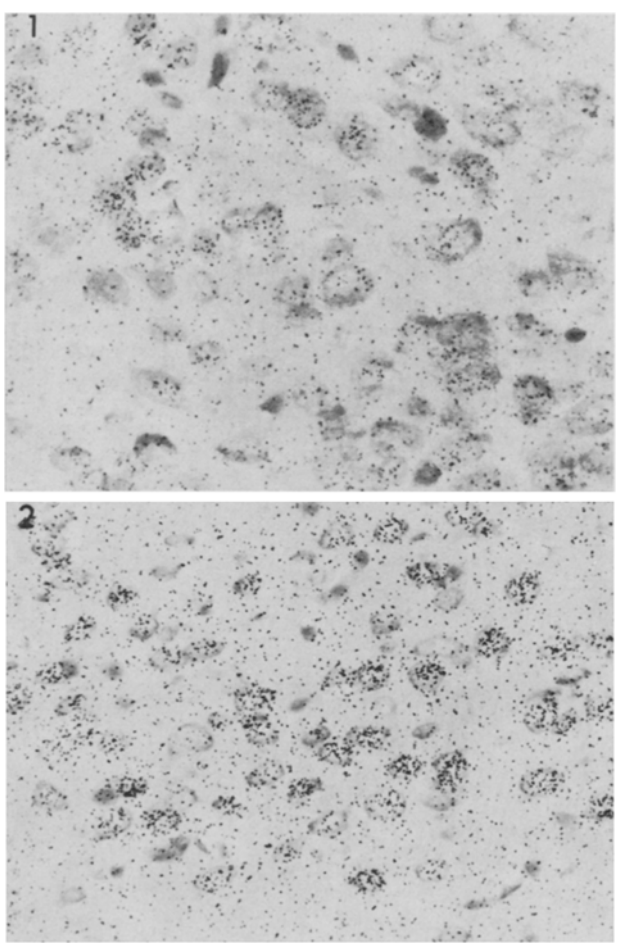
Cellular Localization of Androgen in the Brain and Pituitary After the Injection of Tritiated Testosterone

Evidence has been provided that the control of gonadotropin secretion and sexual behavior in male rats is mediated through the hypothalamus[1]. Lesions and testosterone implants in the basomedial region of the hypothalamus decrease gonadrotropin secretion[2,3] but induce changes in sex behavior when placed in the anterior-hypothalamic preoptic area[4,5]. After the injection of labeled testosterone, radioactivity is accumulated in the brain[6,7]. The distribution of androgen concentrating neurons ('androgen-neurons') could be demonstrated in specific areas of the brain[8] using the dry-mount autoradiographic technique[9].

Materials and methods. 4 immature intact male Sprague-Dawley (SD) rats, 26 days old, and 4 adult male SD rats, castrated 96 h before, were injected s.c. with 0.5–1.0 µg per 100 g body weight of 1,2-^{3}H-testosterone, specific activity 50 Ci/m*M*, dissolved in 10% ethanol in isotonic saline, and killed after 1 h. 2 to 3 mm^3 pieces of brain tissue were excised, placed on a tissue holder, and frozen in liquefied propane. 2 µm serial frozen sections were cut in a cryostat, freeze-dried, and dry-mount autoradiograms were prepared as described[9]. After exposure of 8 to 16 months at −15 °C, the slides were developed, fixed and stained with methylgreen pyronin. Gomori's trichrome stain was used to identify pituitary cell types. Schematic drawings were prepared after serial section autoradiograms and adapted to the coronal and sagittal planes of the atlas of KÖNIG and KLIPPEL[10].



Figs. 1 and 2. Autoradiograms of the nucleus preopticus medialis (Figure 1) and the nucleus medialis amygdalae (Figure 2), frontal plane, showing nuclear concentration of radioactivity in neurons. Prepared at 1 h after s.c. injection of 1,2-^{3}H-testosterone into adult castrated male rats. Stained with methylgreen pyronin, 2 µm. Exposure time 280 days, ×680 (Figure 1) and 300 days, ×440 (Figure 2).

