to both DNP and OA was demonstrated in inbred Chester Beatty hooded rats by administration (i.p.) of 1 μg of DNP_3-OA antigen together with 1 mg $Al(OH)_3$ and 10^{10} *B. pertussis*. It was found that neither $DNP_{0.5}$-OA nor DNP_{20}-OA induce the synthesis of anti-DNP and anti-OA IgE response antibodies; whereas $DNP_{2.8}$-OA and $DNP_{3.8}$-OA were found to be the most effective antigens. The failure of the induction of the formation of IgE response by $DNP_{0.5}$-OA might be due to the lightly modified carrier and low dose of antigen used. On the other hand, when highly substituted conjugated DNP_{20}-OA was employed as antigen for immunization, the failure of the induction of anti-DNP and anti-OA IgE antibodies could be due to the severe modification of the antigenic determinants of the carrier molecule (OA). For better understanding of the nature of the specific immunosuppression of DNP by DNP linked to different rat monoclonal immunoglobulins experiments of pretreatment of rats with different DNP conjugates, i.e; DNP_{10}-IgG_1, DNP_{10}-IgG_{2c}, DNP_{10}-IgG_{2a}, DNP_{10}-IgE, DNP_9-IgA and DNP_9-IgM were conducted. As can be seen from tables 1 and 2, the immunosuppression of anti-DNP IgE response by DNP-IgG was monoclonal immunoglobulin specific. DNP_{10}-IgG_1 was found to be the most effective tolerogen, whereas other DNP conjugates were not effective under the same testing conditions; the immunosuppression was DNP hapten specific, and the anti-OA carrier IgE formation was not affected. Moreover, the pretreatment of rats with administration of 1 mg of DNP-IgG as the tolerogen brought about a complete suppression of anti-DNP IgE response but not OA, and also neither anti-DNP nor anti-OA hemagglutinating antibodies were affected under this treatment. Thus, the essential information presented here is that the administration of hapten linked to a specific monoclonal immunoglobulin brings about the suppression of the induction of the anti-DNP hapten specific response in the rats. In this regard, it is therefore suggested that the immunosuppression model of this study may shed some light on the development of an immunotherapy for some allergic diseases in man by means of coupling a hapten (e.g. penicillin) to human specific monoclonal immunoglobulin, which can be produced in large quantities by using the hybridoma technique.

Table 2. Specificity of immunosuppression of DNP_{10}-IgG_1

DNP-conjugates	Dose mg	PCA titer* DNP	OA
DNP_{10}-IgG_1	0.000	160	120
	0.025	160	120
	0.320	20	120
	0.800	15	120
DNP_{10}-IgG_{2a}	0.000	160	115
	0.092	160	115
	0.277	160	115
	0.500	160	115
DNP_{10}-IgG_{2c}	0.000	160	120
	0.100	160	120
	0.500	160	120
DNP_9-IgA	0.000	150	180
	0.200	150	180
	0.600	150	180
DNP_{10}-IgE	0.000	120	210
	0.500	120	210
	0.900	120	210
DNP_9-IgM	0.000	160	100
	0.500	160	100
	1.000	160	100

* Serum obtained from day 14 was used for measuring the IgE responses.

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Hormonal regulation of a new female-specific serum protein (FP) of the laboratory rat

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Summary. This paper reports evidence for a hormonal regulation of a new rat serum protein (female specific protein, FP) which is only demonstrable in female rats. Male and female rats were treated with testosterone and estrogen. The FP was assayed with immunological methods. The following results were obtained: 1. In testosterone-treated females the serum level of FP is reduced significantly. 2. In estrogenized males the FP was distinctly demonstrable but not in the control males.

Quantitative and qualitative sex-specific differences have been observed in some human plasma proteins and likewise for plasma proteins of different animal species[1]. Apart from merely quantitative differences there are proteins which are demonstrable only in pregnant women (PAPP's = pregnancy associated specific plasma proteins[2]). Such proteins also appear in the serum of rats and mice[3,4]. Besides that, proteins have been discovered which are limited to the female or the male sex under normal conditions. Such sex-specific proteins have been identified in the Syrian hamster[5], in the rat[6] and in the mouse[7].

In a previous paper[8] we described a female-specific rat serum protein which was detected during experiments with specific absorbed antisera. By means of these antisera a

partial immunological identity was demonstrated between sera of female rats and mice. In our studies (rocket-immunoelectrophoresis, Ouchterlony method) no cross-reactivity was obtained between sera of female rats or mice and sera of female Syrian hamsters (unpublished data).

In the course of quantitative determinations of this protein great inter-individual variances were noted. This led us to assume a hormonal regulation of the protein as previously described for the Syrian hamster[5].

