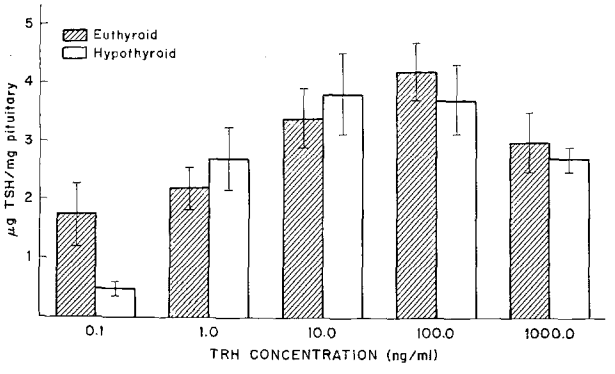
that of hypothyroid tissue ($p < 0.05$). With 1000 ng/ml TRH the mean increase in TSH was less than with 100 ng/ml; however, the difference was not statistically significant. These results are shown in the Figure.

Discussion. The results described above demonstrate that increasing doses of TRH elicit increasing release of TSH from pituitary tissue obtained from euthyroid and 21-day-thyroidectomized rats in vitro. The 0.1 ng/ml concentration of TRH was more effective in causing TSH release from euthyroid pituitaries than from hypothyroid pituitaries, the rate of release of both approaching maximum within 30 min.

Several alternatives might explain this finding of relative unresponsiveness of hypothyroid pituitary tissue to 0.1 ng/ml TRH. First, if it is true that the hypothalamic secretion of TRH increases in hypothyroidism, as has been suggested[6], then repeated exposure of the pituitary to increased concentration of TRH might result in a state of relative refractoriness to TRH. Progressive unresponsiveness to TRH following repeated infusions of TRH has been shown to occur by others[7]. Secondly, it is possible that the pituitary gland requires a minimal amount of thyroid hormone for its routine metabolic processes including the synthesis of TSH. If this is true, then the diminished responsiveness of the hypothyroid pituitary tissue to 0.1 ng/ml TRH might simply reflect a diminished metabolic activity that is overcome by larger concentrations of TRH. Lastly, it is possible that there are 2 'pools' of TSH, a 'labile' one responding to low doses of TRH present primarily in euthyroid pituitary tissue and a second form present in both euthyroid and hypothyroid pituitary tissue.

Euthyroid and hypothyroid pituitaries did not respond differently to the higher concentrations of TRH (1.0, 10.0, 100.0, 1000.0 ng/ml) and both euthyroid and hypothyroid pituitaries continued to release significant amounts of TSH at 60 min. If the pituitary concentration of biologically and immunologically active TSH is decreased in hypothyroidism, as has been shown by several investigators[8–13], and at the higher concentrations of TRH (1.0, 10.0, 100.0 and 1000.0 ng/ml) hypothyroid pituitary tissue is as capable of releasing TSH/mg pituitary as is euthyroid pituitary tissue, then it is possible that the pituitary gland of hypothyroidism possesses an enhanced potential to release TSH, a potential which becomes manifest at higher concentrations of TRH.

Zusammenfassung. Die Kapazität von Rattenhypophysen, auf eine TRH-Stimulation in vitro TSH auszuschütten, zeigt bei hohen TRH-Konzentrationen keine Differenz zwischen eu- und hypothyreoten Tieren, während eine niedrige TRH-Konzentration eine höhrere TSH-Ausschüttung bei Hypophysen euthyreoter Tiere verursacht.



Response of rat pituitary tissue to increasing concentrations of TRH after 60 min incubation in vitro. The results are expressed as mean increase of TSH in the experimental incubation medium minus the control medium per mg of pituitary tissue ± standard error of the mean.

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Loss of Sexual Activity in Rabbits Actively Immunized with Testosterone

Active immunization with testosterone or estrogens leads to hyperplasia and hyperfunction of the interstitial testicular tissue[1,2]. A decrease in the amount of free steroid available to hypothalamo-pituitary receptors caused by antibody binding and a subsequent increase in gonadotrophin secretion, despite high concentrations of total circulating testosterone or estrogen respectively[2], underlies these alterations. In general, it is assumed that steroids which have lost their ability to act on hypothalamo-pituitary receptors have also lost their biological activity. It was, however, recently observed that there is no obligatory correlation between peripheral androgenic effects of a given steroid and its ability to inhibit pituitary LH secretion[3]. A possible corollary to this evidence is that binding of testosterone to circulating antibodies might have different effects on androgen-sensitive receptors in different organ systems. Thus we were prompted to investigate the biological effects of testosterone in rabbits immunized against this androgen. Sexual activity, one of the most characteristic biological effects of testosterone, was chosen as a parameter for this study.