Results. Autoradiograms of the hypothalamus, parolfactory region, preoptic region, hippocampus and amygdala revealed concentration of radioactivity in nuclei of certain neurons (Figures 1 and 2), while not in others. Although silver grains were concentrated over nuclei, silver grains existed also over the cytoplasm and extracellular space. Glial cells and ependymal cells did not concentrate radioactivity. In the brains of both immature intact and mature castred male rats, radioactively labeled neurons showed similar topographic distribution although quantitative differences cannot be excluded.

In the parolfactory region, a few androgen-neurons observed within the nucleus (n.) tractus diagonalis and in the islands of Calleja. In the n. septi lateralis (Figure 3), close to the lateral ventricle, a large number of neurons were labeled. These labeled neurons were continuous with the accumulation of labeled neurons in the n. interstitialis striae terminalis and in the n. preopticus medialis (Figures 1, 3 and 4). The labeling index appeared to be highest in the n. preopticus medialis and in portions of the n. interstitialis striae terminalis. Neurons of the n. suprachiasmaticus proper and of the area hypothalamica anterior did not concentrate radioactivity, except for a few scattered neurons. Neurons of the n. suprachiasmaticus were apparently not labeled, although some neurons of the n. paraventricularis showed labeling. Within the n. periventricularis, labeled neurons existed throughout its course from the preoptic to the arcuate nucleus region. The n. arcuatus contained a number of labeled neurons from its anterior end to its posterior extremity (Figure 4). Radioactivity was concentrated in neurons accumulated in the n. ventromedialis (Figure 4). In the lateral hypothalamus, at the level of the n. ventromedialis, a few labeled neurons were found. Androgen-neurons were also accumulated within the n. premammillaris ventrilas. A few labeled neurons were observed in the n. habenulae lateralis.

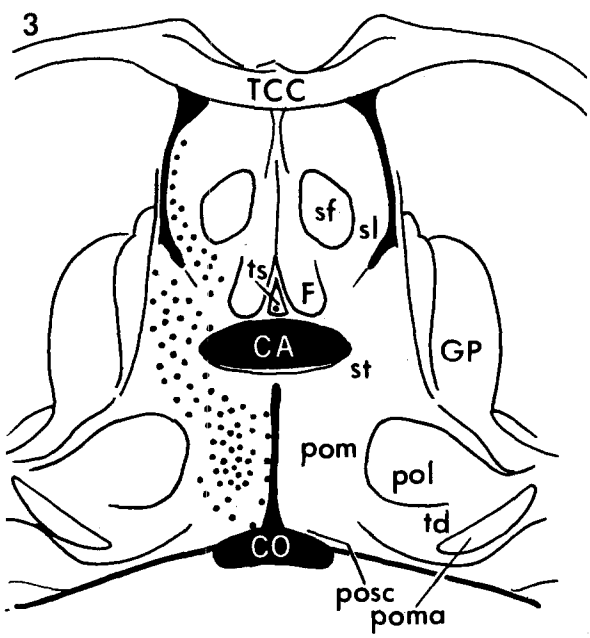
Autoradiograms of the amygdala revealed concentration of radioactivity in neurons of the n. medialis (Figure 2), the n. basalis pars medialis, adjacent to the n. medialis, and the n. corticalis. In the hippocampus, weakly labeled neurons were observed.

