lipo-glycoprotéique, le coagulogène[8,9]; pour un même volume de plasma, à concentration de coagulogène égale, la coagulation est d'autant plus rapide que le nombre d'hémocytes est plus élevé[10–12]. La suite de notre démonstration consiste à obtenir un plasma (liquide dans lequel baignent les hémocytes avant la coagulation) qui reste stable, puis à faire précipiter le coagulogène de ce plasma.

Préparation du plasma. Des larves de dernier stade, sont placées pendant 5 min dans une enceinte à −18 °C. Toutes les opérations de prélèvement sont ensuite effectuées à une température de 4 °C. Après avoir sectionné une patte métathoracique, nous recueillons l'hémolymphe dans un tube à hémolyse; une centrifugation légère (900×g pendant 20 sec) sépare un surnageant jaune, limpide, le plasma, et un culot blanc hémocytaire. Le plasma est immédiatement prélevé par pipetage; isolé des hémocytes, il peut être conservé plusieurs jours sans présenter de coagulation, même à une température de 25 °C. La nécessité de manipuler rapidement ne permet pas de prélever à la fois l'hémolymphe de plus de 2 animaux. Comme nous l'exposons par ailleurs[8,9], la composition de l'hémolymphe n'est pas affectée par la réfrigération des larves. Cette méthode de prélèvement permet d'obtenir un plasma non-dilué et ne contenant aucune substance étrangère à l'hémolymphe, ce qui facilite les analyses qualitatives et quantitatives. De plus des contrôles effectués en microscopie électronique, ont montré que les hémocytes fixés immédiatement après le prélèvement du plasma ne présentent pas encore les transformations concomitantes de la coagulation de l'hémolymphe.

Induction de la coagulation plasmatique par les hémocytes. Du plasma ainsi préparé est divisé en aliquots de 200 µl. Dans certains de ces aliquots on remet en suspension des hémocytes fraîchement obtenus par centrifugation (entre 7×10^5 et $1{,}2\times10^6$ hémocytes par aliquot). Le plasma présente une précipitation, entre 2 et 5 min après la mise en suspension des hémocytes. Des analyses effectuées en électrophorèse et en immunoélectrophorèse montrent que le coagulogène n'est plus présent en solution dans ce plasma; cette précipitation correspond donc bien à une coagulation plasmatique normale. Le plasma des aliquots n'ayant pas reçu d'hémocytes ne présente évidemment aucune coagulation. La coagulation de l'hémolymphe peut donc bien être induite par les hémocytes.

Nous avons essayé d'extraire des hémocytes le (ou les) facteurs capables d'induire la coagulation plasmatique. Pour cela, des broyats d'hémocytes ont été préparés dans des tampons tris-glycine ou véronal à pH 7,2 (entre $1{,}5\times10^6$ et 3×10^6 hémocytes pour 100 µl de tampon). Des volumes de 20 à 100 µl de ces broyats ont été rajoutés à des aliquots de 200 µl de plasma; nous avons dans tous les cas obtenu une coagulation plasmatique; les mêmes volumes de tampon seul restent sans effet sur le plasma. Si l'on effectue une centrifugation de ces extraits (2 min à 2000×g), aucune activité ne peut être mise en évidence dans les surnageants alors que les culots ont permis d'induire la coagulation plasmatique dans le majorité des cas. Il ne nous a donc pas été possible, dans ces conditions, d'extraire des hémocytes le (ou les) facteur(s) induisant la coagulation de l'hémolymphe. Cette coagulation n'a pu être obtenue avec aucun autre tissu de l'insecte (tissu adipeux, hypoderme, chaîne nerveuse, mésentéron), ajoutés intacts ou broyés, au plasma.

