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

estrogen, blocked estrogen-induced increase in uterine RNA and protein content but did not block estrogen-induced uterine eosinophilia or increases in uterine wet weight or glycogen content (table 1). Actinomycin D, when administered 20 h prior to estrogen administration, completely blocked estrogen-induced increases in uterine RNA and protein content, but its effects on estrogen-induced uterine eosinophilia and increases in uterine wet weight and glycogen content were not clear, due to the increase in these parameters under the sole effect of actinomycin D (table 2).

The present study shows that one group of parameters of estrogen stimulation, i.e., increases in uterine eosinophilia, wet weight and glycogen content 6 h after hormone administration is dissociated from another group of responses to estrogens, i.e., increases in uterine RNA and protein content. Estrogen-induced uterine eosinophilia and the increases in uterine wet weight and glycogen content are not modified by a pretreatment with actinomycin D that completely blocks estrogen-induced increases in uterine RNA and protein content. Therefore, the responses of the 1st group seem to be independent from those of the 2nd group. Estrogen-induced increases in uterine RNA and protein content are supposed to be responses dependent on genome activation that follows estrogen stimulation[5]. Assuming that actinomycin D blocked estrogen-induced genome activation, since it blocked the estrogen-induced increases in RNA and protein content, it is possible to speculate that estrogen-induced eosinophil leukocyte mobilization to the uterus and increases in uterine wet weight and glycogen content are non-genomic responses to estrogens.

The present finding of a dissociation of different responses to estrogens under the effect of actinomycin D is coherent with the hypothesis of difference in the action of estrogens in the uterus implying multiple mechanisms of action for this hormone: 1. Estrogen-induced increases in uterine RNA and protein content that were proposed to be mediated by the classical cytosol-nuclear receptor system through genome activation or derepression[5] and not by cAMP[4]. 2. Estrogen-induced increase in uterine glycogen content that might possibly be mediated by cyclic nucleotide levels in the uterus[3] and that could or could not involve the same receptor system. 3. Estrogen-induced migration of eosinophils to the uterus that was proposed to be mediated by estrogen receptors in eosinophil leukocytes[2], independently from genome activation, from cytosol-nuclear receptors[2] or from cAMP[4]. Once eosinophils enter the uterus, they may mediate several parameters of estrogen stimulation, such as edema (water imbibition), increase in vascular permeability and release of histamine[2].

In view of this model, the following properties of eosinophils can be easily integrated in a series of events that leads to the above responses: Eosinophil 'specific granules', known as peroxidasosomes[13], contain high levels of collagenase[14], beta glucuronidase[15], cathepsin[15], arylsulfatase[15], histaminase[16] and a basic protein[17]. In addition, eosinophils were shown to release prostaglandins E_1 and/or E_2[18]. After entering the extravascular compartment of the uterus, eosinophils degranulate and release their content into uterine stroma[19].

Taking the above into consideration, edema (water imbibition) could be easily related to the liberation of collagenase, beta glucuronidase, arylsulfatase and cathepsin, resulting in the previously observed depolymerization of ground sub-

Table 1. Short term effects of actinomycin D on estrogen-induced uterine eosinophilia and other parameters of estrogen stimulation, 6 h after the administration of 30 µg estradiol-17β/100 g b.wt. Actinomycin D (120 or 600 µg/100 g b.wt) was administered 1 h prior to estrogen

Experimental condition	Parameter of estrogen stimulation ± SEM				
	Total No. of uterine eosinophils (in 10^3)	Uterine wet wt in percent of controls	Uterine protein/DNA in percent of controls	Uterine RNA/DNA in percent of controls	Uterine glycogen/DNA in percent of controls
Control	1.0 ± 0.2	100 ± 7	100 ± 6	100 ± 2	100 ± 3
Estrogen	34.8 ± 5.4[c]	248 ± 35[b]	139 ± 7[b]	145 ± 10[b]	135 ± 8[b]
Actinomycin D (120 µg/100 g b.wt)	1.9 ± 0.8	91 ± 6	99 ± 2	93 ± 2[a]	102 ± 6
Actinomycin D (120 µg/100 g b.wt) + estrogen	33.5 ± 6.7[b,e]	196 ± 36[a,d]	114 ± 4[g]	117 ± 4[b,e,g]	166 ± 12[b,e]
Actinomycin D (600 µg/100 g b.wt)	1.9 ± 0.3	106 ± 1	108 ± 7	90 ± 1[b]	97 ± 9
Actinomycin D (600 µg/100 g b.wt) + estrogen	14.3 ± 3.4[c,f,g]	217 ± 28[b,f]	113 ± 8[h]	101 ± 9[g]	201 ± 28[b,e]

Absolute values in controls: Uterine wet wt (both uterine horns) = 109.4 mg; uterine DNA in µg desoxyribose (both uterine horns) = 208.6; uterine RNA/DNA (µg ribose/µg desoxyribose) = 0.67; uterine protein/DNA (µg protein/µg desoxyribose) = 22.9; uterine glycogen/DNA (µg glycogen/µg desoxyribose) = 1.94. [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$ as compared to controls; [d] $p < 0.05$, [e] $p < 0.01$, [f] $p < 0.001$ as compared to animals treated with the same dose of actinomycin D; [g] $p < 0.05$, [h] $p < 0.01$ as compared to estrogen treated.

