DNA Synthesis in the Anterior Pituitary of the Male Rat: Effect of Castration and Photoperiod

Abstract. *Castration increased incorporation of tritiated thymidine into total DNA in the anterior pituitary gland. Furthermore, there was a threefold increase in the percentage of labeled basophils 1 month after castration. Exposure of rats to constant light or dark also changed DNA synthesis; these changes depended on age of the animal and on exposure length. The results reflect physiologically induced mitotic activity in specific classes of pituitary cells and further suggest that neuroendocrine mechanisms may be involved in control of cell turnover in the gland.*

In 1941 Pomerat suggested that the increase in numbers of specific types of cells in the pituitary gland observed in rats under different physiological conditions could be accounted for in part by increased rates of mitotic activity in that specific class of cells (*1*). In fact, some studies indicate that incorporation of the [^{3}H]thymidine into pituitary cell nuclei in vivo increases after castration or adrenalectomy or during estrus in the female rat (*2*). However, the precise relation between pituitary mitoses and the endocrine physiology of the animal is still not well established, probably because of the extremely low turnover of these cells (*3*). Short-term incubation of pituitaries in vitro provides a convenient way to assay incorporation of [^{3}H]thymidine into total DNA in the pituitary. This incorporation reflects mitotic activity and decreases as the animals get older, and approximately 20 percent of the labeled cells are either acidophils or basophils (*4*). Moreover, we have identified labeled somatotropes, gonadotropes, thyrotropes, and chromophobes by quantitative electron microscope radioautography, and have found that the labeling indices obtained by the two methods agree very well (*4*).

Castration or changes in photoperiod affect hormone synthesis and release from the pituitary. We report here that prior castration or prior exposure to continuous light or dark, or both, also changes the incorporation of [^{3}H]thymidine into pituitary DNA and causes shifts in numbers of cell types labeled with [^{3}H]thymidine as well.

Male Holtzman rats (40 to 42 days old, 140 to 160 g) (Madison, Wisconsin) were maintained for 1 week before castration or sham operation. At various periods thereafter, the rats were killed, whole anterior pituitary glands were incubated for 3 hours in Medium 199 containing [^{3}H]thymidine, and DNA synthesis was measured biochemically and radioautographically (*4*).

Incorporation of [^{3}H]thymidine into DNA of pituitaries from castrate rats significantly increased 5 days after the operation (Table 1). This increase was maintained, relative to sham-operated controls of the same age, over the entire period after castration (1 to 4 weeks). Total DNA content per gland was not significantly different than control values 1 week after castration, but increased 18, 12, and 23 percent 2, 3, and 4 weeks, respectively, after the operation. Kraicer reports a 20 percent increase in DNA content per gland in pituitaries taken from animals 32 days after castration (*5*).

Distribution analyses of cell types labeled with thymidine 1 month after castration reveals a threefold increase in the percentage of labeled basophils (Table 2) (labeled castration cells were occasionally encountered). This result correlates well with that of Pomerat who found by differential cell counts that the total number of acidophils decreased slightly while the basophils doubled in the first 2 to 4 weeks after castration (*1*). The increased DNA synthesis in pituitary basophils after castration appears consistent with other cytologic evidence of increased cell number and average cytoplasmic mass per cell leading to higher amounts of gonadotropins (*5*).

In addition to castration, modification of environmental lighting conditions also alters synthesis and release of pituitary hormones, especially the gonadotropins. Rats maintained in constant darkness show delayed onset of sexual maturity, while those kept in constant light show the opposite (*6*). Other results indicate that animals maintained in constant light show increased synthesis and release of gonadotropins, while those in the dark show the opposite effect (*7*). In the adult male rat, there is a diurnal rhythm in the amounts of luteinizing hormone and follicle-stimulating hormone stored in the pituitary; a higher gland content of both hormones is found in late after-

Table 1. Effect of castration on DNA synthesis in male rat anterior pituitary. The results are expressed as disintegrations per minute (dpm) per microgram of DNA (mean ± SEM). The animals were maintained on an LD 12 : 12 photoperiod and were castrated when they were 48 days old. The numbers in parentheses are the number of pituitaries used. Not significant, NS.