[1] J. M. DAVIDSON, in *Neuroendocrinology* (Eds. L. MARTINI and W. F. GANONG; Academic Press, New York 1966), vol. 1, p. 565.
[2] R. D. LISK, Acta endocr., Copenh. *41*, 195 (1962).
[3] J. M. DAVIDSON and C. H. SAWYER, Proc. Soc. exp. Biol. Med. *107*, 4 (1961).
[4] K. LARSSON and L. HEIMER, Nature, Lond. *202*, 413 (1964).
[5] J. M. DAVIDSON, Endocrinology *79*, 783 (1966).
[6] J. A. RESKO, R. W. GOY and C. H. PHOENIX, Endocrinology *80*, 490 (1967).
[7] M. SAR and W. E. STUMPF, 2nd Annual Meeting, Society for Neuroscience, Program and Abstracts, p. 226, 1972.
[8] M. SAR and W. E. STUMPF, Fedn. Proc. *30*, 363 (1971).
[9] W. E. STUMPF, in *Introduction to Quantitative Cytochemistry* (Eds. G. L. WIED and G. F. BAHR; Academic Press, New York 1970), vol. 2, p. 507.
[10] J. F. R. KÖNIG and R. A. KLIPPEL, *The Rat Brain* (Williams and Wilkins, Baltimore 1963).

In the pituitary only a small number of anterior lobe cells were observed to concentrate radioactivity in their nuclei (Figure 5) after ^{3}H-testosterone injection. These cells were identified as basophils, using Gomori's trichrome stain. Radioactivity was not retained in cells of the intermediate and posterior lobes.

Discussion. The present autoradiographic studies demonstrate selective retention and concentration of androgen in neurons in areas of the preoptic parolfactory region, the hypothalamus, the hippocampus and the amygdala, as well as in cells of the anterior pituitary. The nuclear concentration of androgen in specific neurons of the brain and in cells of the anterior pituitary suggests that such retention is related to the action of androgen, as it has been demonstrated for the ventral prostate and seminal vesicles[11,12]. The chemical nature of the radioactivity in tissues at the time of excision has not been identified. In the brain and pituitary the radioactivity is either testosterone or dihydrotestosterone[13–15]. Although the possibility exists that testosterone may be converted to estrogen by peripheral aromatization, such a transformation is unlikely since castrated male rats were used in this study and differences exist in the topographic distribution of radioactivity in the brain and pituitary after the injection of labeled testosterone and estrogen[16].

The sites of androgen-neurons in the brain appear to be associated with the regulation of gonadotropin secretion and sexual behavior as observed by steroid implants and lesion experiments[1]. A nuclear concentration of androgen in anterior pituitary cells also suggests a direct feedback effect of testosterone at the pituitary level. Thus the autoradiographic data support the hypothesis that androgen acts both at the level of the central nervous system as well as the pituitary.



Figs. 3 and 4. Schematic drawings prepared after serial-section autoradiograms from immature intact male and mature castrated male rats 1 h after the injection of ^{3}H-testosterone. Figure 1 coronal section, and Figure 2 sagittal section. The black dots represent areas of concentration of neurons labelled with radioactivity. Abbreviations: ar, nucleus (n.) arcuatus; CA, commissura anterior; Co, chiasma opticum; F, Columna fornicis; f, n. paraventricularis; ha, n. anterior hypothalami; hdv. n. dorsomedialis hypothalami ventralis; hp, n. posterior hypothalami; hvm, n. ventromedialis hypothalami; I, infundibulum; pom, n. preopticus medialis; posc, n. preopticus suprachiasmaticus; sc, n. suprachiasmaticus; sl, n. septi lateralis; st, n. interstitialis striae terminalis; TCC, truncus corporis callosi; ts, n. triangularis septi. For other abbreviations see the atlas of König and Klippel[10].