Table 2. Long term effects of actinomycin D on estrogen-induced uterine eosinophilia and other parameters of estrogen stimulation, 6 h after the administration of 30 µg estradiol-17β/100 g b.wt. Actinomycin D (120 µg/100 g b.wt) was administered 20 h prior to estrogen

Experimental condition	Parameter of estrogen stimulation ± SEM				
	Total No. of uterine eosinophils (in 10^3)	Uterine wet wt in percent of controls	Uterine protein/DNA in percent of controls	Uterine RNA/DNA in percent of controls	Uterine glycogen/DNA in percent of controls
Control	1.0 ± 0.2	100 ± 7	100 ± 6	100 ± 2	100 ± 3
Estrogen	34.8 ± 5.4[b]	248 ± 35[a]	139 ± 7[a]	145 ± 10[a]	135 ± 8[a]
Actinomycin D	3.7 ± 0.4[b]	171 ± 25[a]	104 ± 1	96 ± 2	157 ± 12[a]
Actinomycin D + estrogen	6.8 ± 1.2[b,c,e]	221 ± 28[a]	103 ± 4[d]	103 ± 1[d]	162 ± 15[a]

[a] $p < 0.01$, [b] $p < 0.001$ as compared to controls; [c] $p < 0.05$ as compared to animals treated with actinomycin D; [d] $p < 0.05$, [e] $p < 0.01$ as compared to animals treated with estrogen.

stance mucopolysaccharides and collagen and in changes in osmotic pressure that follow[2]. Similarly, vascular permeability may be modified by depolymerization and dissagregation of basement membrane collagen fibrils in small uterine blood vessels, noted following estrogen treatment, and by released prostaglandins[2]. Histamine release from uterine mast cells, in turn, may be influenced by released prostaglandins[20] and/or the basic protein fraction liberated from eosinophils, in a similar way to that described for peritoneal mast cells[21].

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Effect of light at night on the pineal rhythm in N-acetyltransferase activity in the Syrian hamster *Mesocricetus auratus*

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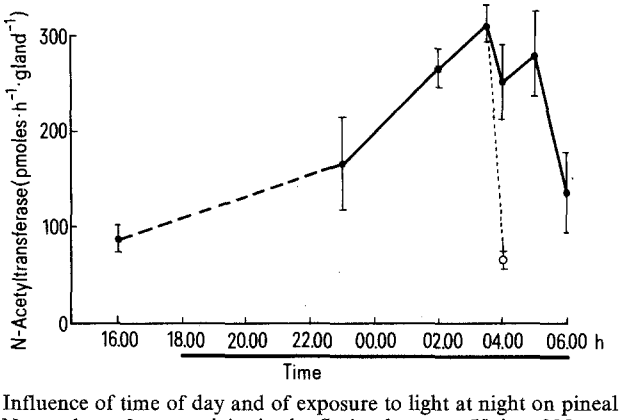
Summary. Pineal N-acetyltransferase activity in the male Syrian hamster exhibited a daily rhythm; the maximal night-time value was 3.5-fold higher than the day-time value. When hamsters were exposed to light at night N-acetyltransferase declined within 30 min to 1/5 of its former activity. These results indicate that in the Syrian hamster the pineal melatonin rhythm may be regulated at least partly via changes in N-acetyltransferase activity.

The enzyme N-acetyltransferase (acetyl-CoA arylamine N-acetyltransferase, EC 2.3.1.5) (NAT) produces the melatonin precursor N-acetylserotonin[2]. It is supposed that the daily rhythm in the melatonin content in the rat pineal gland is driven by rhythmic changes in NAT activity[3], as both NAT activity and melatonin concentration change in the same way throughout the day[4] and respond similarly to light at night[5–8]. In Djungarian hamsters the melatonin rhythm is probably also driven by the rhythm in NAT activity. The ratio of night to day pineal NAT activity[9,10], as well as the ratio of night to day pineal concentration[11] is about 30–50. After exposure to light at night, both NAT activity[9] and melatonin content[11] decline rapidly. Different relations between NAT and melatonin may exist in Syrian hamsters. Though the ratio of night to day pineal melatonin content is about 10–20 in Syrian hamsters[12,13], Tamarkin et al.[13] did not find any difference between day and night NAT activity. Moreover, the rapid decline in melatonin concentration in response to light at night was not accompanied by any decrease in NAT activity[13]. These observations led Tamarkin et al.[13] to the supposition that the regulation of changes in melatonin content in Syrian hamsters may not be affected by changes in pineal NAT activity. To find out whether a real difference exists in the regulation of pineal melatonin content between this species and other nocturnal rodents such as rats and Djungarian hamsters, we studied the NAT rhythm and the response of NAT activity to light at night in Syrian hamsters.



Influence of time of day and of exposure to light at night on pineal N-acetyltransferase activity in the Syrian hamster. Units of N-acetyltransferase activity are defined as pmol N-acetyltryptamine formed in 1 h/pineal gland. ●, hamsters killed from 16.00 h to 06.00 h in the normal lighting regimen, with lights off from 18.00 h to 06.00 h. ○, hamsters exposed to light at 03.30 h and killed 30 min later with light on. Points are means ± SEM of 4–5 determinations from 8–10 animals. The solid line indicates the duration of the dark period. Differences between groups were analyzed for significance using Student's t-test. Night N-acetyltransferase activity was significantly higher than the day activity measured at 16.00 h at 02.00 h ($p < 0.001$), 03.30 h ($p < 0.001$), 04.00 h ($p < 0.02$) and 05.00 h ($p < 0.02$). The activity in hamsters exposed to light at 03.30 h was significantly lower than the activity at 03.30 h ($p < 0.001$) and 04.00 h ($p < 0.01$) in darkness and did not differ significantly from the day value at 16.00 h.