and invasive behaviour of this transplantable tumour of human origin makes the study of such questions imperative.

Zusammenfassung. Zweidimensionale Immunodiffusionsteste nach OUCHTERLONY zeigten, dass das im Hamster überpflanzte menschliche Ovarialkarzinom, GW-127, nach über 5 Monaten Tierpassagen noch humanes Antigen beibehalten hat. Die Möglichkeit einer Hybridisation von Humantumor- mit normalen Hamsterchromosomen wird zur Diskussion gestellt.

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Autoradiographic Localization of Testosterone-^{3}H in the Female Rat Brain and Estradiol-^{3}H in the Male Rat Brain

It is well known that male sex hormones trigger sex behavior in males, and female hormones trigger sex behavior in females of many species, including rats[1]. Sex hormones also affect sex behavior when injected into rats of the opposite sex. Testosterone injections increase the frequency of masculine mating responses and alter the strength of feminine mating behavior in ovariectomized or normal female rats[2]. Complementary effects are seen when estrogens are injected into castrated male rats[3].

As part of the analysis of sex hormone effects on reproductive functions, testosterone uptake in the brain of the male rat has been described[4,5], as has estradiol uptake in the female brain[6–9]. The present report describes testosterone-^{3}H uptake in the female rat brain and estradiol-17β-^{3}H uptake in the male rat brain.

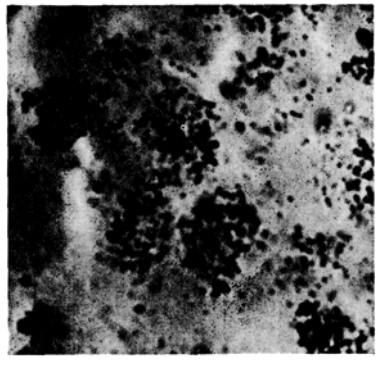
Testosterone-1,2-^{3}H (200 μc; sp. act. 46.5 c/mM; New England Nuclear Corp.) was injected i.v., dissolved in 0.05 cm^3 25% ethanol, into each of two 200 g female rats, ovariectomized 2 weeks previously. Estradiol-17β-6,7-^{3}H (200 μc; sp. act. 42.4 c/mM; New England Nuclear Corp.) was injected i.v., dissolved in 0.05 cm^3 25% ethanol, into each of two 200 g male rats, castrated 2 weeks previously. 2 rats were killed $^1/_2$ h after injection (1 of each sex) and 2 rats were killed 2 h after injection (1 of each sex). The brain of an uninjected male rat was prepared in the same way as the experimental brains, to check for the presence of autoradiographic artifacts.

The brains were frozen quickly after removal, and 8 μ serial frozen sections were cut in a cryostat, mounted directly onto slides and rapidly dried. Then the tissue was fixed by immersion in 1% osmium tetroxide and 10% neutral formalin. Other details of the histological procedure are described elsewhere[9]. The fixed and dried sections were coated with Kodak NTB-3 emulsion, redried and exposed in lightproof boxes packed with a drying agent, for 11 months at 5 °C. The slides were then developed for 7 min in Kodak D19, cleared in hypo, and some were stained with Mayer's haematoxylin. The numbers of reduced grains over cell bodies were counted in 22 brain regions, including about 100 cells for each brain region in each animal. In other brain regions, uptake was estimated qualitatively, rather than by counting. Care was taken to insure that sampling from brain regions was comparable for all animals.

Radioactive hormone was taken up in cells throughout the female brain $^1/_2$ h after testosterone-^{3}H injection (e.g. Figure). Most limbic-hypothalamic structures showed greater uptake than most non-limbic structures (Table). No such grain reduction was seen over cells in the brain of the uninjected control animal.

In most regions of the female brain, testosterone-^{3}H uptake was much lower 2 h after injection than it was $^1/_2$ h after injection (Table). However, in certain anterior limbic structures – the preoptic area, prepiriform cortex, olfactory tubercle, septum and olfactory bulb granule cells – and in the cingulate gyrus, uptake after 2 h remained high. Prolonged uptake of this sort is characteristic of steroid hormone target structures[10].