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Effect of active immunization with testosterone or estrogens on male rabbits

	Weight (g)	Antiserum titer	Testosterone in serum (ng/100 ml)	Binding (%) of testosterone and estradiol		Sexual activity
Normal rabbits[a] ($n = 5$)	3310 ± 240	–	408 ± 172	90.4 ± 1.5	81.6 ± 2.5	+
Testosterone-immunized rabbits						
Nr. 1	3100	1/ 8000	3662	99.3	89.1	–
2	3680	1/32000	8388	99.4	94.7	–
3	3050	1/ 6400	9706	99.3	91.4	–
4	3850	1/12000	10820	99.6	92.7	–
5	3420	1/ 7000	8254	99.3	–	–
6	3650	1/ 6000	11186	99.1	89.9	–
Mean ± S.D.	3460 ± 330	–	8669 ± 2733	99.3 ± 0.2	91.6 ± 2.2	
Estrogen-immunized rabbits						
Nr. 1	3050	1/16000	293	93.7	98.9	+
2	3450	1/14000	342	94.4	98.7	+
3	4050	1/ 1200	144	89.5	98.8	+
4	3500	1/15000	559	91.7	98.7	+
5	3100	1/ 7000	770	88.2	98.9	+
6	3340	1/ 7000	595	93.9	98.9	+
7	3680	1/ 4000	405	89.7	99.0	+
Mean ± S.D.	3450 ± 340	–	444 ± 211	91.6 ± 2.5	98.8 ± 0.1	

[a] Mean values ± S.D.

Methods. Two groups of 12-week-old male white New Zealand rabbits were immunized with testosterone-3-albumin or estrone-17-albumin conjugates by intradermal injections[4]. No booster injections were administered. After 16 weeks they were exposed to receptive female rabbits for 15 min and the sexual activity (chasing, number of mountings and ejaculation) during this period was recorded. Thereafter blood was drawn from an ear vein on the same day and the antiserum titer (i.e. the dilution at which 50% of 20,000 dpm tritiated testosterone or estradiol are bound), the concentration of total circulating testosterone[5] and the percentage of binding of testosterone and estradiol[6] were determined in the serum samples. 5 rabbits of the same sex and age served as a control group.

Results. The results are shown in the Table. Testosterone in the rabbits immunized with this steroid exceeded normal values up to 30-fold. The percentage of binding of testosterone to plasma proteins or circulating antibodies respectively was increased from 90.4 ± 1.5% (mean value ± S.D.). in the controls to almost 100%. None of these animals displayed any signs of sexual activity towards females. The animals immunized with estrone, however, showed normal sexual activity by chasing the females, mounting them 5 to 15 times and ejaculating. The testosterone levels and the percentage of binding of testosterone in these animals was normal.

Discussion. Complementary to these findings we have observed in a limited number of rabbits that the accessory reproductive glands (prostate, paraprostate and seminal vesicles) are atrophic in testosterone-immunized, but not different from normal in estrogen-immunized animals[2]. All these findings suggest that the binding of testosterone to antibodies renders the hormone not only unobtainable to hypothalamo-pituitary but also to other receptors. It appears unlikely that there are graded effects on receptors in different organ systems. Hence, active immunization with testosterone results in neutralization of the steroid's biological activity and induces a condition identical to castration in biological effect, despite increased number and activity of Leydig cells. Considering the fundamental function of the immune system in providing defense and protection for the organism, the effects of active immunization with testosterone give a striking example of the fascinating phenomenon that the immune system can be inverted by active immunization with hormones to incapacitate the very organism which it is supposed to protect.

Zusammenfassung. Die aktive Immunisierung mit Testosteron führt trotz bis zu 30fach erhöhten peripheren Testosteron-Konzentrationen und Leydig-Zell-Hyperplasie zu einem Verlust der sexuellen Aktivität männlicher Kaninchen. Als Ursache wird die Neutralisation der biologischen Aktivität des Testosterons durch Bindung an Antikörper angesehen.

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