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Reappearance of HCG-receptors in immature rat ovary after HCG-treatment is not due to receptor synthesis[1]

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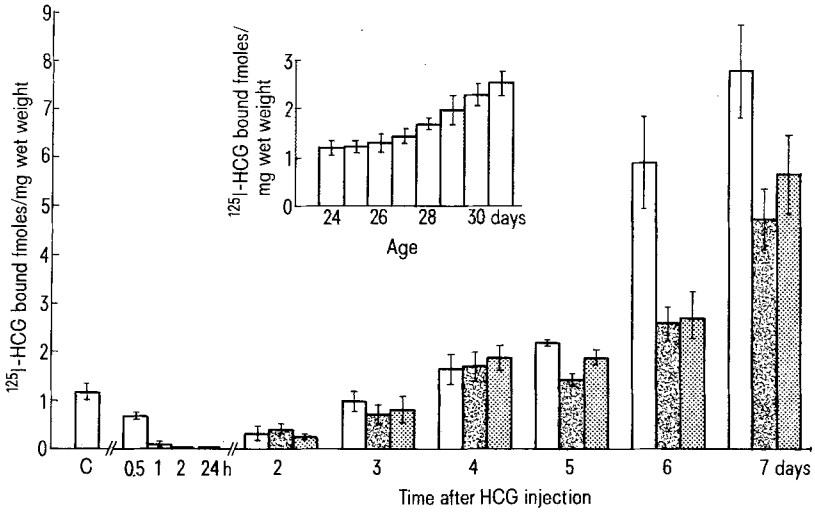
Summary. In 24-day-old rats the reappearance of ovarian HCG-receptors after the injection of 200 IU HCG is independent of protein synthesis. The reutilization of occupied receptors in immature female rats is due to dissociation of the receptor-hormone complex.

Evidence is accumulating that chronic exposure of target cells to high levels of hormone results in a decrease in sensitivity of the target cells to the hormone. Desensitization of target cells has been demonstrated for insulin[3,4], growth hormone[5], thyrotropin releasing hormone[6], catecholamine[7,8] and human chorionic gonadotropin (HCG)[9–12]. The effect of the hormones is mediated at the level of the target cell receptors resulting in a decrease in the number of free hormone receptors. This decrease could be due to an occupation of the binding sites by the hormone and/or to an actual loss of the surface receptors. We here report that in the immature rat ovary desensitization after high doses of HCG is caused by hormone occupancy of the receptors and not by their loss. Furthermore, the restoration of hormone binding starting at the 2nd day after the HCG-injection was found to be due to increasing numbers of dissociated hormone-receptor complexes and does not seem to depend upon receptor synthesis.

Material and methods. 24-day-old female rats of the strain SIV-50 were injected i.p. with 200 IU of HCG (Primogonyl, Schering AG, Berlin West) and the HCG-binding capacity of the ovaries was determined 0.5, 1, 2, 24 h and daily up to 7 days after the injection; 24- to 30-day-old untreated rats served as controls. In a 2nd experiment HCG-treated rats were injected i.p. with 3 doses of actinomycin D (1.5, 1, 1 µg/g b.wt) or cycloheximide (2 µg/g b.wt per injection). Since the toxicity of these substances does not allow a



Ovarian HCG-binding capacity in rats treated with HCG at the 24th day of age (open columns), in rats treated with HCG and cycloheximide (solid columns) and in rats treated with HCG and actinomycin D (hatched columns). Rats between the first and the 7th day after the HCG-injection were treated with the respective toxin only for 1 day and killed 2 h after the last of 3 toxin injections. C: HCG-binding capacity in untreated rats at the 24th day of age. Each column is the result of at least 5 experiments. The mean values±SE are included. In the insert the specific uptake of ^{125}I-HCG by ovarian homogenates from rats, 24–31 days old, are given.