Radioactivity was detected in cells throughout the male brain $^1/_2$ h after estradiol-17β-^{3}H injection. Uptake tended to be higher in limbic-hypothalamic structures than in non-limbic structures, but it was not uniformly so (Table). Estradiol-^{3}H uptake after 2 h was high in the preoptic area, prepiriform cortex, olfactory tubercle, septum and cingulate gyrus, a pattern similar to testosterone-^{3}H distribution in the female brain. However, in contrast to the testosterone-^{3}H distribution, estradiol-^{3}H



Autoradiograph showing labeling of 5 cells in the lateral preoptic area of a female rat injected with testosterone-^{3}H. The section is unstained. The clumps of reduced grains are known to be located over cell bodies, from examination of adjacent, stained autoradiograms. × 1200.

[1] F. A. BEACH, *Hormones and Behavior* (Hoeber, Harper & Bros., New York 1948).
[2] F. A. BEACH, Endocrinology *31*, 673 (1942).
[3] F. A. BEACH, Endocrinology *31*, 679 (1942).
[4] J. ALTMAN and G. DAS, J. comp. Neurol. *124*, 319 (1965).
[5] D. W. PFAFF, in manuscript.
[6] A. J. EISENFELD and J. AXELROD, J. Pharmac. exp. Ther. *150*, 469 (1965).
[7] J. KATO and C. A. VILLEE, Endocrinology *80*, 567 (1967).
[8] R. P. MICHAEL, Br. Med. Bull. *21*, 87 (1965).
[9] D. W. PFAFF, Endocrinology *82*, 1149 (1968).
[10] E. V. JENSEN and H. I. JACOBSON, Recent Prog. Horm. Res. *18*, 387 (1962).

Heterologous sex hormone uptake in the rat brain

Brain structures	Testosterone-^{3}H in female brain		Estradiol-17β-^{3}H in male brain	
	Time after injection		Time after injection	
	$^1/_2$ h	2 h	$^1/_2$ h	2 h
Olfactory bulb				
Granule cell layer	22.9 ± 1.8	12.0 ± 1.0	14.0 ± 1.2	3.0 ± 1.9
Mitral cell layer	15.0 ± 1.1	7.2 ± 1.2	25.1 ± 3.9	8.8 ± 0.9
Limbic-hypothalamic system				
Prepiriform cortex	18.0 ± 1.3	15.6 ± 2.3	19.0 ± 1.0	12.7 ± 2.0
Preoptic area	12.3 ± 1.4	15.5 ± 1.7	9.7 ± 2.1	15.8 ± 1.8
Olfactory tubercle	12.9 ± 1.6	13.2 ± 2.1	9.8 ± 1.5	12.8 ± 2.0
Septum	7.4 ± 1.4	11.3 ± 1.6	7.0 ± 0.9	10.0 ± 2.1
Cingulate gyrus	13.8 ± 2.3	11.6 ± 1.1	21.0 ± 1.4	15.1 ± 2.1
Amygdala	29.4 ± 5.1	7.2 ± 0.9	12.2 ± 2.1	10.2 ± 1.5
Hippocampus	17.4 ± 1.3	8.6 ± 1.1	9.3 ± 1.8	3.5 ± 1.2
Ventromedial hypothalamus	18.5 ± 3.0	8.0 ± 0.9	21.3 ± 2.5	9.8 ± 1.1
Entorhinal cortex	15.3 ± 1.6	7.9 ± 0.9	10.9 ± 0.7	3.0 ± 1.0
Non-limbic structures				
Sensory-motor cortex	15.1 ± 2.9	6.8 ± 1.2	16.3 ± 2.1	5.0 ± 2.3
Cerebellar granule cells	10.6 ± 1.7	6.1 ± 0.8	4.9 ± 0.5	2.8 ± 0.9
Superior colliculus	7.8 ± 1.2	3.9 ± 0.8	8.5 ± 1.3	2.4 ± 1.2
Ventrobasal thalamus	6.3 ± 0.8	4.9 ± 1.1	7.7 ± 1.2	4.2 ± 1.2
Spinal cord	6.9 ± 0.9	7.9 ± 1.0	10.4 ± 1.6	1.8 ± 1.6