Zusammenfassung. Nach Injektion von ^{3}H-Testosteron wurde mit Hilfe der Trocken-Autoradiographie eine selektive Aufnahme und Speicherung von Radioaktivität in Kernen spezifischer Neurone im Hypothalamus, in der präoptisch-parolfaktorischen Region, im Septum, im

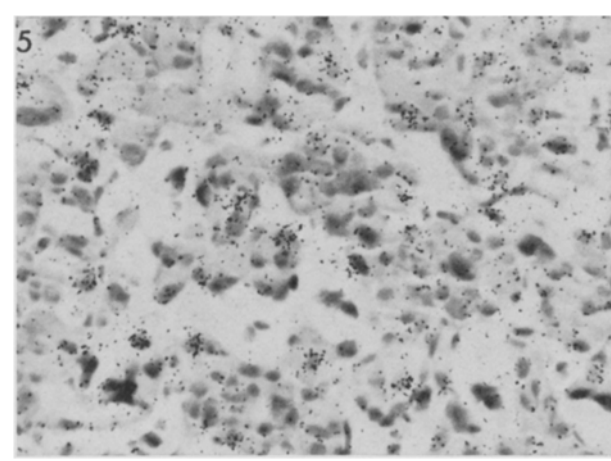


Fig. 5. Autoradiogram of pituitary prepared 1 h after s.c. injection of 0.5 µg/100 g body weight of 1,2-^{3}H-testosterone into an adult castrated male rat. Stained with Gomori's trichome stain. Exposure time 230 days, 2 µm, × 550. Note the nuclear concentration of radioactivity in a few cells of anterior pituitary. These cells are identified as basophils.

[11] M. Sar, S. Liao and W. E. Stumpf, Endocrinology *86*, 1008 (1970).
[12] S. Liao and S. Fang, Vitams and Horm. *27*, 17 (1969).
[13] J. M. Stern and A. J. Eisenfeld, Endocrinology *88*, 1117 (1971).
[14] Z. Kniewald, R. Massa and L. Martini, Excerpta med. *210*, 59 (1970).
[15] P. Jouan, S. Samperez, M. L. Thieulant and L. Mercier, J. Steroid Biochem. *2*, 223 (1971).
[16] W. E. Stumpf, Am. J. Anat. *129*, 207 (1970).

Hippokampus und in der Amygdala beobachtet. Im Hypophysenvorderlappen war Radioaktivität in den Zellkernen von Basophilen nachweisbar. Die autoradiographischen Ergebnisse unterstützen das Konzept eines doppelten Androgen-«Feedbacks» auf der Ebene des Zentralnervensystems und der Hypophyse.

MADHABANANDA SAR and W. E. STUMPF[17]

Laboratories for Reproductive Biology, Departments of Anatomy and Pharmacology, University of North Carolina, 111 Swing Building, Chapel Hill (North Carolina 27514, USA),
20 March 1972.

[17] We thank Mrs. GERDA MICHALSKY and Mrs. ANU TURNBULL for their technical assistance. This study was supported by PHS grant No. AM14929 to W.E.S. and a grant from the Rockefeller Foundation to the Laboratories for Reproductive Biology, Chapel Hill, North Carolina.

Plasma Adrenocorticosteroid Concentrations Immediately after Birth in the Rat, Rabbit and Guinea-Pig

Circulating plasma concentrations of the principle adrenocorticosteroid are elevated in the period immediately after birth in the lamb[1,2] and calf[3]. In both these species the concentration falls over the next few days. There are few reports of very early steroid levels in the rabbit and guinea-pig in the neonatal period[4], and investigations on the rat have generally been made on animals one or more days after birth. Different methods of assay have yielded very variable results. Fluorimetric techniques, in particular, appear to result in very high values[5,6].

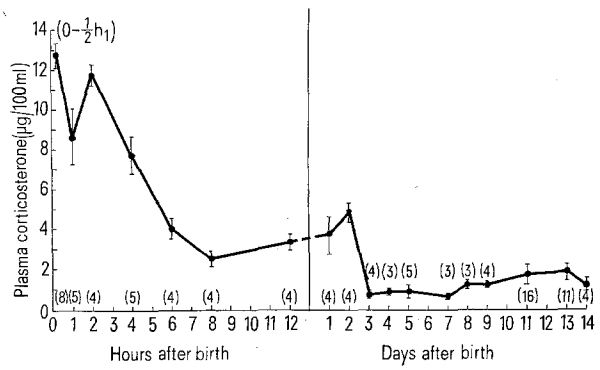


Fig. 1. Plasma corticosterone concentration in the young rat. Experimental points represent mean values ± S.E.M. (Number of animals in parentheses). Ordinate: plasma steroid concentration µg/100 ml. Abscissa: Age after birth in h and days.