continuous treatment over several days with effective protein synthesis inhibiting doses, the following mode of injection was used: 24-day-old rats were injected with 200 IU HCG at 8 a.m. and received the toxin at 2 p.m., 10 p.m. and at 6 a.m. of the next day and were killed at 8 a.m. The next series of rats treated with the hormone at the 24th day of age started to be injected with the toxin at 2 p.m. of the first day after the HCG-injection, treated with the toxins at intervals of 8 h, and were killed at 8 a.m. of the 2nd day after the HCG-injection. This mode of injection was used also in rats killed at the 3rd to 7th day after the hormone injection. From the toxin-treated rats, ovaries were prepared for HCG-binding studies. - Our experimental procedure used for the demonstration of HCG-binding to ovarian homogenates has been described in detail recently[13]. The ovaries were homogenized 1:10 (w/v) in cold Tris-HCl buffer (0.04 moles/l, pH 7.4), containing $MgSO_4$ (0.005 moles/l) using 6 strokes of a glass Teflon homogenizer. After centrifugation at $100\times$ g for 20 min, duplicate aliquots of the supernatant, corresponding to 5 mg wet tissue, were incubated at 37 °C for 30 min in homogenization buffer containing 0.1% bovine serum albumin (BSA) and varying amounts of ^{125}I-labelled HCG and unlabelled HCG in a final volume of 1 ml; the standard binding assay contained 8 ng ^{125}I-HCG. The reaction was stopped by the addition of 1 ml ice-cold buffer, and the incubates were immediately filtered with suction through cellulose acetate filters (pore size 0.45 µm; Sartorius, Göttingen), previously washed with 10 ml 4% BSA to reduce nonspecific binding. Then the filters were washed with 10 ml cold homogenization buffer and the radioactivity on the filters was counted in a gamma-spectrometer. For each experimental point, the nonspecific binding was determined in the presence of a 1000-fold excess of unlabelled HCG. The specifically bound radioactivity was calculated by substracting the nonspecifically bound from the totally bound radioactivity. Specific radioactivity of the ^{125}I-labelled HCG (biological activity: 11,000 IU/mg) was 30–50 µCi/µg. - Dissociation constants (K_D) of the hormone-receptor complex were obtained by Scatchard[14] analysis.

Results and discussion. The number of HCG-binding sites in 24-day-old and 30-day-old control rats was 2.56×10^{-15} and 4.99×10^{-15} moles/mg wet weight, respectively. As can be seen from the figure, 1 h after the administration of 200 IU of HCG, the binding capacity of the ovary for I-HCG is only about 10% of the control and is no longer detectable 2 h after the injection. It can be assumed that at this time HCG-receptors of the ovary are totally occupied by the hormone. The receptor begins to recover at the 2nd day after the injection and reaches the values obtained in control rats at the 4th to 5th day (28th day of development). 6 and 7 days after the HCG-injection to the immature rats, a drastic increase of ovarian HCG-binding capacity (250% and 350% of the controls, respectively) was seen (figure). The K_D for the hormone-receptor complex in control animals and after recovery of the receptor varied between 2.47 and 2.90×10^{-10} M^{-1} and is similar to previously determined values[13].

The question arises if the reappearance of HCG-binding capacity at the 2nd day after injection is due to dissociation of hormone-receptor complexes or due to newly synthesized HCG-receptors. As can be seen from the figure, during the first 4 days after the HCG-injection, the pattern of HCG-binding capacity of actinomycin D or cycloheximide treated rats corresponds exactly to that in toxin-untreated rats. This result indicates that the reappearance of HCG-binding at the 2nd day after the injection and the increase of binding capacity till to the 5th day is not due to synthesis of new receptors but due to an increasing amount of dissociating receptors. A reduction in HCG-binding capacity in the toxin-treated rats as compared to the untreated rats, is first observable at the 5th day after the HCG-administration, and more drastically at the 6th and 7th day (figure).

The assumption that the HCG-receptors demonstrable from the 2nd to the 5th day after HCG-treatment represent receptors previously occupied by the hormone is further supported by our results obtained after acid treatment of ovaries of HCG treated rats. HCG and luteinizing hormone (LH) are released after treatment of the homogenates with 0.025 M acid formic at pH 2.8 for 30 min at 4 °C. The hormones are not inactivated by the acid elution and can be determined by radioimmunoassay[10]. The amount of released hormone is a measure for the number of occupied receptors. Between day 1 and day 4 after the injection, the total number of receptors (calculated as the sum of free receptors plus the amount of extractable HCG) corresponds to that in untreated control rats (calculated as the sum of free receptors plus the amount of tissue bound LH). On the contrary, in rats at the 6th day after the HCG-injection, the total amount of receptors is about 6 times higher than in control rats, apparently due to receptor synthesis in consequence of the luteinization of the ovary (data not shown).

