Effects of α-Amanitin on Nucleolar Structure and Metabolism in *Drosophila hydei*

The substance α-Amanitin specifically inhibits the action of Mn^{2+}-dependent (chromosomal) RNA polymerase in vitro[1]. The activity of Mg^{2+}-dependent RNA polymerase which, in the intact cell, resides in the nucleolus, is unaffected by the toxin if tested in an in vitro system[2-4].

However, in intact mammalian[5,6], as well as insect cells[7], the toxic octapeptide causes changes in nucleolar morphology and metabolism: fragmentation of the nucleoli[7-9] and an impairment of the processing of nucleolar RNA[7,8] were recently reported. The present paper describes the effects of α-amanitin on nucleolar morphology and RNA metabolism in polytene salivary gland nuclei of *Drosophila hydei* after injection of the toxin into mid-third instar larvae.

Methods. Mid-third instar larvae of a laboratory stock of *Drosophila hydei* were injected with various concentrations of α-amanitin (Boehringer) in *Drosophila*-Ringer[10]. At various time intervals, from 90 min to 20 h after injection, the incorporation of ^{3}H-uridine into chromosomes and nucleoli of explanted glands was investigated autoradiographically (sea below). These experiments revealed that, at 5 h after injection of 0.5 μg α-amanitin per larva, a maximal reduction of chromosomal incorporation was achieved[11]. All observations reported here were obtained following the application of 0.5 μg α-amanitin per larva. At 5 h after injection of the toxin, salivary glands were dissected and incubated for 15 min in Ringer's solution[10] containing 0.05 mCi/ml ^{3}H-uridine (spec. act. 24 Ci/m*M*) and 100 μg/ml α-amanitin. Autoradiographs were prepared as described previously[12] and exposed for 7 days.

The diameters of compact nucleoli were measured with an ocular micrometer and labelling density was established on the basis of grain numbers per surface area.

The incorporation of ^{3}H-uridine into newly synthesized RNA was assessed by sucrose gradient centrifugation of RNA extracted from 100 salivary glands according to the method of PELLING[13]. The isotope was incorporated in *vivo* during 30 min, 5 h after the injection of 0.5 μg/larva α-amanitin. Larvae were injected with 0.5 μCi ^{3}H-uridine. Carrier RNA was extracted with SDS-phenol from 0.5 g frozen-powdered larvae. Radioactivity was measured with a Philips scintillation spectrometer and calculated after quench correction as disintegrations per min.

Results and discussion. Among 30 pairs of glands prepared for autoradiography, 18 revealed varying degrees of fragmentation or dispersion of the nucleolus in 20 to 90% of the nuclei (Figure 1a, b). The diameters of compact nucleoli were significantly increased as compared with the controls not submitted to an α-amanitin treatment. The average diameter was 37.4 ± 6.0 μm ($n = 27$) in α-amanitin-treated animals compared to 22.1 ± 8.2 μm ($n = 26$) in the controls. Moreover, the nucleoli of α-amanitin-treated salivary gland cells were labelled more densely than those in the controls (Table), suggesting a defect in the mechanism(s) involved in the release of RNA from the nucleolus.

