Results. As can be seen in the tables 1 and 2, neonatal androgenization increases the FSH-levels without altering LH-concentrations in prepuberal male and female rats of 30 days of age. Table 1 shows that, while normal female rats treated with estrogens, showed 24 h later higher levels of LH and FSH than the controls, the administration of estrogens induced in the androgenized group a significant decrease in the levels of both gonadotrophins. On the other hand, the administration of estrogens to prepuberal male rats, 30 days old (table 2), produced a reduction in the LH- and FSH-levels in normal and androgenized rats, but the inhibition in the FSH-levels observed in the androgenized group, was significantly greater than in the controls (89% vs 57% respectively). Consequently, FSH-concentrations were lower in the androgenized male rats treated with estrogens than in the controls with a similar treatment.

Discussion. The results here reported showed that neonatal androgenization increases the FSH-levels in prepuberal male and female rats. According to the current concepts on the sexual differentiation[3], it could be postulated that the increase in the FSH-levels observed in the androgenized female rats is connected with the development of a tonic or male control of gonadotrophin secretion, since the prepuberal stage that characteristically shows higher levels of FSH than the female type of control[4]. On the other hand, the higher levels of FSH observed in the androgenized male rats as compared with the normals, gives an additional support to the hypothesis that the presence of androgens in the first days of life is responsible for the higher levels of FSH that characterize the male control of gonadotrophin secretion, and also shows that an additional dose of testosterone during the first days of life is able to develop in the prepuberal male a type of pituitary control with higher levels of FSH than those observed when physiological levels of androgens are present. While the administration of estradiol to prepuberal female 30-day-old rats induced an increase in the LH and FSH, there was a significant reduction of both gonadotrophins in androgenized female rats as well as in normal and androgenized male rats. These results appear to indicate that neonatal androgenization in the female rats, besides increasing FSH-levels, altered the normal mechanisms implicated in the development of the positive feed-back effect of estrogens on gonadotrophins secretion. Since estrogens produced a similar reduction in FSH- and LH-levels in normal male and androgenized female, it could be considered that the positive feed-back effect of estrogens is also a sexually differentiated function and its development is impaired by the presence of testosterone in the first days of life.

The androgenization of male rats increased the FSH-sensibility to the negative feed-back effect of estrogens. This fact could be additional evidence that the mechanism of control of FSH is a relevant process that takes place during the sexual differentiation of hypothalamus, influenced by the presence of sexual hormones in the first days of life.

Radioprotective effects of cyproterone acetate

J. Ševčík, J. Wilhelm and J. Šonka[1]

Laboratory for Endocrinology and Metabolism, Faculty of Medicine, Charles University, 128 21 Prague (Czechoslovakia), 12 April 1977

Summary. The pretreatment of irradiated mice by cyproterone acetate had a better radioprotective effect in comparison with orchidectomy, although the weight of the thymus was lower in the cyproterone acetate group. The radioprotective mechanisms involved in both cases were discussed.

The reduced immunocompetence accompanying the thymolytic effect of androgens is expected to enhance the sensitivity of mammals to ionizing radiation[2]. Nevertheless, the reports on the radiosensitizing effects of androgens (and on the radioprotection provided by estrogens) are rather controversial[3]. This may be due to the role of the time-interval between irradiation and the intervention in the hormonal status – during this period, the regeneration of the thymus and the immunocompetence may be restored. Male mice were therefore pretreated by the antiandrogen cyproterone acetate. After a subsequent irradiation, the mortality was compared with that of irradiated mice having no pretreatment. The expected radioprotective effect of cyproterone acetate was obtained, but the administration of this drug to irradiated mice was accompanied at the age of 14 weeks by a decrease in weight of the thymus, not observed in irradiated mice pretreated by orchidectomy. The mechanism of radioprotection induced by cyproterone acetate therefore remains to be explained.

Materials and methods. 5-week-old male mice (Velaz breeding) were kept in groups of 10–20 animals in plastic boxes. The mice were daily exposed to light for 12 h, the room temperature was maintained at 24 ± 2 °C. In group 1 castration was performed in ether narcosis at the age of 5 weeks. After the ligation of the ductus deferens, the testes and epididymis were removed and the wound was powdered with neomycin and bacitracin (Framykoin, Spofa). In the control group (2), a sham operation (opening of the scrotum) was executed. In group 3 cyproterone acetate (6-chloro-17-hydroxy-1,2-methylene-pregna-4,6-dien-3,20-dione acetate, Androcur Schering AG, Berlin) was added to the food at the age of 7 weeks in a daily dose of 1 mg/100 g b.wt. The drug was administered for 14 days, the amount of food consumed by 1 animal was in general 3 g a day. At the age of 10 weeks (5 weeks after castration, 3 weeks after the beginning of cyproterone acetate administration and 1 week after stopping this medication), all 3 groups were irradiated by ^{60}Co. A 4th group with no other intervention was also irradiated at the age of 10 weeks. The exposure was 700 rad with a

1 Acknowledgment. We are indebted to Dr Sastre from Schering AG, Berlin, for the generous supply of cyproterone and cyproterone acetate.

2 M. Poláčková, J. Pěknicová and M. Vojtíšková, Fol. biol., Praha *19*, 13 (1973).

3 E. H. Betz, in: Contribution à l'étude du syndrôme endocrinien provoqué par l'irradiation totale de l'organisme, p. 172. Masson, Paris 1956.

