98.8% of ^{3}H-activity, found in form of neutral steroids, 89.6% were isolated as DHEA, followed by androstendione with 7.5% and minute quantities of ring-D hydroxylated compounds. In the fraction of free phenolic steroids estradiol and estriol contained the major portion of ^{3}H-activity, while E_{x1} and E_{x2} were not detectable.

From these results, however, no conclusions can be drawn as to the preference of any biosynthetic pathway,

Table II. Metabolites in the fraction of free steroids

Steroid	dpm ^{3}H	% of ^{3}H-activity
DHEA	135,500	89.604
Androstendione	11,120	7.492
Testosterone	1,200	0.811
Androstendiol	1,350	0.911
Androstentriol[a]	50	0.030
Estrone[a]	45	0.030
Estradiol	1,140	0.770
Estriol	520	0.352

[a] Not properly identified.

leading from DHEA or its sulphate to androgens and estrogens in ovarian tissue. The assumption that, in the ovary, sulphoconjugated C_{19}-steroids primarily undergo hydroxylation in Ring D[8,9] prior to direct aromatization, still requires the appropriate experimental support[10].

Zusammenfassung. Nach Inkubation von menschlichem Ovarialgewebe mit 7α-^{3}H-DHEA-^{35}S-sulfat konnten als Metaboliten 5 verschiedene C_{19}- und 2 phenolische C_{18}-Steroide isoliert und identifiziert werden, die als Sulfokonjugate einen gegenüber dem Substrat unveränderten ^{3}H/^{35}S-Quotienten aufwiesen.

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Persistence of the Levator Ani Muscle in Female Rats

The levator ani muscle (MLA) has been considered as part of the genital tract of male rats. It has been generally assumed that this muscle exists only in the male rat[1]. However it could be shown that the levator ani muscle of the rat develops in both sexes from a uniform blastem. Only from the 18th day of embryological development, there occurs a progressive involution of this muscle in the female rat and the muscle cannot be found in female adult rats[2]. The homologous embryonic blastems of the levator ani in both sexes suggested that it might be possible to ensure its maintenance in female rats, if androgens are applied before or immediately at the time of birth, i.e. before the involution of the muscle anlage.

Material and methods. Experiments were undertaken to test the possibility of achieving persistence of the levator ani muscle in female rats. From 2 litters of newborn rats the female rats were selected and testosterone propionate (1 mg twice a week) was injected s.c. for the period of 1 and 2 months respectively. The muscles were weighed 1 and 2 months after birth and compared with the muscles of male untreated rats selected from the same litters. Both the absolute and relative (i.e. weight of muscle expressed as $^0/_{00}$ of body weight) weights of MLA were determined.

Results. The Table shows that testosterone treatment maintains the levator ani muscle in female rats. There is no difference in the weight of the muscles against those of the male control animals 1 month after birth, but 2 months after birth the muscles of the male control animals show a greater weight increase than those of the testosterone-treated female animals. It can also be seen that one month's treatment is not sufficient to ensure further growth of the muscle as in the muscles of animals in which treatment was continued for both months. It is, however, of interest that the relative muscle weight of the MLA appears to be stationary after cessation of treatment and these relations deserve further study.

Absolute and relative (i.e. weight of muscle expressed as $^0/_{00}$ of body weight) weights of the MLA of 1- and 2-month-old rats. The female rats received 1 mg of testosterone propionate (TP) twice weekly, beginning with the day after birth

Type of experiment	Female treated rats MLA (mg)		Male non-treated rats MLA (mg)	
	absolute weight	relative weight	absolute weight	relative weight
1-month-old 1 month TP treatment ($n = 6$)	19.6 ± 1.9	0.201	19.7 ± 1.4	0.210
2-month-old 2 months TP treatment ($n = 6$)	68.0 ± 10.6	0.321	97.5 ± 9.4	0.541
2-month-old 1 month TP treatment, 1 month cessation ($n = 5$)	38.6 ± 4.5	0.216		