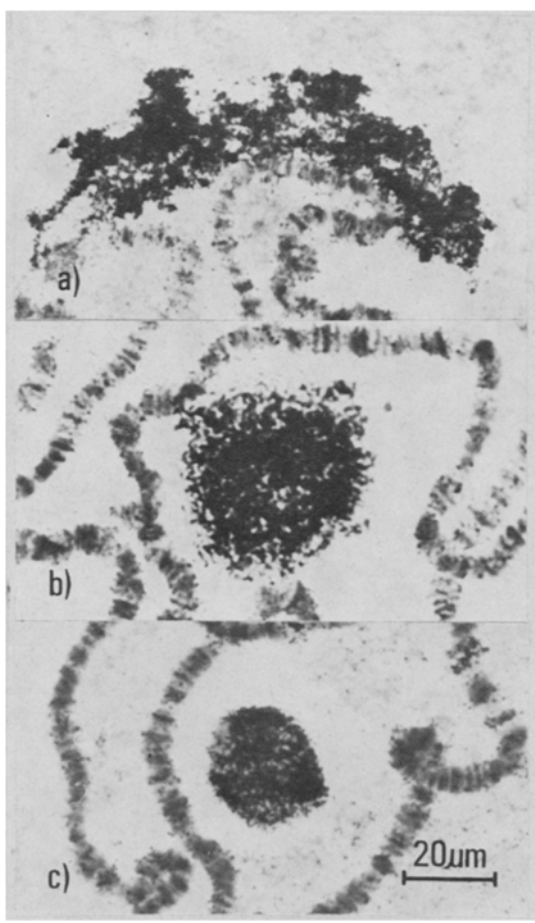

Fig. 1. ^{3}H-uridine incorporation in nucleoli of *Drosophila hydei* salivary glands. a) and b) nucleoli from larvae treated for 5 h with 0.5 μg α-amanitin (injected) and subsequent incubation of the salivary glands (15 min) in Ringer's solution containing 0.05 mCi/ml ^{3}H-uridine and 100 μg/ml α-amanitin. c) nucleolus of a control animal injected with Ringer's solution, 5 h before dissection of the glands and 15 min incubation with 0.05 mCi/ml ^{3}H-uridine.

Grain density of ^{3}H-uridine labelling in salivary gland nucleoli of α-amanitin-treated larvae of *D. hydei* and in nucleoli of control animals which were injected with Ringer's solution[10] instead of α-amanitin

	Nucleoli of α-amanitin-treated ($n = 160$)	Nucleoli of controls ($n = 191$)
Heavy labelling (no grain counts possible)	89 (56%)	56 (29%)
Medium labelling (more than 10 grains per μm²)	47 (29%)	64 (34%)
Weak labelling (less than 10 grains per μm²)	24 (15%)	71 (37%)

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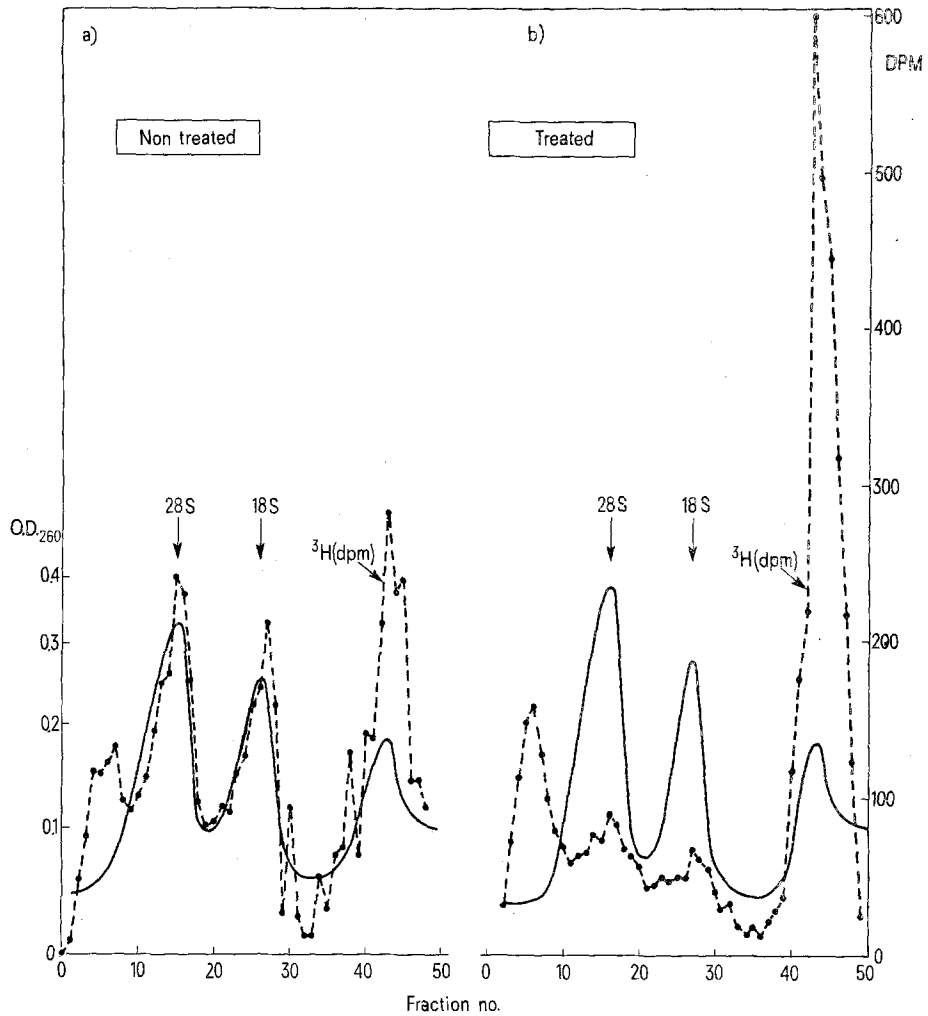

