water and the mesonephric blastemae dissected out very carefully so that contamination from the adjacent tissues was avoided. Equal number of blastemae of the control and hormone-treated larvae were homogenized in 0.5 ml 5% trichloroacetic acid (TCA) and centrifuged at 750 g. The supernate plus one wash of 0.5 ml 5% TCA provided acid soluble pool fractions. DNA of the pellet was hydrolyzed with 1 ml of 5% TCA at 90 °C for 20 min. The TCA soluble pool fractions were extracted with ether to remove the TCA and then each fraction was brought to 1 ml volume with distilled water. One aliquot was used for a DNA determination by the diphenylamine reaction following BURTON's method[5], while 0.1 ml of the DNA and acid soluble pool fractions were added to 10 ml of a 10% Bio-Solv (Beckman) toluene cocktail for counting in a liquid scintillation spectrometer.

The levels of DNA synthesis (Total cpm DNA/Total cpm acid-soluble pool ÷ Total μg DNA) are higher for the mesonephric blastemae of larvae treated with a masculinizing concentration of methyltestosterone, but feminizing and masculinizing concentrations of estradiol do not change the levels of DNA synthesis of the mesonephric blastemae significantly from that of controls (Table). Although methyltestosterone stimulates DNA synthesis and presumably cell divisions in the mesonephric blastemae, the failure to detect any effect of the feminizing or-partly masculinizing concentrations of estradiol of DNA synthesis suggests that more evidence is necessary before the suggestion of MITTWOCH[3] is accepted that the number of cell divisions controls the type of sex determination[6].

*Résumé.* L'addition d'une concentration masculinisante de méthyl testostérone aux larves de *Rana pipiens* stimule la synthèse de l'ADN dans leurs blastèmes mésonéphrétiques. On n'observe aucun effet semblable sur la synthèse de l'ADN quand on emploie la concentration masculinisante ou féminisante de l'estradiol.

D. C. VERMA and R. A. FLICKINGER

*Department of Biology,*
*State University of New York at Buffalo*
*(N.Y. 14214, USA), 3 June 7377.*

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## α-Amanitin: its Effect on RNA Synthesis in Polytene Chromosomes

α-Amanitin, the toxic octapeptide from the mushroom *Amanita phalloides*[1], has become a valuable tool in exploring the mechanism of RNA synthesis in eucaryotic cells. The initial work of FIUME et al.[2] demonstrated the inhibition of RNA synthesis in mouse liver nuclei via an influence on the $Mn^{2+}$-dependent RNA polymerase[3,4] located in the nucleoplasm. The nucleolar $Mg^{2+}$-dependent RNA polymerase remained almost unaffected[3,4]. A number of further investigators confirmed these results[5–8]. At present the view is widely accepted, that α-amanitin binds specifically to the nucleoplasmic RNA polymerase (polymerase II or A)[9]. The toxin is a very potent inhibitor with an approximate stoichiometry of 1:1 between enzyme and inhibitor[10]. It seems to allow chain initiation but blocks abruptly chain elongation[7,10]. In the view of all these data, recent results of NIESSING et al.[11] and JACOB et. al.[12] were somewhat surprising since they indicated that after application of α-amanitin to rats in vivo the synthesis of heterogenous RNA as well as ribosomal RNA were inhibited. These findings prompted us to study the influence of α-amanitin on RNA synthesis in the polytene chromosomes of the *Chironomus thummi* salivary gland.

*Materials and methods.* Fourth instar larvae of *Chironomus thummi*, raised in the laboratory, were used in all experiments. For in vitro experiments, dissected salivary glands were incubated in CANNON's insect medium[13] as modified by RINGBORG et al.[14] and supplemented with α-amanitin[15] and/or $^3$H-uridine (25.9 C/m*M*, ÚVVVR, Praha, Czechoslovakia) as required at concentrations specified later on in the text. In in vivo experiments, larvae were placed in an aqueous solution of α-amanitin. The incorporation of $^3$H-uridine into the chromosomes was followed by autoradiography[16].

*Results.* In a first series of experiments we examined the influence of 0.5 μg/ml α-amanitin on salivary glands, explanted for 60, 120 or 180 min in inactive and for further 60 min in radioactive medium with α-amanitin. The sister gland of each larva was treated in exactly the same way but omitting the toxin from the medium.

As shown in Figure 1a, in the amanitin-treated gland only the nucleous shows heavy incorporation of $^3$H-uridine. The weak labelling of the chromosomes, though clearly above background, is distributed in a diffuse fashion with no accumulation above puffs or balbiani rings. One may speculate that the cause for this diffuse label is

