Masculinization and Tumour-Inducing Activity of Pregneninolone in the Female Gonads of the Viviparous Toothcarp *Xiphophorus helleri*.

In experimental sex inversion by the use of sex hormones in viviparous Toothcarps the duration of the experiments plays an important role. A high percentage of *Lebistes* males treated from birth with oestrogenic substances, appeared to develop ovotestes[1]. The expected result of the treatment with androgenic hormones would be the development of ovotestes in the treated females. When young *Lebistes* females were treated with pregneninolone from birth for a period of two to three months, the ovaries showed a considerable regression without losing their specific female structure[2,3]. After the cessation of the treatment, the ovaries returned to their normal state[3]. Treating young *Lebistes* from birth for six months with pregneninolone (Esthistérone, UCLAF, high dose of 0.03 mg, low dose of 0.015 mg, three times a week) out of 55 treated fishes 37 male gonads, 15 hermaphrodite gonads and 2 female gonads were observed[4]. Out of 16 control animals, 11 gonads were male and 5 female. As we personally could demonstrate masculinization and tumour-inducing activity of pregneninolone in the female gonads of *Lebistes*[5], it was desirable for us to perform a corresponding investigation with another Toothcarp. As experimental material we chose the swordtail *Xiphophorus helleri*. 90 young fishes from the same litter were treated from birth during a 12 month period with pregneninolone (0.03 mg, three times a week). The microscopical examination of the gonads revealed that 69 treated fishes were males, 18 had hermaphrodite gonads and 3 were females. Out of 21 control animals from the same litter 18 were of the male and 3 of the female sex. In 6 out of the 69 males a testicular tumour had developed. Type seminoma: masses of large, rounded, polyhedral cells with distinct cell boundaries, having large spherical vesicular nuclei and pale, poorly-staining cytoplasm, plentiful mitoses, stroma consisting of strands of connective tissue with blood vessels and lymphoid regions.

The masculinization of the fishes corresponded very well with that obtained in *Lebistes*[4,5].

Zusammenfassung. Es wird die maskulinisierende und Tumor erzeugende Aktivität von Pregneninolon beim lebendgebärenden Zahnkarpfen *Xiphophorus helleri* beschrieben.

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Histological Laboratory, Free University, Amsterdam (The Netherlands), July 31, 1961.

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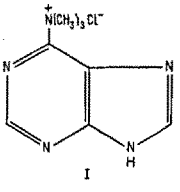
Purin-6-yltrimethylammonium Chloride: A New Purine Antimetabolite[1]

Examination of the literature fails to disclose a single example of a purinyltrialkylammonium salt in the relatively wide spectrum of purine derivatives that have been synthesized and screened as potential antimetabolites.



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It was found, in the course of a related study, that 6-chloropurine is readily transformed (78% yield) to purin-6-yltrimethylammonium chloride (I), m.p. 179–180°C[2], on treatment with trimethylamine in N,N-dimethyl-formamide. The toxicity and the effect of I on transplantable mouse tumor is herein described.

Acute toxicity in mice. The LD_{50} was determined in 6 months old A_f/Sp mice. Two equal doses dissolved in sterile saline were administered intraperitoneally 6 h apart. The volume of the solution was 0.5 ml at each injection. The acute LD_{50} was 500 mg/kg. All animals died within 12 h after the second injection. Liver, kidney, spleen, lungs, bone marrow and proximal jejunum were examined histologically in each animal. In all animals central portions of the liver lobules showed hyperemia and small areas of necrosis. No mitotic figures could be seen in the crypts of Lieberkühn. Other organs showed no abnormalities. Daily white blood counts in surviving animals showed a moderate drop in neutrophils (Figure 1).

Chronic toxicity. Six mice were treated with 150 mg/kg. The compound was administered intraperitoneally daily in 0.5 ml of sterile saline. All treated animals died between the 18th and 25th day of treatment. Before death all animals developed diarrhea. White blood counts, obtained every three days from a tail wound, showed disappearance of circulating neutrophils by the 15th day of treatment. Histological examinations showed aplastic bone marrow in all animals, focal hemorrhagic enteritis in 5 animals and lung abscesses in 2 animals.

Effect on Ehrlich Ascites tumor. Two groups of eighteen 6-months-old Ehrlich Ascites tumor carrying male A_f/Sp mice were treated intraperitoneally daily for one week with 150 mg purin-6-yltrimethylammonium chloride. One group of animals was started on treatment four days after intraperitoneal injection of 0.2 ml of non-hemorrhagic ascites containing 4 million tumor cells. The treatment of the second group was started the day after the tumor inoculation. Equal numbers of tumor-inoculated mice of the same strain, age, and sex served as controls. The results are summarized in Figure 2. All animals in the control group had large amounts of ascites at death as did the animals dying between 5 and 9 days after tumor inoculation in the treated groups. Only two of the animals

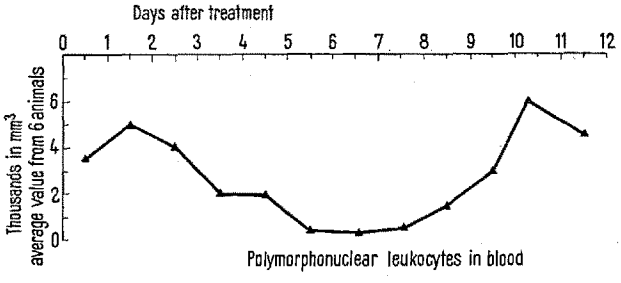

Fig. 1

1 Supported in part by grants CY-2903 aud CY-4519 from the National Cancer Institute of the U.S.P.H.S.
2 Anal. Calc. for $C_8H_{12}N_5Cl$: C, 44.97; H, 5.66; N, 32.78. Found: C, 44.96; H, 5.83; N, 32.98.