Fig. 2. Radioactivity profile of ^{3}H-uridine labelled RNA extracted from salivary glands from midthird instar larvae of *Drosophila hydei* which were injected with 0.5 µCi/larva ^{3}H-uridine at 5 h after injection of 0.5 µg α-amanitin/larva. The glands were dissected 30 min after injection of the isotope. In each of 3 replicate experiments 100 pairs of salivary glands were used for RNA extraction as described by PELLING[13]. a) Profile of RNA from control glands obtained from animals which received an injection of Ringer's solution instead of α-amanitin. b) Profile of the RNA of in vivo α-amanitin-treated salivary glands.

A comparison of the radioactivity profiles of salivary gland RNA extracted from α-amanitin-treated and control animals (Figure 2) revealed obvious differences in the relative amounts of 28 S, 18 S and low molecular weight 3–7 S) RNA.

The relative quantity of a fraction a molecular weight greater than 28 S, which should include the 38 S precursor of ribosomal RNA[14], was unaffected in the α-amanitin-trated animals. On the basis of total radioactivity in RNA extracted from salivary glands of larvae treated with α-amanitin in comparison with that of the controls, it seems that transcription of nucleolar RNA is not quantitatively impaired by the toxin. This suggestion finds support from the autoradiographical data. On the other hand, accumulation of the radioactivity in the nucleolus and the decrease in 18 and 28 S RNA species indicate that the processing of the ribosomal precursor RNA may be impaired following the application of the toxin in vivo. The similar indications obtained in studies on the nucleolar RNA of CHIRONOMUS[7,15] in which the toxin was applied to isolated salivary glands, are probably not artifacts of the in vitro conditions. Because, in the experiments described, whole salivary glands were extracted, the origin of the strongly increased relative quantity of low molecular weight RNA in the 3–7 S region in α-amanitin-trated glands remains unknown. It is reasonable to assume unimpaired transcription of 4 S and 5 S genes[7], to which have been added small molecules from other sources, possibly breakdown products of ribosomal RNA of which the normal processing has been impaired.

Zusammenfassung. Fünf Stunden nach Injektion von 0.5 µg α-Amanitin in Larven des III. Stadiums von *Drosophila hydei* zeigen viele Nukleolen der Speicheldrüsenzellen eine Fragmentierung und die meisten eine erhöhte Markierung mit ^{3}H-Uridin. In Tieren, die mit α-Amanitin behandelt wurden, ist der Grad des ^{3}H-Uridin-Einbaus in 28 S- und 18 S-RNS geringer, während der Einbau in niedermolekulare RNS (3–7 S) im Vergleich mit Kontrolltieren stark zugenommen hat.

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