

Cell Proliferation in the Rat Pituitary.* (30127)

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There are few precise observations on factors that influence cell growth dynamics in the anterior pituitary. This may be partly due to the fact that conventional counts of the mitotic index lack sensitivity in organs, such as pituitary, which have a low rate of cell division. A more accurate assessment can be obtained by administration of tritiated thymidine to label nuclei in the DNA synthetic phase of the nuclear cycle. The present results are from rats with various forms of experimental endocrine hypertension in which the nuclei utilizing ^3H -thymidine for DNA synthesis were visualized by autoradiography(2,3). Hypertension was produced in sensitized rats (unilateral nephrectomy, high NaCl load) with desoxycorticosterone (DOC), cortisone, or by adrenal regeneration(4,5).

Materials and methods. Sprague-Dawley rats were maintained in individual cages at 24-26°C. Some were tube-fed a medium carbohydrate diet (MCOH) containing a normal or 4% content of sodium chloride (Table I). Others had a standard Archer pellet diet to eat and 1% NaCl to drink *ad libitum*.

The right kidney was removed from all rats by clean surgical technique. Mature males fed the low and high NaCl diet were given either 3 mg desoxycorticosterone acetate or 5 mg cortisone acetate subcutaneously each day for 6 weeks. Appropriate dietary controls were maintained (Table II). Adult and immature females had the right adrenal removed and were given pellets and 1% saline for 6 weeks. In some the left adrenal was enucleated at the same time(5) and allowed to regenerate for a similar period. The blood pressure was measured in the lightly etherized animal by direct manometry using heparinized saline as the anticoagulant.

Tritiated thymidine (1 $\mu\text{C/g}$ final body weight) was injected intraperitoneally. A 4-hour labeling time (9 a. m.-1 p. m.) was used. At autopsy the relevant organs were fixed in 4% neutral formaldehyde after weighing. Autoradiographs were prepared from 5 μ paraffin sections with stripping film from Kodak AR 10 plates. The exposure time was 6 weeks at 4°C in dry air. The image was developed with Kodak D 19 b and sections were stained through the film with 1% aqueous neutral red. Approximately 1500 epithelial nuclei were counted in each pituitary and the percentage that were radioactive was calculated.

Results. The results are in Table II. The anterior pituitary of mature male rats tube-fed the MCOH diet contained approximately 2 labeled nuclei per 1000 epithelial cells (label index 0.21). Radioactive nuclei showed a fairly uniform distribution throughout the gland. Further labeling was seen in the flattened nuclei of capillary endothelium but cells of this type were excluded from the counts. Addition of 4% NaCl to the diet or administration of DOC to rats on MCOH caused minor shifts in pituitary labeling in different directions (0.1 and 0.29 respectively). A marked rise in blood pressure with severe hypertensive cardiovascular-renal

TABLE I. Medium Carbohydrate Diet.

Constituent	Grams
Cellu flour (Chicago Dietetic Supply House)	60
Osborne and Mendel salt mixture	.40
Dried yeast (Pabst)	100
Wheat germ oil	10
Cod liver oil	10
Vitamin K	.1
Mazola oil	200
Casein (Labco)	160
Starch	200
Dextrin	190
Sucrose	200

Water to a total volume of 2000 ml. High salt diet contains 4% NaCl as a percentage of the dry constituents.

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TABLE II. Effect of Age, Sex, Diet and Hormones on Numbers of DNA-Synthesizing Nuclei in Rat Anterior Pituitary. Means and standard error.

Exp	No. of rats	Sex	Body wt (g)		B.P. (mm/Hg)	Left adrenal (mg)	Thymus (mg)	Nuclei per pituitary sample		Pituitary label index
			Initial	Final				Total	Labeled	
MCOH	7	♂	285 ± 1	368 ± 3.6	134 ± 1.75	36.6 ± 2.12	475 ± 35	1508 ± 39	3.14 ± .59	.21 ± .04
4% NaCl MCOH	5	♂	277 ± 2.7	354 ± 5.4	141 ± 3.7	38.2 ± 1.1	392 ± 26	1797 ± 95	2.2 ± .97	.1 ± .05
DOC, MCOH	6	♂	280 ± 0	384 ± 3.8	152 ± 4.3	26 ± 1.03	507 ± 37	1572 ± 37	4.5 ± 1.2	.29 ± .08
" 4% NaCl MCOH	6	♂	283 ± 4	358 ± 3	177 ± 5.1	29.8 ± .95	332 ± 20	1628 ± 129	15.17 ± 2.86	.92 ± .13
Cortisone, MCOH	6	♂	274 ± 3	254 ± 5.7	147 ± 3.6	14.8 ± .83	22 ± 1.5	1769 ± 76	.67 ± .33	.038 ± .02
" 4% NaCl MCOH	7	♂	277 ± 2.4	237 ± 5.2	148 ± 5.5	10.3 ± .7	27 ± 2.7	1532 ± 85	.29 ± .18	.016 ± .009
Uni-adrenx, 1% NaCl	6	♀	81 ± 1.8	184 ± 5.4	136 ± 2.7	43.8 ± 3.1	312 ± 14.9	1229 ± 128	14.67 ± 1.93	1.27 ± .24
Adren-regen, 1% NaCl	9	♀	78 ± .7	179 ± 7.5	193 ± 8.5	38.2 ± 1.04	351 ± 53.8	1258 ± 79	11.67 ± 2.24	.98 ± .2
Uni-adrenx, 1% NaCl	6	♀	247 ± 3.7	258 ± 3.5	128 ± 3.4	49.5 ± 2.4	182 ± 6.5	1752 ± 108	9.83 ± 3.95	.55 ± .21
Adren-regen, 1% NaCl	7	♀	252 ± 3.5	272 ± 4.35	147 ± 4.7	50 ± 3.5	167 ± 7.5	1704 ± 147	6.57 ± 2.82	.35 ± .13