Our results reveal that, after the injection of a high dose of HCG, ovarian HCG-receptors in immature rats are totally

occupied by the hormone for at least 24 h. Similar results have been obtained by Conti et al.[10] in luteinized ovaries. The occupancy of the receptors in luteinized ovaries result in the desensitization of the ovary to further hormonal stimulation and apparently in the degradation of the occupied receptors[10,15]. Therefore the reappearance of free receptors in luteinized cells is due to receptor synthesis. However, as we have shown here, in the ovaries of immature rats, where luteinized cells are not present, the reappearance of receptors at the 2nd day after the injection is independent of protein synthesis but due to the dissociation of the receptor-hormone complex. From these observations it can be assumed that the modulation of LH/HCG-receptors in luteinized cells to HCG is different from the receptor modulation in follicles and interstitial tissue of immature rat ovaries. A more detailed description of our results will be published elsewhere.

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Anti-estrogen inhibition of testosterone-stimulated aggression in mice[1]

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Summary. Testosterone-stimulated intermale aggression in castrated mice can be reversibly inhibited by anti-estrogen administration suggesting that estrogen formation and actions in the brain may be required for testosterone's behavioral actions.

Numerous recent studies support the hypothesis that metabolic conversion of testosterone to estradiol in the brain is an essential step in eliciting the behavioral actions of this androgen. For exemple, in rats and mice biochemical studies have clearly established the presence of the aromatization enzymatic pathway in the brain[2,3], while behavioral studies have shown that male sexual behavior can be stimulated by both androgens and estrogens and inhibited by anti-estrogens[4-7]. Since intermale aggression in castrated male mice can also be stimulated by both androgens and estrogens[8-10], the present study was designed to test the effects of the potent anti-estrogen, CI-628 on this behavior. In previous work with both rats and mice, CI-628 has been shown to be very effective in blocking the nuclear binding of estrogen in preoptic and medial-basal hypothalamic brain regions[2,11].

25 individually housed adult CD-1 mice (Charles Rivers) were castrated and started on a daily s.c. injection schedule of 200 µg testosterone dissolved in 0.05 cm^3 benzylbenzoate-oil (20:80, v:v). Beginning 1 week later all males were placed in $12 \times 28 \times 28$ cm glass testing chambers and given their first 10 min behavioral test. All testing was conducted in the dark phase of the lighting cycle. Males were allowed to habituate to the chamber for 10 min prior to each test. Tests were initiated by the introduction of a group-housed nonaggressive male mouse to the chamber with the test male and all biting and other forms of aggression were scored as described in our previous work[9,12]. After 5 min the group-housed male was removed and replaced with another male for an additional 5-min test. Mice were retested at 3- to 4-day-intervals for the next 46 days. Following these baseline tests 10 males which had displayed active biting during the last 4 tests were selected for further study. For the next 2 weeks these males received additional daily injections of 2 mg CI-628 dissolved in 0.05 cm^3 3% ethanol-saline. Males were tested at 7, 10 and 14 days of CI-628 treatment. For the next week males were given vehicle injections (in addition to testosterone) and tested at 3 and 7 days.

As shown in the table the CI-628 treatment produced a dramatic drop in testosterone-stimulated intermale aggression. By the end of the 2-week period only 1 male was still exhibiting biting attacks ($p < 0.005$, Fisher Exact Probability test). Within 1 week after cessation of CI-628 treatment 80% of the males had resumed biting attacks ($p < 0.01$, compared to last CI-628 test). These data are entirely consistent with the recent report that the placental aromatase blocker, 4-androsten-3,6,17-trione, inhibits testosterone- but not estradiol-stimulated fighting[13]. Thus there is increasing support for the hypothesis that testosterone aromatization to estradiol is an important step in androgen-induced aggression in male mice.

Number of testosterone-treated (200µg/day) male mice exhibiting attacks during the last 4 baseline tests. 3 CI-628 (2 mg/day) and 2 vehicle tests

	Baseline tests				CI-628 tests			Vehicle tests	
No. of days of testosterone	35	39	42	46	53	56	60	63	67
No. exhibiting biting attacks	10/10	10/10	10/10	10/10	5/10	4/10	1/10	5/10	8/10