In the investigations reported here, animals were maintained under standard conditions and killed by decapitation. Individual guinea-pig samples or pooled blood from at least 5 rats or 2 rabbits were analyzed by competitive protein binding after Sephadex LH 20 separation as described previously[6].

Corticosterone is the main circulating adrenocorticosteroid in the rat. In the first 30 min of extra-uterine life, mean plasma levels were 12.6 ± 0.8 µg/100 ml (Figure 1). Thereafter, the plasma steroid concentration declined steadily over the first 8 h. A further drop occurred between days 2–3.

HOLT and OLIVER[7], using a fluorimetric assay, reported a mean plasma corticosterone concentration in the rat of 200 µg/100 ml 1–5 h after birth. The values observed at unspecified times during the first day after birth by 3 other groups of workers were about 18 µg/100 ml[8–10]. The only previously published values which approach our observed ranges are those of BARTOVA[11] who reported a concentration of 5 µg/100 ml on day 2 of extra-uterine life.

Our finding that cortisol is the major adrenocorticosteroid in the newborn rabbit has not been previously recorded. Concentrations were highest 3–10 min after birth (Figure 2) and had fallen by the end of the first day to levels which remained relatively constant until day 14, at which time the mean value was 0.5 ± 0.1 µg/100 ml. At this age, THORNTON et al[4] found a mean plasma cortisol concentration of 11 µg/100 ml.

Figure 3 demonstrates plasma cortisol levels in the guinea-pig from birth to 14 days. Cortisol is the principle circulating adrenocorticosteroid in this species. Values

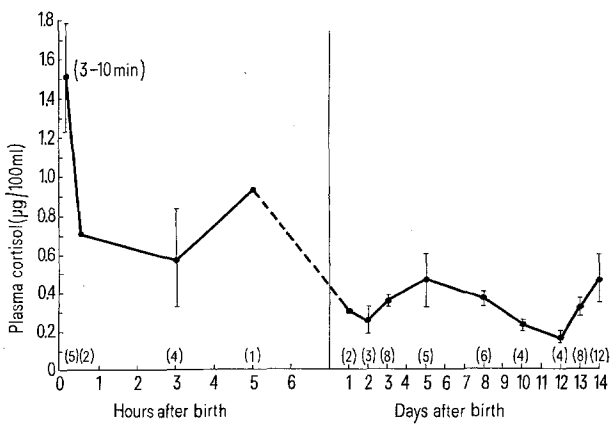


Fig. 2. Plasma cortisol concentration in the young rabbit. Experimental points represent mean values ± S.E.M. (Number of animals in parentheses). Abscissae and ordinates as Figure 1.

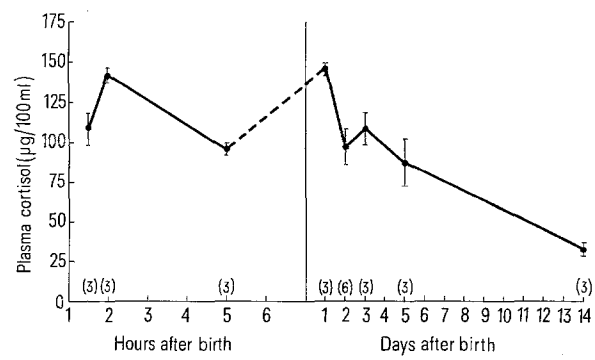


Fig. 3. Plasma cortisol concentration in the young guinea-pig. Experimental points represent mean values ± S.E.M. (Number of animals in parentheses). Abscissae and ordinates as Figure 1.