Days after castration	DNA synthesis (dpm/μg DNA)		P*
	Noncastrates	Castrates	
1	1701 ± 28 (4)	1694 ± 86 (3)	NS
3	1152 ± 159 (8)	1298 ± 76 (12)	NS
5	991 ± 102 (8)	1293 ± 57 (8)	$<.05$
7	748 ± 43 (12)	1016 ± 47 (12)	$<.001$
11	623 ± 52 (8)	1038 ± 160 (8)	$<.05$
14	578 ± 58 (16)	977 ± 56 (14)	$<.001$
21	417 ± 35 (12)	676 ± 50 (12)	$<.001$
28	333 ± 20 (12)	602 ± 50 (8)	$<.001$

* Student's t-test.

Table 2. Labeling indices and distribution of label among cell types in pituitaries removed from (i) male rats castrated 14 or 28 days and (ii) noncastrate male rats exposed to cyclic light (LD 12 : 12), constant light (LD 24 : 0), or constant dark (LD 0 : 24), for 14 days. The labeling indices (number of cells labeled per 100 cells counted ± SEM) were obtained by counting 30,000 cells per two to three glands per treatment.

Treatment	Days	Photoperiod	Labeling index	Labeled cell type/total labeled nuclei (%)		
				Acidophils	Basophils	Chromophobes
Noncastrate	14	LD 12 : 12	0.51 ± .04	13.7	5.7	80.6
Castrate	14	LD 12 : 12	.90 ± .09	13.3	6.7	80.0
Noncastrate	28	LD 12 : 12	.38 ± .06	13.7	4.1	82.2
Castrate	28	LD 12 : 12	.68 ± .17	6.1	14.9	78.6
Noncastrate	14	LD 12 : 12	.51 ± .04	13.7	5.7	80.6
Noncastrate	14	LD 24 : 0	.32 ± .02	24.2	2.3	73.5
Noncastrate	14	LD 0 : 24	.36 ± .09	15.4	12.2	72.4

Table 3. Synthesis of DNA in pituitaries removed from noncastrate and castrate animals after exposure to cyclic light (LD 12 : 12), constant light (LD 24 : 0), or constant dark (LD 0 : 24), for 3 days to 4 weeks. All animals were 48 to 50 days old when placed in constant light or constant dark. Those values (marked with an asterisk) which are significantly different ($P < .01$) when compared with the cyclic photoperiod are: noncastrates, light versus cyclic light (14 days) and dark versus cyclic light (7 and 14 days); castrates, dark versus cyclic light (7, 14, and 21 days).

Exposure time (days)	Animals per group (No.)	DNA synthesis (dpm/μg DNA)		
		LD 12 : 12	LD 24 : 0	LD 0 : 24
		Noncastrate		
3	4	832 ± 62	965 ± 63	790 ± 43
7	4	636 ± 42	654 ± 44	428 ± 14*
14	8	407 ± 38	266 ± 13*	220 ± 15*
21	4	392 ± 43	414 ± 22	350 ± 26
28	8	252 ± 19	332 ± 22	293 ± 56
		Castrate		
3	6	1316 ± 63	1138 ± 90	1351 ± 100
7	6	1084 ± 49	1030 ± 46	742 ± 72*
14	4	814 ± 45	831 ± 43	602 ± 22*
21	4	636 ± 21	784 ± 59	754 ± 21*

noon (*8*). A diurnal cycle for storage and release of pituitary thyroid-stimulating hormone, prolactin, and adrenocorticotropin is also suggested from other studies (*9*).

