taking the animal out and its decapitation being not more than 5 sec. Trunk blood was collected and centrifuged. Individual samples of plasma were frozen and kept for subsequent determination of corticosterone.

Immediately after decapitation, the adrenal glands were dissected and weighed to an approximation of 0.1 mg, homogenized in a saline-alcohol solution and then frozen until corticosterone was determined. Bulbectomy was performed according to previously described technique[2]. Adrenal and plasma corticosterone was determined by means of the Rerup and Hedner method[3]. Results were evaluated by Student's t-test.

Results and discussion. Results obtained may be seen in the table. In the first instance, when comparing normal animals, handled with non-handled, it may be seen that adrenal and plasma corticosterone values are significantly less in handled rats, both male and female. In every case, the drop was over 50%. When considering bulbectomized animals, however, we have deemed it more convenient to make a separate analysis of results obtained in females and in males.

In bulbectomized females, handled animals reveal adrenal and plasma corticosterone values significantly lower than in non-handled. Furthermore, as may be seen in the table, the removal of olfactory bulbs produces a lowering in levels of both parameters in the handled and non-handled lots. Comparing bulbectomized with normal animals, it may be observed that the difference in adrenal corticosterone levels is statistically significant in the handled animal lot ($p < 0.010$). In non-handled animals, despite the drop of more than 50%, this does not become significant owing to the great dispersion of data. Respecting the plasma corticosterone, the differences with the normal animals are significant in both lots, i.e. handled ($p < 0.020$) and non-handled ($p < 0.005$).

Regarding males, the handled animals also show significantly lower values than non-handled (adrenal corticosterone, $p < 0.010$ and plasma corticosterone $p < 0.005$). On the other hand, the removal of olfactory bulbs in no way modifies the adrenal or plasma corticosterone either in handled or non-handled animals.

The effects of bulbectomy in females confirms previous findings in this laboratory[1,2,4] regarding relationship between olfactory bulbs and adrenal glands in rats. The fact has been confirmed by other investigators as being observed not only in rats[5,6] but also in dogs[7]. The fact that bulbectomy has no effect on the levels of corticosterone in males reveals a sexual difference in the effects of bulbectomy on adrenal activity. While studying other parameters in bulbectomized rats, we have, at the same time, been able to observe sexual differences in the results, together with insulin sensitivity test[8] and levels in serum free fatty acids[9].

These results indicate that handling reduces adrenal corticosterone synthesis in normal and bulbectomized rats. The exact nature of the mechanism, whereby handling produces diminishing corticoadrenal activity, we cannot explain at present. Perhaps variation in the levels of adrenal steroids produced by handling might cause alterations in sensitivity in hypothalamic areas which control the adrenal function, as Levine and Mullins[10] suggest when referring to rats handled during prepuberal age.

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'Adrenalectomy, ovarian HCG-binding and the onset of female puberty in the rat*

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Summary. The delay of puberty onset in female rats adrenalectomized before the 25th day of age is due neither to changes in the number of ovarian HCG-receptors nor to an altered hormone affinity of these receptors. It is assumed that glucocorticoids act on an intracellular level in the ovarian cells, possibly by alterations of cyclic AMP-dependent phosphodiesterase activity.

Involvement of the adrenal glands in the maturation and function of the reproductive system of the female rat has been implied in previous studies. Although adult rats without adrenal function maintain ovulatory cycles and can reproduce, some irregularities of the cycle have been noted[3]. According to Peppler and Jacobs[4], the adrenal glands are necessary for normal follicular development as well as for the normal complement of eggs to be shed. In the immature female rat, the adrenals play a definite role in determining the time of onset of puberty (for review see Ramaley[5]). Cory and Britton[6] induced precocious puberty with adrenocortical extracts. Bilateral adrenalectomy in rats younger than 25 days of age delays the normal appearance of vaginal opening and ovulation[7–9]. A restoration can be achieved by the administration of low doses of corticosterone[10], or by adrenal autotransplantation[8].

Adrenalectomy has been found to impair the sensitivity of immature female rats to gonadotrophins[11–13], with cortisone returning response to normal[11]. Hypophysectomized immature rats showed an impaired response to human chorionic gonadotrophin (HCG) unless adrenocorticotrophic hormone (ACTH) was also given[14]. Since the sensitivity of the ovary to gonadotrophins is dependent on the specific membrane-bound gonadotrophin receptors, one might speculate that the effects caused by adrenalectomy in female rats are due to the impairment of these receptors. In order to test this possibility, we studied the ovarian HCG-binding in adrenalectomized rats during sexual maturation.