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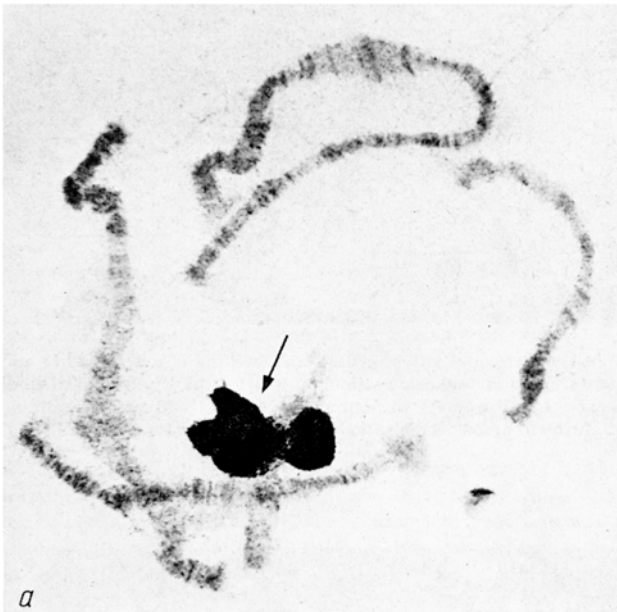
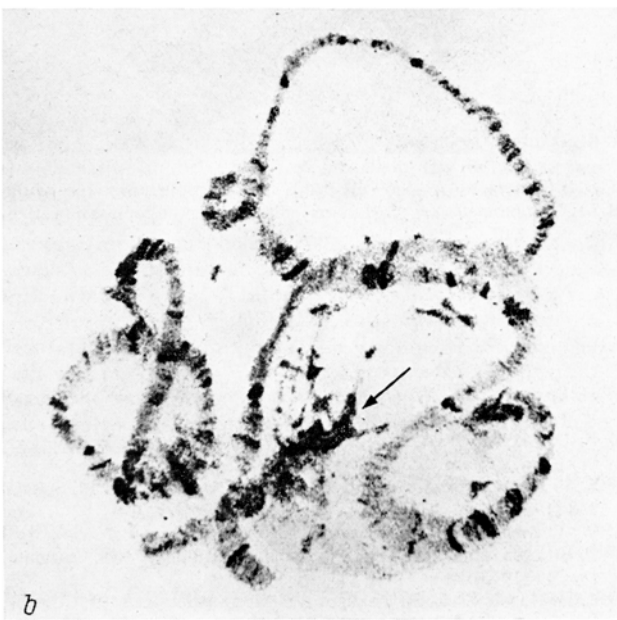
either RNA of nucleolar origin[14, 17] or RNA synthesized by a 3rd distinct polymerase[6]. The chromosomes of the untreated sister gland are strongly labelled above many puffs (Figure 1 b). If one compares labelling above nucleoli in experimental and control glands, no striking quantitative difference is observed. This fact may indicate that nucleolar RNA synthesis remains unimpaired. In a small number of autoradiographs of glands treated with amanitin, 2 to 5 distinct regions in 10–20% of the long chromosomes I, II and/or III are clearly and sometimes strongly labelled. Whether this finding is of any significance, remains to be established.




a) Autoradiograph of an orcein-acetic acid stained chromosome set of a *Chironomus thummi* salivary gland incubated 120 min in CANNON's modified insect medium[14] containing 0,5 µg/ml α-amanitin, followed by a further 60 min incubation in the same medium supplemented with $^3$H-uridine (25 µC/ml). Exposure time was 34 days at 3 °C. The arrow points to the total labelled nucleolus in chromosome IV.




b) Autoradiograph of a chromosome set of a sister gland treated in exactly the same way as the experimental gland in Figure a) but omitting α-amanitin from the medium. The arrow points to the strong labelled nucleous slightly disrupted by squashing.

In a second set of experiments, glands were explanted for 2 or 4 h in radioactive medium with 5 and 0.5 µg/ml amanitin, respectively, and the sister glands without the toxin. No difference from the results described above could be found. The high concentration of 5 µg/ml amanitin did not prevent the slight and diffuse labelling of the chromosomes.

In a preliminary experiment, larvae were placed for 22–24 h in an aqueous amanitin solution (1 µg/ml). Afterwards, glands were dissected and incubated for 15 min in radioactive medium without amanitin. The results were negative, i.e., in the glands of treated animals, too, chromosomal puffs and balbiani rings were labelled in addition to the nucleous. It remains uncertain, however, whether there is a slight general inhibition of RNA synthesis. The in vivo action of amanitin in *Chironomus* needs further investigation. No clear indication of a nucleolar fragmentation observed in tissue culture cells[18], and after injection of the toxin in mice[19] and rats[12], could be found.

*Discussion.* At the cytological level, our experiments demonstrate that α-amanitin in explanted salivary glands of *Chironomus thummi* larvae inhibits chromosomal RNA synthesis while nucleolar RNA synthesis remains uninfluenced. This observation corresponds to in vitro experiments on RNA polymerase activities from sea urchin[6] and mammals[3–9]. In intact *Chironomus* larvae, however, amanitin seems to have no clear-cut effect. Similarly, amanitin administered in vivo to rats[12] and mice[11] failed to inhibit selectively nonribosomal RNA synthesis but impaired the synthesis of all species of RNA, including nucleolar RNA. JACOB et al.[12] interpreted this observation on the basis of an extranucleolar control mechanism sensitive to amanitin. The specific action of amanitin in an intact organ, the salivary gland, makes this possibility unlikely, at least for *Chrionomus*. We rather suppose a metabolic conversion of the toxic substance in the haemolymphe.

The effect of α-amanitin on chironomids is presently being investigated in more detail by seperating labelled RNA of isolated chromosomes after treatment of explanted glands with the toxin.

*Zusammenfassung.* Autoradiographisch wurde festgestellt, dass α-Amanitin in explantierten Speicheldrüsen von *Chironomus-thummi*-Larven die Synthese der chromosomalen RNA hemmt. Die nukleoläre RNA-Synthese bleibt unbeeinflusst. Behandlung der Larven für 22–24 h in einer wässrigen Amanitin-Lösung ergab bisher keinen spezifischen Effekt.

U. WOBUS, R. PANITZ and E. SERFLING

*Zentralinstitut für Genetik und Kulturpflanzenforschung der Deutschen Akademie der Wissenschaften, DDR-4325 Gatersleben (DDR), 27 April 1971.*

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