Discussion. Though the animals studied are few, it is quite evident that autophagic vacuoles appear following cyclophosphamide treatment, and there may be a hypertrophy of the Golgi system. There thus appear to be changes in the lysosomal system, which is generally considered to be responsible for the autophagic vacuoles. Further studies, including lysosomal enzymes, are desirable. Early changes were not observed in the rough-surfaced endoplasmic reticulum, free ribosomes and polysomes and the nuclei including nucleoli.

It is interesting to note that lysosomes have been found in odontoblasts in large numbers and have been considered to take part in the formation of dentine[4].

The literature on the ultrastructural effects of cyclophosphamide is scant and mainly consists of a few studies on tumour cells. Most of these papers[5-8] describe a hypertrophy of the lysosomal system and/or appearance of autophagic vacuoles, though the relationship between these alterations is not always stressed. Vitamin A, a lysosomal labilizor, potentiated this effect[6,7]. Interestingly, hypervitaminosis A has been described as decreasing the appositional growth of the rat incisor dentine[9], but this change was later considered to be due to decreased food intake[10].

These observations suggest that the effects of cyclophosphamide, or some of them, may be exerted via the lysosomal system. Mitoses are found during treatment, and it may be the interphasic cells that are sensitive[6]. The questions arise, primarily, whether tumour cells rich in lysosomes are especially sensitive to cyclophosphamide, and if that is of importance in the treatment of human malignancies; and secondly, if the use of lysosomal labilizors, for example in perfusion treatment, may potentiate the therapeutic effect of cyclophosphamide. A third question is to what extent the deleterious effects of cyclophosphamide on normal cells (cf. [2]) are mediated via the lysosomal system, and if the effects can be counteracted by lysosomal stabilizors. Finally, it should be emphasized that even if lysosomes are involved early by cyclophosphamide, they are not necessearily the primary target.

Zusammenfassung. In vorläufigen Untersuchungen akuter Effekte von Cyklophosphamid wurden in der Rattenzahnpulpa elektronenoptisch autophagische Vakuolen gefunden, was auf eine Aktivierung der Lysosomen hinweist.

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The Effect of Methyltestosterone and 17β-Estradiol on DNA Synthesis of Mesonephric Blastemae of Frog Larvae

Previous studies have indicated that frog larvae can be masculinized or feminized by hormonal treatments[1]. In *Rana pipiens* administration of methyltesterosterone (0.1 µg to 5 mg/l of aquarium water) masculinizes the larvae. Estradiol (20 µg to 500 µg/l of aquarium water) feminizes, but above 500 µg/l of aquarium water, estradiol masculinizes the larvae and in addition causes adrenal hyperplasia[2]. It is also known that adrenal hyperplasia and sex reversal are independent responses to estradiol administration. According to Witschi[1], the mesonephric tubules, medullary and efferent parts of the sex glands and the cortical part of the adrenal have a common origin from the mesonephric blastemae. Mittwoch[3] has suggested that sex differentiation may be determined by a quantitative difference in number of cell divisions of the undetermined gonads. The present experiments were designed to learn if hormonal treatments that cause sex reversal in *Rana pipiens* affect DNA synthesis of the mesonephric blastemae.

The methyltestosterone and 17β-estradiol were prepared as stock solutions in absolute alcohol (5 mg and 2.5 mg per ml of absolute alcohol, respectively). Approximately 90 larvae (stage 25 of Shumway[4]) were raised for a period of 2 weeks in bubbled tap water, methyltestosterone (1 mg/l) and 2 concentrations of estradiol (500 µg and 250 µg/l). The solutions were changed daily and the animals fed each day with precooked high protein cereal. Concentrations of estradiol above 500 µg/l were not used since the growth of the larvae was reduced.

The larvae were stage 27 at the conclusion of 2 weeks. At this stage they were washed 3 times with boiled tap water and incubated for 24 h at 20 °C in 2.5 µg/ml of H^3-thymidine with the respective hormones present in the tap water. The larvae were washed 3 times in boiled tap

DNA synthesis in mesonephric blastemae of hormone-treated larvae

Treatments	Biological effect (Chang and Witschi[2])	Total cpm DNA/Total cpm acid-soluble pool ÷ Total µg DNA
Controls	----------	$9.92 \times 10^{-2} \pm 1.84 \times 10^{-2}$
Methyltestosterone (1 mg/l)	Complete masculinization	$14.51 \times 10^{-2} \pm 1.52 \times 10^{-2}$
17β-Estradiol (500 µg/l)	Partial masculinization	$9.05 \times 10^{-2} \pm 3.47 \times 10^{-2}$
17β-Estradiol (250 µg/l)	Complete feminization	$8.81 \times 10^{-2} \pm 2.33 \times 10^{-2}$

3 separate experiments were performed and the average values are reported.

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.

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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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