Material and methods. Wistar female rats were received at 20 days of age and divided into control (N = 28) and experimental (N = 16) groups. Bilateral adrenalectomy was performed by an abdominal approach with the ani-

Binding of ^{125}I-labelled HCG by ovarian homogenates from adrenalectomized and control rats at various stages of development tested in the range of 2 to 70 ng of free hormone

Age (days)	Number of binding sites mol/mg wet weight [$\times 10^{15}$]		Dissociation constants K_D [$\times 10^{10}$]		Ovarian weight (mg)		Body weight (g)	
	Adrenal-ectomized rats	Controls	Adrenal-ectomized rats	Controls	Adrenal-ectomized rats	Controls	Adrenal-ectomized rats	Controls
23	–	3.46	–	5.90	–	4.93 ± 1.00	–	37.98 ± 4.86
28	5.61	5.02	5.38	5.12	6.72 ± 0.77 ns	8.90 ± 1.22	43.5 ± 3.34 [c]	49.10 ± 6.75
34	7.33	8.80	5.23	4.90	8.40 ± 0.87 [b]	10.51 ± 1.22	53.50 ± 4.27 [a]	71.43 ± 6.70
42	6.10	6.61	5.15	4.95	9.65 ± 0.96 [d]	17.32 ± 4.10	74.00 ± 5.4 [c]	112.85 ± 10.46

For comparison of body and ovarian weight between experimental and control animals the t-test was done; [a] $0.02 > \alpha > 0.01$, [b] $0.01 > \alpha > 0.002$, [c] $0.001 > \alpha > 0.0001$, [d] $0.0001 > \alpha$, ns = non significant. $\alpha \approx 0.2$. Mean values ± SE are included.

mals under ether anesthesia. Adrenalectomized rats were provided a 0.9% saline solution instead of drinking water at libitum. All rats were housed in air-conditioned and light-controlled animal rooms (light period from 6 a.m. to 9 p.m.). From the 32nd day of life onwards, control and adrenalectomized rats were checked for vaginal opening. Ovarian HCG-binding was studied in rats 23, 28, 34 and 42 days of age.

At autopsy, body and ovarian weights were recorded. The removed ovaries were trimmed of fat, frozen at −175 °C, and stored at −75 °C for no longer than one week. The experimental procedure used for the demonstration of HCG-binding was very similar to that of Leidenberger and Reichert[15] and Lee and Ryan[16]. Purified HCG (biological activity: 11,000 IU/mg) was iodinated by the modified method C of Leidenberger and Reichert[15] with an exposure to chloramine-T of 20 s. Specific radioactivity of the ^{125}I-labelled HCG was 30–50 μCi/μg. Binding studies were performed essentially as described by Lee and Ryan[16]. The ovaries were homogenized 1:10 (w/v) in ice-cold Tris-HCl buffer (0.04 mol/l, pH 7.4 containing 0.005 mol/l $MgSO_4$) and subsequently centrifuged at 100 × g for 20 min. Aliquots of the supernatants corresponding to 5 mg tissue wet weight were incubated in duplicate in the homogenization buffer containing 0.1% bovine serum albumine (BSA) and varying amounts of labelled and unlabelled HCG, in a final volume of 1 ml, at 37 °C for 30 min. 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, BRD) previously washed with 10 ml 4% BSA. Then the filters were washed with 10 ml of the cold homogenization buffer and the radioactivity on the filters was counted in a liquid scintillation spectrometer. The amount of specifically bound ^{125}I-HCG was determined as the difference between the radioactivity bound in samples containing a 1000fold excess of competing unlabelled HCG at each concentration of labelled hormone and in parallel samples with ^{125}I-HCG alone.

Results and discussion. The observation of different authors[7–10, 13] that the onset of puberty is delayed in adrenalectomized female rats is confirmed by our results. Vaginal opening occurred around days 38 to 39 in Wistar control female rats, while it occurred only around days 44 to 45 in rats adrenalectomized at the 23rd day of age. Furthermore, the adrenalectomized rats showed a significant reduction in body and ovarian weight as compared to control rats of the same age (table). According to Gorski and Lawton[8], the reduction in b.wt in adrenalectomized rats might reflect a generalized effect of the adrenals on maturation besides their effect on the reproductive system as the ovary.