Table 1. Effects of cyproterone acetate feeding, orchidectomy and gamma irradiation on the mortality of mice. The time sequence is represented on the figure

	Number of mice in experiments	Number of dead mice	Death rate	Mortality reduction
1 Castration and irradiation	79	12	15%	25%
2 Sham operation and irradiation	76	29	38%	64%
3 Cyproterone acetate and irradiation	25	2	8%	13.6%
4 Irradiation	32	19	59%	0%

Using the χ^2 test for evaluation the death rate in different groups, the following statistical significance was obtained: groups 1 and 2 $\chi^2 < 0.01$; 1 and 4 $\chi^2 < 0.01$; 2 and 4 $\chi^2 < 0.05$; 3 and 4 $\chi^2 < 0.01$.

Table 2. Effects of cyproterone acetate feeding, orchidectomy and gamma irradiation on the weight of testes, thymus and spleen of mice

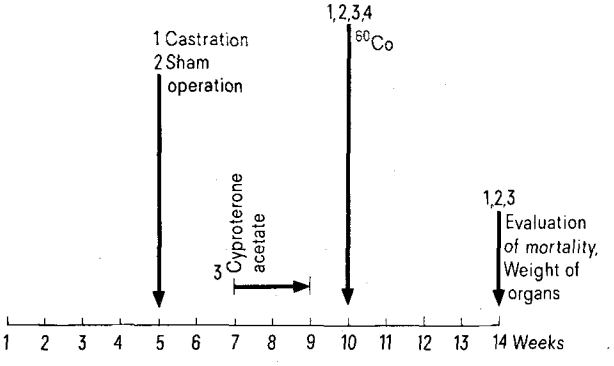
	Mean weight ± SEM (g) Testes	n	Thymus	n	Spleen	n
1 Orchidectomy and irradiation	–	–	0.049 ± 0.014	12	0.147 ± 0.036	13
2 Sham operation and irradiation	0.053 ± 0.006	14	0.035 ± 0.011	6	0.149 ± 0.037	7
3 Cyproterone acetate and irradiation	0.049 ± 0.006	20	0.034 ± 0.008	9	0.144 ± 0.033	10

For comparison of the thymus weights the t-test was used. The statistical significance of the differences between the groups 1 and 2 is $0.05 < p < 0.02$; for groups 1 and 3 $p < 0.01$.

dose rate 27 rad/min. The irradiation was performed in a turning cage to assure field homogeneity. The animals in all 4 groups were observed for 30 days. At the end of this period, the survival was evaluated and the weight of the thymus, speen and testes was measured in the surviving animals. All experiments were repeated several times. A total of 212 mice were sacrificed, for statistical analysis the χ^2 and t-test were used. The time sequence of the interventions is represented on the figure.

Results. The effects of cyproterone acetate, sham operation or castration and gamma irradiation on the mortality of mice are represented in table 1. These results may also be expressed as a reduction of the mortality caused by irradiation only and considered as 100% (the last column). The dose 700 rad administered by ^{60}Co decreased the survival of nontreated animals to 40%. Castration, and especially the feeding of cyproterone acetate, led to a strong reduction of mortality in irradiated mice (to 25 and 13% respectively) if compared to the death rate observed in nontreated, irradiated animals.

The weight of the testis, thymus and spleen at the age of 14 weeks is given in table 2. The thymus weight was greater in irradiated and castrated mice than in irradiated and sham operated, or in irradiated and cyproterone acetate pretreated animals. The weights of the spleen and testes did not differ significantly.



The time sequence of different interventions. The numbers 1–4 correspond to the experimental groups indicated in tables 1 and 2.

Discussion. Orchidectomy performed in mice at the age of 5 weeks leads, according to Castro[4], to a delay of thymic involution, accompanied by an enhanced immunological response which may provide radioprotection. The feeding of cyproterone acetate was started at the age of 7 weeks in order to prevent the production of the nuclear dihydrotestosterone-protein-chromatin complex in androgen dependent tissues, as decribed by Neumann and Steinbeck[5]. In both instances (orchidectomy or cyproterone acetate administration), a similar thymic enlargement was expected.

However, as may be seen in table 2, cyproterone acetate pretreatment did not prevent thymus involution in irradiated mice, while orchidectomy secured the protection of the thymus. The weight decrease of the thymus following cyproterone acetate administration was already reported in intact rats by Neumann and Steinbeck[5]. This effect was not related to a stimulation of adrenals due to a decrease of their weight. The reduction of the weight of the thymus and its depletion of lymphocytes in cyproterone acetate-treated mice was also reported by Vojtíšková et al.[6]. These authors suggested an accelerated migration of lymphocytes from the thymus. The immunocompetence of the treated mice was not tested before irradiation, but it may be suggested that the rapid destruction of the thymus is a stimulus for an enhanced thymus regeneration or lymphocyte production in some other organ, such as the spleen or the liver. Also a massive liberation of antigens from the disintegrated T-lymphocytes may enhance, at a given time, the defence against the radiation bacteriemia.

It may be of interest that free cyproterone, given i.p. in a dose of 1 mg/100 g 1 day before irradiation was toxic and led to a 100% death rate. A preliminary orchidectomy did not change the mortality rate in cyproterone-treated and irradiated mice. The impressive radioprotective effect of oral cyproterone acetate is a stimulus for further studies.

4 J. E. Castro, J. Endocr. *62*, 311 (1974).
5 F. Neumann and H. Steinbeck, Handb. exp. Pharmak. *35*, 424 (1974).
6 M. Vojtíšková, M. Poláčková and V. Viklický, Experientia *32*, 1202 (1976).