

THE ANABOLIC EFFECTS OF ESTROGENS ON MOUSE-LIVER AND THEIR INHIBITION BY CLOMIPHENE

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(Received 18 April, 1969; accepted 27 June 1969)

Abstract—0.5 mg/kg/day clomiphene-citrate given to 6-weeks old female white mice for 7 days led to a significant decrease in liver weight and RNA- and protein-content of the liver. This effect of clomiphene is not found with bilaterally ovariectomized animals of the same age. Two mg/kg estradiol applied once in form of estradiol undecylate caused a significant increase in weight, protein, RNA- and DNA-content as well as in the cellcount of the liver. This effect of exogenous estradiol is inhibited completely by 0.5 mg/kg/day clomiphene.

ESTROGENS have a stimulating effect on the growth of uterus and vagina and a weaker anabolic effect on the liver of rodents.¹ Especially under the influence of exogenous estradiol but also under the effects of the placental estrogens in pregnancy the fresh and dry weight of the liver, its cell count and its DNA-, RNA- and protein-contents do increase.^{1–5} The primary targets of estrogens are perhaps the specific hormone-receptors (proteins or nucleoproteins),⁶ from which the estrogen molecule can be competitively displaced by antiestrogens. The next step of estrogenaction is the activation of genes followed by an increased synthesis of enzymes. This synthesis depends on DNA- and RNA-coupled processes which can be inhibited by actinomycin and puromycin.^{7, 8} Clomiphene, an 1(*p*- β -diethylaminoethoxy-phenyl)-2,2-diphenyl-2-chloro-ethylen-citrate, which can be derived from stilbene, is a substance with many different pharmacological effects on the species (humans or animals). In the presence of estrogens it has antiestrogenic effects which are manifested in a diminished action of estrogens on the uterus and in a blockade of estrogenspecific receptors in the hypothalamopituitarian area.^{9–11} In women clomiphene enhanced the action of the gonadotrophins on the ovaries, the glands weight, the number of follicles, ovulations, cysts and pregnancies are increased. In animal experiments on the other hand it could be seen that clomiphene inhibited the synthesis and secretion of gonadotrophins in the pituitary gland. In absence of estrogens clomiphene shows estrogenic effects itself.^{11–13} In our investigations we tried to clear up the influence of clomiphene on the anabolic action of endogenous and exogenous estrogens in mice livers. There may be three different types of action:

- (1) In presence of estrogens clomiphene may act like an antiestrogen.
- (2) In absence of estrogens it may show no effects at all if it has only antiestrogenic qualities in the liver and
- (3) it may show anabolic effects because clomiphene itself acts in some cases like an estrogen.

METHODS

For our investigation we used 6-weeks old female white mice strain NMRI and with 22 g body weight. The animals were kept at $24 \pm 1^\circ$ room temperature and were fed with standard diet (Altromin) and water *ad lib*. The first group of animals was injected with 0.5 mg/kg/day clomiphene-citrate i.p. for 7 days. Another group of animals once received 2 mg/kg estradiol as estradiolundecylate subcutaneously shortly before the clomiphene treatment was started. For the experiments on castrated animals mice with a body weight of 14 g were ovariectomized bilaterally. Three weeks after the castration the animals received 0.5 mg/kg/day clomiphene citrate for 7 days. After 7 days of treatment the animals were sacrificed and the weights of the livers, its number of cell-nuclei and the DNA and RNA contents were estimated and compared with those of the control animals of the same age and weight. The controls to the pretreated ovariectomized mice were castrated animals of the same age. The nucleic acids were purified by the method of Ogur and Rosen,¹⁴ then they were