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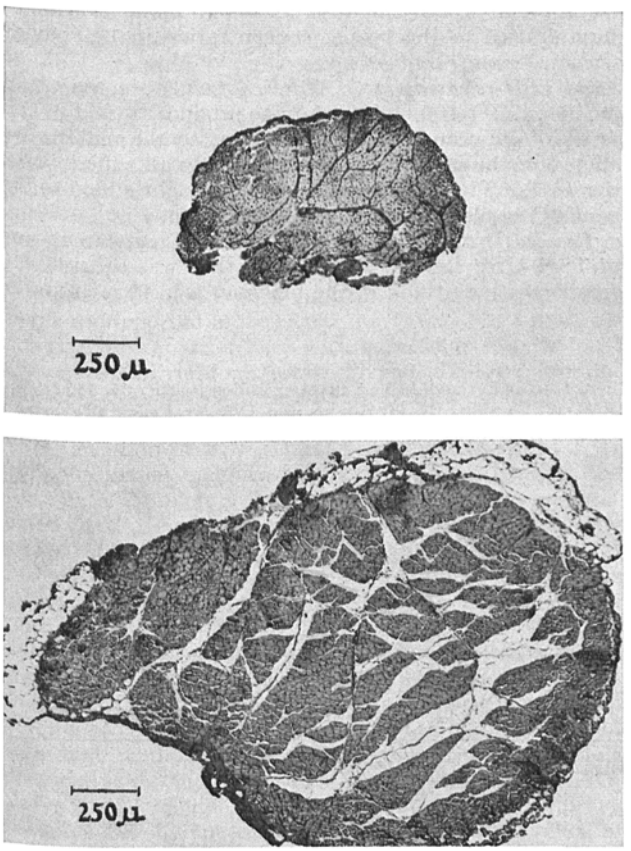
Discussion. Wasting of the MLA after castration can be prevented by testosterone[3] and a series of papers have shown that androgens, in addition to their responsibility for development and maintenance of the male accessory sex organs, stimulate an increase in body weight and growth[4]. Indeed the increase in weight of the MLA of castrated male animals after testosterone has been suggested and widely used as a biological indicator of the myotropic activity of androgens[5]. However, the use of the MLA as a test for anabolic activity in castrated male animals has been criticized[6] and the lack of a standard method which would allow determination or comparison of the relations of myotropic and androgenic effects of anabolic steroids, has made therapeutical applications difficult[7]. The persistence of the MLA in female rats has the character of a qualitative finding and may help to find new methods of evaluation of androgenic and myotrophic actions of anabolic steroids.



Cross section of the largest part of MLA in a female rat 1 and 2 months after birth. During this time testosterone propionate 1 mg was injected s.c. twice weekly.

Zusammenfassung. Testosteronapplikation bei neugeborenen weiblichen Ratten (1 mg Testosteron propionat zweimal wöchentlich s.c.) führt zur Erhaltung des M. levator ani, der normalerweise sich bei beiden Geschlechtern gleichartig entwickelt, bei fortlaufender Reduktion vom 18. Tag der Embryonalentwicklung an. Die Möglichkeit der Erhaltung des Muskels als Test für die Wirkung anaboler Steroide wird diskutiert.

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Presence of Calcium Ions as a Requisite for the in vitro Stimulation of TSH-Release by Hypothalamic TRF

We have recently undertaken a series of experiments to explore the mechanisms of action of the hypothalamic factor TRF (TSH-releasing factor) when it stimulates acutely the secretion of adenohypophysial TSH (thyroid-stimulating hormone). Thus, we have already observed that neither cycloheximide nor actinomycin-D prevent the in vitro release of TSH induced by TRF, in conditions in which we could demonstrate the efficacy of the antibiotics to inhibit protein and RNA synthesis[1,2]. In vivo we have shown[3] that TRF injected i.v. stimulates release of TSH in an extremely rapid manner, evidence for increasing plasma TSH concentration being observed less than 120 sec following injection of the hypothalamic material. These results have led us to several working hypotheses considering a possible effect of TRF on the cellular membrane potential in its stimulating release of TSH. One of the corollaries of these hypotheses would be that alteration of the K^+, Ca^{++} and Mg^{++} concentrations of the incubation milieu should modify the activity (release of TSH) of the mediator (TRF).

The results presented here will show that indeed the effect of TRF in stimulating the release of TSH when added to pituitary tissues incubated in vitro[1,4] can be completely prevented by prior incubation of the pituitary in Ca^{++}-free medium. Experiments in which Ca^{++} is re-added to the medium show further that the responsiveness to TRF is restored when Ca^{++} is re-introduced in the incubation fluid.

Materials and methods. (1) *Incubation of the pituitary glands.* The anterior pituitary gland of rats (males, body

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