disease resulted when DOC was administered to rats on high salt diet(2). In these circumstances, pituitary cell labeling was more frequent and the index rose to 0.92 ($t = 5.349$; $p < 0.001$).

Cortisone caused marked thymic and adrenal atrophy but the rise in blood pressure was less than produced by DOC. There was significant depression of pituitary labeling in rats given cortisone and MCOH (mean 0.038; $t = 3.659$; $p < 0.01$), and addition of 4% NaCl to the diet caused a further fall (mean 0.016; $t = 4.863$; $p < 0.001$). This effect of NaCl was also noted in rats given MCOH diet without hormone.

Female rats used for adrenal regeneration experiments showed higher indices of pituitary labeling. The highest index (1.27) was by young females with a single adrenal hypertrophied 6 weeks after unilateral adrenalectomy. Regenerating adrenal cortical tissue in young females caused severe hypertension and a slight fall (mean 0.98; $t = 0.919$; $p > 0.3$) in the number of labeled pituitary cells. The pituitary label index was lower in mature female rats and lowest in those with regenerating adrenals, although the hypertension was slight. In all female animals the scatter of labeling indices in any one experimental group, and the corresponding standard errors of the mean (Table II), were greater than in male rats. This variability did not correlate with level of the blood pressure, presence or absence of cardiovascular-renal disease, or the adrenal state, but appeared to relate to the frequency of DNA-synthesizing nuclei in the generative organs, for the lowest pituitary label indices were in those females with the lowest frequency of labeled nuclei in the endometrium and uterine muscle.

In all groups labeling of cells in the posterior pituitary lobe was of a much lower order and no counts were made.

Discussion. Although the frequency of mitotic division in the anterior pituitary is low, the thymidine autoradiographic technique is sufficiently sensitive to make reliable assessments of pituitary cell turnover but does not permit separate analysis of the contribution made by individual pituitary

cell types to the labeled population.

Age, sex, and hormonal state are some of the factors that influence the numbers of anterior pituitary nuclei in DNA synthesis. Female rats had higher labeling indices than males and young females showed more frequent pituitary labels than adult females with their growth phase completed. Cortisone treatment of adult males produced a 6-fold depression in the labeling index of the anterior pituitary. Previous studies with cortisone or hydrocortisone have shown a similar reduction in the numbers of DNA-synthesizing nuclei in a variety of tissues including the zona fasciculata of the adrenal, articular and growth-plate cartilage(6) and various cells of the cardiovascular-renal system(2). An exception is the pancreas, for when steroid diabetes is produced the islet tissue increases in bulk and thymidine labeled nuclei in islet cells become more numerous(1).

DOC has no comparable effect on pituitary labeling but when it is combined with an increased dietary load of sodium chloride in unilaterally nephrectomized rats and hypertension is produced there is a significant increase in the pituitary labeling index. This is coupled with an increase in the numbers of DNA-synthesizing nuclei in the hypertensive damage that occurs in the heart, arteries, and kidneys of such rats(2). It is not clear that this alteration in pituitary cell turnover represents a response to the increased blood pressure, for when a severe degree of hypertension was established by the adrenal regeneration procedure of Skel-

ton(5) the general level of pituitary labeling is decreased as compared with control animals. A comparison of the effect of hypertension in these circumstances is not necessarily valid since the age and sex of the 2 groups of hypertensive rats differed, and these factors and the stage of the oestrus cycle seem to have important effects on the magnitude of cell turnover in the anterior pituitary.

Summary. The numbers of nuclei in the DNA synthetic phase of the nuclear cycle were estimated in the rat anterior pituitary by ^3H -thymidine autoradiography. Age, sex, and hormonal state produced significant shifts in the labeling frequency. Radioactive nuclei were more numerous in young females and less frequent in adult males. The low labeling frequency in adult male rat pituitary was further depressed by cortisone administration.

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Effect of Alternating Acid and Alkaline Digestive Fluids on the Gastrointestinal Tract of the Rat.* (30128)

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The formation of gastric and duodenal ulcer in man is usually attributed to effects of acid-pepsin on susceptible areas. A few authors have suggested that tryptic origin of ulcer may be possible(1-3). In earlier experi-

ments one of us has found that a frog's leg exposed for a short period of time to acid or

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