The aim of this study was to investigate the presence and quantitative differences of the rat FP in males and females under hormonal treatment in order to gain an insight into regulatory mechanisms affecting this protein. The understanding of such regulatory mechanisms is important with respect to pharmacological or toxicological experiments because the FP of rats may be of diagnostic significance in certain investigations. According to our observations the serum concentration of the FP is diminished in tumour-bearing rats (unpublished data).

Materials and methods. Laboratory animals. The rats used were 16 weeks old inbred male and female Wistar rats from the conventional breed Falcke-Barby. The animals were housed in groups of 10 rats in PVC cages (53×34×9 cm) with a wire cover. Operated animals were housed individually (7 days) up to removal of the surgical clips. All animals were supplied with pellets (Rehbrücke diet schedule) and water ad libitum under conventional keeping conditions. The experiments were started after a habituation period of 3–4 weeks.

Sera and antisera. We obtained the sera from male and female rats by cardiac puncture under ether narcosis and centrifugation of the blood samples after clotting. The samples were stored at −20 °C before use. Preparation of a monospecific anti-FP was performed in 2 steps. The 1st step was the production of a polyspecific anti-female rat antiserum in rabbits according to Harboe and Ingild[9]. In the 2nd step this antiserum was absorbed with an adequate amount of male rat serum obtained from 2- to 3-month-old animals.

Immunological method. For the quantitative determination of the FP we chose the rocket-immunoelectrophoresis according to Weeke[10]. The values are given in mm precipitate height.

Experimental procedure. Testosterone treatment (female rats). The control and test groups (10 female rats per group) were examined according to the following scheme:

Group 1, control without any handling. Group 2, injection of 0.1 ml sunflower oil per animal i.m. Group 3, injection of 250 µg testosterone enanthate (Testosteron-Depot®, VEB Jenapharm) per animal i.m. in a volume of 0.05 ml oil solution. Group 4, injection of 500 µg testosterone enanthate per animal i.m. in a volume of 0.1 ml oil solution.

Control group 2 is necessary because the high testosterone enanthate concentration in the commercial preparation must be diluted with oil.

The rats were treated every 2nd day for 3 weeks, thereafter the animals were killed and exsanguinated as described above.

Estrogen treatment (male and female rats). We examined 3 female and 3 male animal groups (10 animals per group) according to the following scheme:

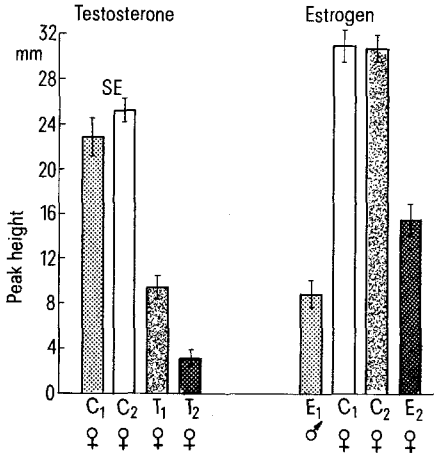
Group 1, control without any handling. Group 2, sham operation (the surgical procedure consisted of cutting a skin bag in the back skin which was closed with a clip; in test groups the skin bag contained the hormone pellet). Group 3, s.c. implantation of a dienestroldiacetat pellet (Oestrasid® C, VEB Berlin Chemie, 25 mg per pellet), surgical treatment as in group 2. Blood samples were removed 21 days after pellet implantation.

Results and discussion. As indicated in the figure, the serum level of FP in testosterone-treated females is diminished at both hormone concentrations. The injection of oil alone has no influence on the rat FP. In estrogen-treated male rats the FP becomes demonstrable but not in the sham operated males. The estrogen-implanted females have significantly lower FP serum levels than the sham-operated animals. There are no remarkable differences between control group 1 and 2. We assume that biorhythmic processes may be the reason for the significant differences ($a < 0.01$, Mann-Whitney test) between the controls of the testosterone and the estrogen experiment. The experiments were performed at different seasons (June and September).

Our results confirm the suggestion of a hormonal regulation of the rat FP which appears to be similar to the mode of action described for the Syrian hamster[5]. This regulatory uniformity might be attributed to the close phylogenetic relationships between cricetidae (hamsters) and muridae (rats). Therefore, it can be assumed that similar proteins are present also in other species of these families.

Our results are also in accordance with the findings of Bryson and Bischoff[11]. These authors have observed that estrogenized male mice have a reduced body weight as well as a lower serum testosterone level. Furthermore, they have observed a positive correlation between testosterone levels and testes weight in the estrogenized mice. Therefore, in the present study, it was not surprising to find that the estrogen-treated male rats also had reduced body weights and smaller testes (macroscopic observation) in comparison to the control animals. Consistent with these observations and in analogy with a similar FP of hamster[5], the appearance of FP in the serum of estrogenized male rats might be explained as a result of a direct inhibitory effect of estrogen on the testes and the consequent suppression of the synthesis of testosterone, which was thus unable further to inhibit FP synthesis or release into the serum. In other words the inhibitory action of testosterone on FP synthesis is apparently suppressed by estrogen. Consequently, the serum FP in males rises to a level which becomes detectable with our methods.