An increase in blood concentration of estradiol has been found to precede vaginal opening and the initial ovulation in rats[17]. Furthermore, the onset of puberty is inducible in prepubertal rats by estrogens[18, 19]. Thus, a decrease in ovarian sensitivity to gonadotrophins after adrenalectomy, as observed by Mandl[11] and Ramaley[12], might lead to a delayed or lower ovarian steroid hormone production and result in a delay of pubertal onset. Our results, shown in the table, clearly indicate that the decrease in ovarian sensitivity to gonadotrophins in adrenalectomized prepubertal rats is not due to the loss of specific LH-/HCG-receptors or an altered hormone affinity of these receptors. The number of ovarian HCG-binding sites in 42 days old control rats amounted to 6.61×10^{-15} mol/mg wet weight and to 6.10×10^{-15} mol/mg wet weight in 42 days old rats adrenalectomized at day 23 of age. Scatchard[20] analyses of the results obtained in control and adrenalectomized rats at the 28th, 34th and 42nd

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day of life revealed very similar dissociations constants (K_D) of the receptor-hormone complex in both groups, namely ranging between 4.95 and 5.12×10^{-10} M^{-1} in control rats and between 5.15 and 5.38×10^{-10} M^{-1} in adrenalectomized rats.

The decrease in ovarian gonadotrophin sensitivity after adrenalectomy can be restored by glucocorticoids[10,11]. The question arises in which way glucocorticoids may influence ovarian gonadotrophin sensitivity; glucocorticoids have been found to modulate the action of various hormones onto their target cells[21,22]. Recently Engel and Frowein[23] and Schmidtke et al.[24] presented evidence that in the rat testis this 'permissive effect' of the glucocorticoids is possibly due to an inhibitory effect onto the cyclic-AMP phosphodiesterase, which is engaged in the hydrolysis of cyclic-AMP. If glucocorticoids act in a similar way in the ovarian cells, the lack of glucocorticoids in adrenalectomized prepubertal rats may result in a lowered intracellular cyclic-AMP level and, in consequence of this, in a reduced gondal steroidogenesis.

The interplay of the adrenals and the ovaries during female rat development is rather short[9]. Sexual maturation is only impaired if the adrenalectomy is performed before the 25th day of age, but even in these rats vaginal opening and ovulation occur spontaneously, although delayed. Furthermore, adult adrenalectomized rats maintain ovulatory cycles and can reproduce[3]. If the glucocorticoids act in the ovarian cells by inhibition of cyclic-AMP phosphodiesterase, as suggested above, their action should be restricted with respect to developmental time. Cyclic-AMP phosphodiesterases have been found to exist in the pre- and postpubertal ovary in 2 molecular forms exhibiting distinct kinetic properties (J. Schmidtke, personal communication). The question is now under study of whether these molecular forms (isozymes) differ from each other in their inhibition by glucocorticoids before and after the 25th day of age.

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Behavioural consequences of vasectomy in the mouse

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Summary. Vasectomy was found to have no influence on the sexual activity of male mice. Testis and seminal vesicle weights were similary not influenced by this operation although a significant increase in epididymus weight was observed.

In order to demonstrate the acceptability of vasectomy as a means of sterilization, the influence of this operation on long term sequelae such as auto-immune disease[2-4] and granuloma development[5] as well as sexual activity and libido must be established. While there has been no shortage of research on the physiological status of vasectomized animals[5,6], the behavioural changes induced by this treatment have been largely neglected despite their importance. Rodgers and Ziegler[7] observed a slight increase in the frequency of human intercourse following vasectomy, although the interview and questionnaire procedure used in this, and similar clinical studies, may have been influenced by motivated distortions of recall and reporting by the subjects. Unfortunately, few attempts have been made to obtain more objective data with laboratory species. McGlynn and Erpino[8] compared various aspects of coital behaviour in intact and vasectomized rats, but did not investigate coital frequency in these animals. In a clinical context, however, it is the frequency rather than the normality of copulation which would seem the more valid criterion of sexual activity. A study was therefore designed to assess the influence of vasectomy on coital frequency in the mouse. The mouse was chosen for this experiment because the presence of a vaginal copulation plug in this species permits the ready identification of mated females.

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Table 1. Coital frequencies (No. of females covered in each 10-day-period) observed in sham-operated and vasectomized mice

Treatment		Duration of pre-operative test period (days)	Pre-operative coital frequency (± SE)	Duration of post-operative test period (days)	Post-operative coital frequency (± SE)	Number of mice
Experiment 1	Sham	23	5.61 ± 0.52	78	5.55 ± 0.31	10
	Vasectomized	23	4.78 ± 0.52	78	5.61 ± 0.35	11
Experiment 2	Sham	51	6.58 ± 0.64	155	5.04 ± 0.73	9
	Vasectomized	51	7.09 ± 0.62	155	5.11 ± 0.76	12