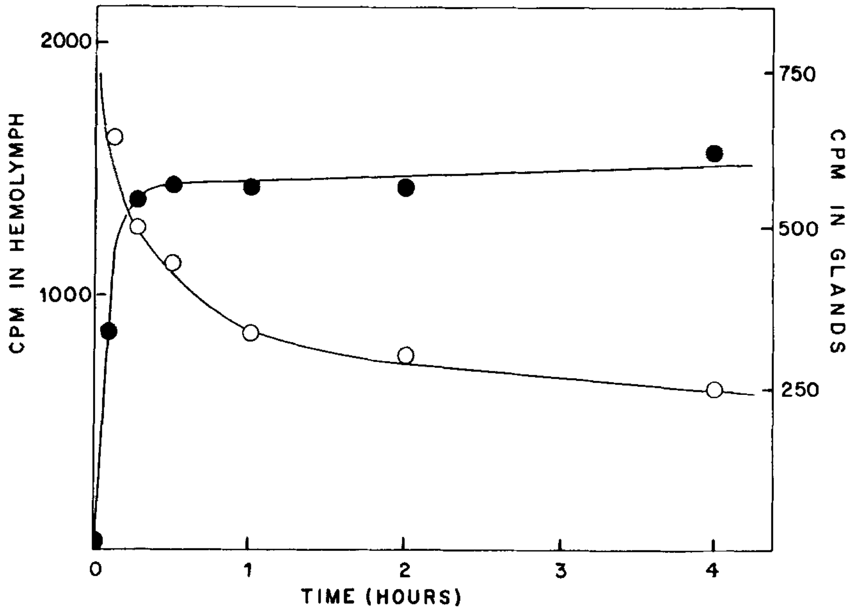

Figure 2. Actinomycin D levels in hemolymph and salivary glands subsequent to injection in R. angelae larvae. Female larvae in 3rd period were injected with 1.2 λ of actinomycin D-H^3 (0.05mC/ml, 0.3 C/mM). At the indicated time the salivary glands from three larvae and 5λ of hemolymph of one larva were extracted and radioactivity was determined as described in the text. Each point represents the average of three independent measurements.

● ——— ● cpm in glands

0 ——— 0 cpm in hemolymph

---

in Figure 2, the intracellular level of actinomycin rises sharply after its administration, reaching and maintaining a high value in spite of a considerable drop in the actinomycin level in the hemolymph. This suggests that intracellular actinomycin is not freely diffusible, very likely due to complex formation with DNA.

As shown in Figure 3, the recovery of incorporation capacity of uridine-H^3 after actinomycin administration is not observed with 5 th period larvae. It may be also seen that the incorporation is not linear. These data indicate that at this larval period the synthesis of unstable RNA is predominant.

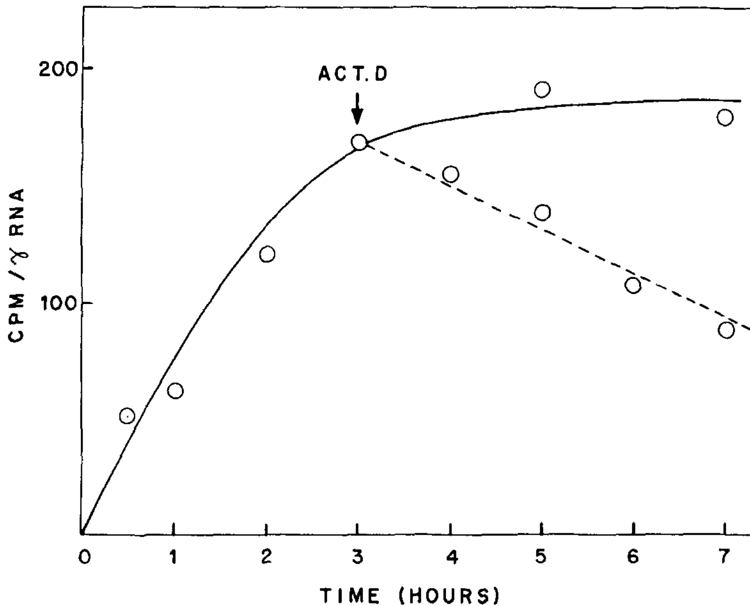

Figure 3. Kinetics of uridine-H^3 incorporation into RNA and inhibition by actinomycin in 5th period larvae. The experimental conditions are the same as in Fig. 1.