The decline of the FP serum level in estrogenized females



Effect of hormone treatment on the appearance of female specific protein (FP) in rats. Testosterone treatment. C 1, untreated control (females); C2, oil control (females); T1, testosterone treated females (250 µg); T2, testosterone treated females (500 µg). Estrogen treatment. E1, estrogenized males; E2, estrogenized females; C1, untreated controls (males and females); C2, sham-operated controls (males and females). The values are given as the mean of at least 10 animals ± SE. The differences between control and test groups are significant at the $a < 0.01$ level (t-test). In C1 and C2 males no FP was detectable with our method.

may be explained by a negative feedback mechanism activated by the high hormone dose in the pellet.
Another sex-dependent protein, α_{2u}-globulin, has been described for the male rat[6]. The synthesis of this protein is subject to complex regulation including hormonal[13,14] and dietary[15] control. Therefore, there are some similarities between the regulation of FP and α_{2u}-globulin, but the synthesis of α_{2u}-globulin is inhibited by estrogens[12,13] whereas, according to our results, the appearance of FP is suppressed by testosterone.
Summarizing the results of our experiments and from the literature[5,11,12] we can establish the following facts: A female-specific serum protein is present at least in the Syrian hamster[5], in the mouse[7] and in the laboratory rat[8]. The serum concentration of this protein is decreased under the influence of testosterone in females and becomes demonstrable in estrogenized males (in rat and hamster). These results are interpreted to mean that testosterone inhibits the synthesis or secretion of FP into the blood and that estrogen, by acting directly on the testes[12] and thereby decreasing the synthesis of testosterone, releases the FP synthesizing tissue from testosterone repression. Further work is required to elucidate the regulatory mechanism in more detail and to assess the biological function of FP.

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Dissociation of separate mechanisms of estrogen action by actinomycin D[1]

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Summary. Pretreatment with actinomycin D 1 h before estrogen administration completely blocks estrogen-induced increases in uterine RNA and protein content, but does not counteract estrogen-induced uterine eosinophilia, edema and increase in glycogen content.

The effects of estrogens in the rat uterus may be separated into 2 groups of parameters: 1 group includes the increases in uterine RNA and protein content; the 2nd group includes estrogen-induced migration of eosinophil leukocytes to the uterus, edema, increase in vascular permeability and release of histamine[2]. The existence of a 3rd group, including estrogen-induced increase in uterine glycogen content and some other estrogenic responses, has also been envisaged[3,4]. The hypothesis was considered[2] that each group of responses could be mediated by a separate mechanism: the 1st one by the classical 2-step interaction of estrogen with the receptor[5], the 2nd one by the eosinophil leukocyte receptor[2], of which the existence was demonstrated in previous work[6]. The 3rd group of responses could be mediated by cyclic AMP[3].
Genomic activation can be blocked at the level of transcription by actinomycin D. To elucidate which of the various responses to estrogens are dependent on or independent from estrogen-induced genome activation, the effects of actinomycin D upon several parameters of estrogen stimulation were investigated.
Female, adult adrenalectomized and ovariectomized Sprague-Dawley rats were used in the present experiments. Ovariectomy and adrenalectomy were performed 10 days before the experiment, and the rats were given saline physiological solution to drink ad libitum. Adrenalectomized animals were chosen in the present study to avoid actinomycin D-induced increase in endogenous glucocorticoids[7], which may interfere with some of the parameters of estrogen stimulation under investigation[8]. A solution of estradiol-17β in 5% ethanol-saline was injected into the jugular vein under ether anesthesia, using a dosage of 30 µg/100 g b.wt. The control animals were similarly injected with equal amounts of the vehicle. Actinomycin D was i.v. injected at a dose of 120 or 600 µg/100 g b.wt 1 or 20 h prior to estrogen or vehicle injection.
The animals were killed 6 h after estrogen (or vehicle) administration and the uteri excised. The right uterine horn was used for biochemical studies and the left uterine horn was fixed in neutral formalin for subsequent histological studies[8].
The following parameters were measured for each animal: uterine wet weight, DNA[9], RNA[10], protein[11] and glycogen[12] content and total number of uterine eosinophils[8]. The increases in uterine wet weight, RNA per unit of DNA, protein per unit of DNA and glycogen per unit of DNA were expressed as percent change over the controls. The uterine eosinophilia was expressed as the total number of eosinophils in the uterus.
Tables 1 and 2 show the effects of a pretreatment with actinomycin D upon various parameters of estrogen stimulation. Actinomycin D, when administered 1 h before