To assess the possible effect of different photoperiods on pituitary DNA synthesis, we exposed newly castrated or sham-operated animals to cyclic light [12 hours light, 12 hours dark (LD 12:12)], continuous light (LD 24:0) or continuous dark (LD 0:24) for 3 days to 4 weeks. Continuous light was produced by one 150-watt incandescent bulb placed 1.0 m above the cage floor. No significant changes in cage temperature were noted. All animals were killed between 11:00 and 11:45 a.m., Eastern Standard Time. Incorporation of [^{3}H]thymidine into pituitary DNA changed significantly, especially in the animals exposed to dark only (Table 3). After 7 days of exposure to LD 0:24, a 33 percent decrease in DNA synthesis in pituitaries from castrates and noncastrates was repeatedly observed. This depressive effect of the dark was also found in the groups exposed for 14 days (castrate, 26 percent; noncastrate, 46 percent), but was not encountered in animals exposed for longer periods. A 35 percent depression in the noncastrates exposed to light for 2 weeks was also observed. No consistent changes in DNA content per gland were found in any of these treatments.

Exposure of noncastrate animals to 2 weeks of continuous light or dark also resulted in changes in distribution of thymidine-labeled cell types (Table 2). A twofold increase in the percentage of labeled basophils was found in the group exposed to dark, while a twofold increase in percentage of labeled acidophils was observed in the group exposed to light. Furthermore, the percentage of labeled basophils decreased in response to constant light.

Because the fluctuating responses seen in animals maintained for longer periods in the light or dark could reflect additional age-dependent changes (*10*), we looked at the response of adult animals of different ages to a constant exposure period (1 week).

The depressive effect of the dark was consistently seen in animals castrated when they were 48 days old and then maintained in an LD 12:12 cycle for 1, 2, or 3 weeks before exposure to total dark. However, exposure of noncastrate rats (55 to 69 days old) to constant light or dark for 1 week caused no significant changes in total DNA synthesis in the pituitary. This result may account for the lack of effect seen in the normal animals maintained in light or dark for 3 and 4 weeks (Table 3).

It seems possible that alterations in secretion rates of pituitary hormones brought about by modification of environmental lighting might explain some of the observed differences in DNA labeling. For example, decreased release of pituitary gonadotropins in animals exposed to dark could result in cell division in some basophils. In fact, differential cell counts in pituitaries taken from rats exposed to constant light or dark for 1 month or 1 year showed that the most striking and repeatable change was a doubling of the basophils in rats kept in darkness for 1 month (*10*).

Selective enhancement of mitotic activity in thyroidectomy cells of the mouse pituitary after radiothyroidectomy has been reported, as determined by electron microscopic radioautography (*11*). Our results also reflect physiologically induced changes in mitotic activity within certain classes of pituitary cells and suggest neuroendocrine control of cell turnover in the gland.

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References and Notes

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Countercurrent System in the Renal Cortex of Rats

Abstract. *Tubules on the surface of the renal cortex are intertwined with the capillaries. Micropuncture experiments on rats show that generally the flow of tubular fluid is against the flow of blood in the peritubular capillaries. A countercurrent flow system, therefore, exists not only in the renal papilla but also in the superficial cortex.*

Although little doubt remains today about the existence of the countercurrent system in the renal papilla (*1*), its physiological mechanisms are still under discussion. Recent work indicates a countercurrent system also exists in the superficial renal cortex of rats (*2*). Here, in contrast to the papilla, the countercurrent system involves tubular (urine) flow and peritubular (blood) flow. We report our results of studies concerning that system. In order to demonstrate it, we combined incident-light photomicrography, micropuncture techniques, and the Lissamine green (LG) method as previously described (*3*). We used male Wistar rats (body weight, 250 to 300 g) which we anesthetized with Inactin (sodium pentobarbital). The decapsulated kidney was rinsed with physiological saline or mineral oil at body temperature.