The difference between the incorporation kinetics found in the two larval periods agrees with previous results from this laboratory (Lara and Hollander, 1967), which have been recently confirmed in further detail (Armelin, Meneghini and Lara, unpublished results). These authors demonstrated that in the 3rd period the larvae synthesize primarily r-RNA until the stage of giant puffs when synthesis of polydisperse and soluble RNA is predominant. This could explain the notable difference between the stability of RNA synthesized at the two stages that we have demonstrated here.

The differences observed between the responses to the antibiotic at the two different larval periods might possibly be accounted for by different rates of DNA synthesis at these two periods. As shown previously (Lara and Hollander, 1967) the rate of DNA synthesis is very high in the 3rd period , whereas at the 5th period, the level of DNA in the gland, after having reached a maximum value, starts to drop. It is conceivable that during the 3rd period as the DNA duplicates, the disturbance of its secondary structure

resulting from this process, even if restricted, might lead to the release of actinomycin bound to it, since, at least in vitro, helical configuration is essential for the complex stability (Goldberg, Rabinowitz and Reich,1962). The new copies formed could be transcribed, since the free actinomycin in the gland — which must reflect that present in the hemolymph — should have dropped to a level much lower than that present immediately after its injection. It is interesting to note, however, that the feature of the incorporation curve during the recovery period (Fig.1) suggests that synthesis of unstable RNA is then predominant, contrary to what occurs in the absence of actinomycin when one notes a predominance of stable RNA synthesis. If the above premisses are accepted, we are led to conclude that during this period only restricted portions of the genome replicate. It is possible that the activity of these portions, surely important to cell life at that moment, might be responsible for the synthesis of the so-called metabolic DNA (Pavan and Breuer, 1955; Pavan, 1965). Work under progress at this laboratory is aimed at further clarification of this phenomenon.

Acknowledgements

This work was supported by a grant from the U.S.Army Research Office. We wish to acknowledge the expert technical help of Miss Hiromi Tamaki, the assistance of Mr. A.G. Bianchi in some experiments and of Mrs. H.S.Lafayette in the preparation of the manuscript.

References

Fleck, A. and Munro, H.N.(1962), Bioch.Biophy.Acta,55,571.

Goldberg, I.H., Rabinowitz, M. and Reich, E.(1962), Proc.Nat.Acad.Sci.U.S., 48,2094.

Guaraciaba, H.L.B. and Toledo, L.F.A.(1967), Rev.Brasil.Biol.,27,321.

Lara, F.J.S., Tamaki, H. and Pavan, C.(1965),Amer.Natural.,99,189.

Lara, F.J.S. and Hollander, F.M.(1967),Natl.Canc.Inst.Monogr.27,235.

Pavan, C. and Breuer, M.E.(1955),in Schreiber, G., Simposium on Cell Secretion. Belo Horizonte, Instituto de Biologia da U.M.G.,p.90.

Pavan, C.(1965), Natl.Canc.Inst.Monogr.,18,309.

Perry, R.P.(1963), Exp.Cell Res.,29,400.

Scherrer, K., Latham, H. and Darnell, J.E.(1963), Proc.Nat.Acad.Sci.U.S., 49,240.

Scherrer, K., Marcaud, L., Zajdela, F., Breckenridge, B. and Gross, F.(1966), Bull.Soc.Chim.Biol.,48,1037.