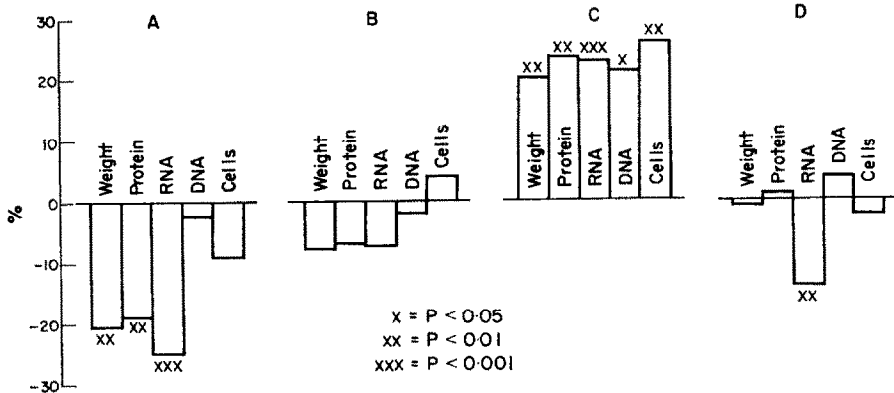


FIG. 1. The changes expressed as percentage of the liver in weight, cellcount and its content of protein, RNA and DNA of

- (A) normal female white mice (startweight 22 g) after a 7 days treatment with 0.5 mg/kg/day clomiphene citrate to untreated normal controls,
- (B) bothsides ovariectomized animals after a 7 days treatment with 0.5 mg/kg/day clomiphene citrate to untreated bothsides ovariectomized controls,
- (C) normal female mice, 7 days after a single application of 2.0 mg/kg estradiolundecylate to untreated controls,
- (D) normal female white mice, 7 days after a single application of 2.0 mg/kg estradiolundecylate and a following 7 days treatment with 0.5 mg/kg/day clomiphene citrate to animals only treated with estradiolundecylate.

extracted according to the method of Schneider-Hogeboom with 0.6 N perchloric acid.¹⁵ The colorimetric estimation was performed by means of diphenyl and orcin reagents which give specific colour-reactions with desoxyribose or ribose respectively. For control measurements u.v.-absorption at $268 \text{ m}\mu$ were performed. For the estimation of protein the Biuret-method was used. The number of the liver cells was determined by counting the nuclei in a Zeiss-Thoma counting chamber, details are given in another paper.¹⁶

RESULTS

As Fig. 1 shows 0.5 mg/kg/day clomiphene citrate leads on normal animals to a significant decrease of liver weight and of its protein and RNA contents (A). The weight and the cell-count of the livers of the castrated animals and their DNA, RNA and protein contents were not significantly diminished by clomiphene treatment (B). When normal female mice of 22 g body weight were injected once with 2 mg/kg estradiol 7 days later the weight of the liver and its cell count, DNA, RNA and protein contents had increased significantly as compared with the controls (C). These increases cannot be observed in the estradiol treated animals which received 0.5 mg/kg/day clomiphene. The content of RNA in the liver is even significantly diminished (D).

DISCUSSION

The effects of 0.5 mg/kg clomiphene on growth and synthesis of DNA, RNA and protein in the liver

(a) of normal animals

(b) of those treated with 2 mg/kg estradiol and

(c) of ovariectomized white mice

can be explained in the same manner by its antiestrogenic property although clomiphene may have many different actions.

- (1) The estrogens formed in the ovaries of young still growing mice exert an anabolic effect on the liver which manifests itself in a stimulation of growth, a formation of new proteins and a neosynthesis of RNA and perhaps DNA which can be inhibited by clomiphene, perhaps by competitive displacement of estrogens from their binding sites at the hormone specific receptors.
- (2) After bilateral ovariectomy the physiological conditions for the effectiveness of an antiestrogenic together with an antianabolic effect of clomiphene on the liver are no longer present. The differences of untreated castrated animals are no longer significant.
- (3) Two mg/kg of estradiol applied to young mature female white mice have a great growth stimulating effect on liver tissue, which can be inhibited quantitatively by the antiestrogenic clomiphene.
- (4) The observation that all animals that received clomiphene responded with the greatest inhibition of RNA synthesis can be explained with the main effect of estrogens on the genetic system of the nucleus which consists in the DNA dependent synthesis of RNA.
- (5) An estrogenic that means an anabolic effect of clomiphene on the liver of female white mice could not be observed. This effect may be restricted to the sexual organs because estrogens have different modes of action in their target organs.

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