All results are expressed as average numbers of reduced grains/cell, ± S.E. of the mean.

uptake also remained high in the amygdala and ventromedial hypothalamus 2 h after injection.

The results of this experiment, showing a pattern of longer-lasting (2 h) uptake of testosterone in the female rat brain and estradiol in the male, can be compared with previously determined patterns of uptake for testosterone in the male, and estradiol in the female rat brain. Testosterone-^{3}H is highly concentrated in the male rat brain for at least 3 h by the preoptic area, prepiriform cortex, olfactory tubercle, septum and olfactory bulb and is concentrated to some extent by the cingulate gyrus, prefrontal cortex and caudate[5]. Estradiol-^{3}H is highly concentrated in the female rat brain for at least 2 h by the preoptic area, prepiriform cortex, olfactory tubercle, septum, cingulate gyrus and by some more posteriorly placed limbic-hypothalamic structures, including the amygdala and ventromedial hypothalamus[9].

That is, regions which retain both testosterone in the male brain and estradiol in the female brain are the preoptic area, prepiriform cortex, olfactory tubercle, septum and cingulate gyrus. These are the same regions which retained both testosterone in the female rat brain and estradiol in the male rat brain after 2 h in the present experiment. In addition, long-lasting testosterone uptake in the female olfactory bulb resembles levels of testosterone uptake in the male olfactory bulb; and long-lasting estradiol uptake in the male amygdala and hypothalamus resembles estradiol uptake in the female amygdala and hypothalamus.

Thus, some anteriorly placed limbic structures concentrate and retain either type of sex steroid hormone in either sex brain. These are not structures which have been shown to concentrate substances from the bloodstream indiscriminately, in studies using other substances[11-13]. In contrast to these more 'universal' sex hormone uptake areas, the olfactory bulb apparently shows more affinity for testosterone in either sex animal, and the amygdala and ventromedial hypothalamus show more affinity for estradiol in either sex animal.

In all brain regions and in all animals studied in this experiment, radioactive hormone levels in the brain tissue would appear to be due primarily to levels of uptake by cell bodies, because the concentration of reduced grains over cell bodies was much higher than that outside cell bodies. For both testosterone-^{3}H and estradiol-^{3}H, all identifiable types of nerve cells and glial cells took up the radioactive hormone. There was no evidence for exclusive uptake, or absence of uptake by any particular type of neuron or glial cell[14].

Zusammenfassung. Eine halbe Stunde nach der i.v. Injektion wurde Testosterone-^{3}H von den Nerven- und Glialzellen innerhalb des Gehirns der weiblichen Ratte und Estradiol-^{3}H innerhalb des Gehirns der männlichen Ratte aufgenommen. Zwei Stunden nach der Injektion war die Aufnahme in hohem Masse nur noch in gewissen Teilen des hypothalamisch-limbischen Systems vorhanden.

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Rockefeller University, New York (N.Y. 10021, USA), 16 April 1968.

[11] F. Chevallier and L. Petit, Expl Neurol. *16*, 250 (1966).
[12] L. J. Roth and C. F. Barlow, Science *134*, 22 (1961).
[13] R. J. Wurtman, *Catecholamines* (Little, Brown & Co., Boston 1966).
[14] This investigation was supported by grants from NASA (No. NsG 496) and the John A. Hartford Foundation to Dr. H.-L. Teuber, and from the U.S. Atomic Energy Commission to Dr. J. Altman, both of the Massachusetts Institute of Technology, and the work was performed while the author was at M.I.T.