In an arbitrary field viewed under the microscope (see Fig. 1) we first photo-

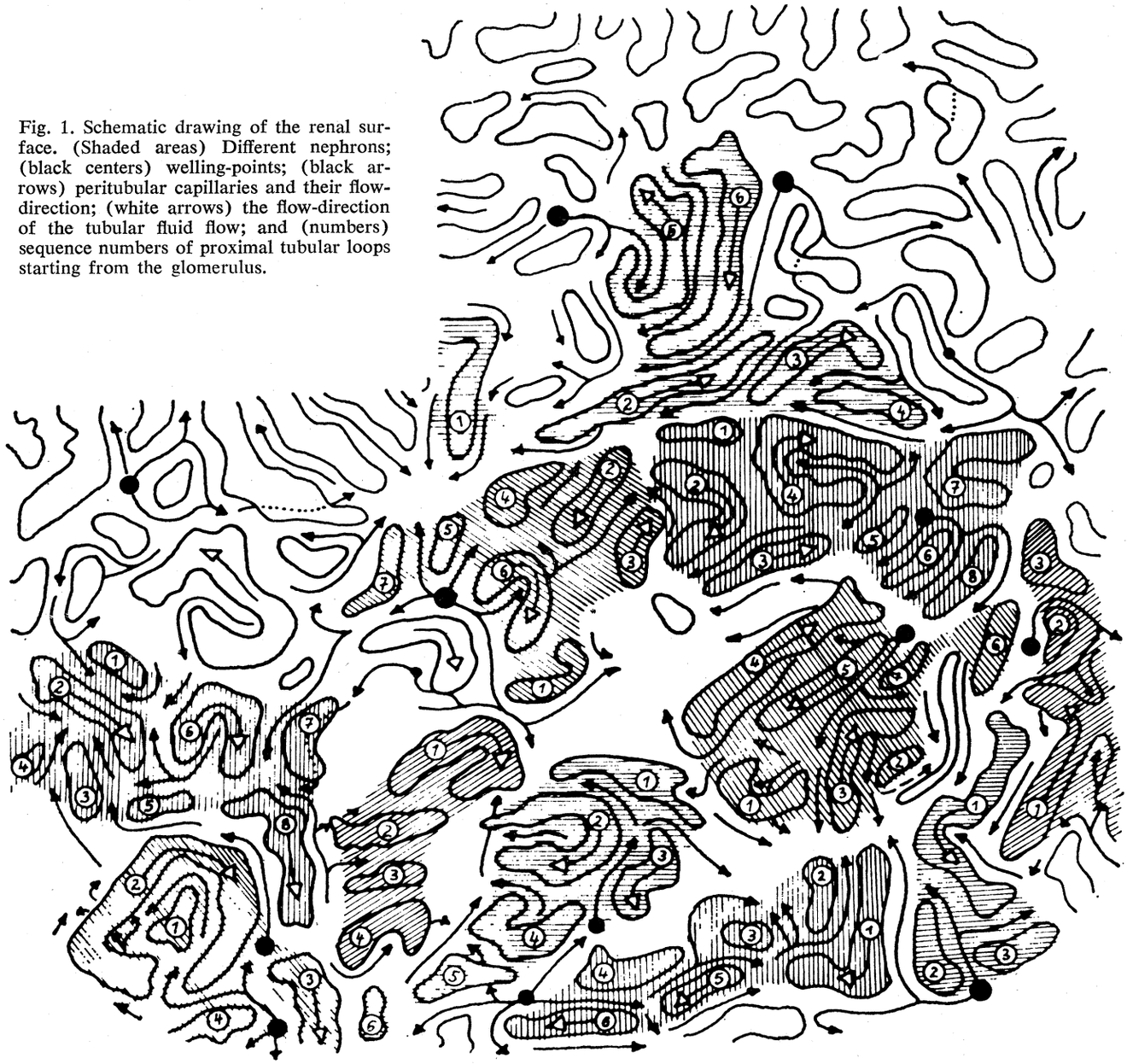

Fig. 1. Schematic drawing of the renal surface. (Shaded areas) Different nephrons; (black centers) welling-points; (black arrows) peritubular capillaries and their flow-direction; (white arrows) the flow-direction of the tubular fluid flow; and (numbers) sequence numbers of proximal tubular loops starting from the glomerulus.