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Blood Flow in Diethylstilbestrol-Induced Anterior Pituitary Gland Hyperplasia (42434)

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Abstract. Anterior pituitary hyperplasia was developed in female Fisher 344 rats by subcutaneously implanted slow-release diethylstilbestrol (DES) capsules. Blood flow was measured in two separate areas of the adenohypophysis using hydrogen clearance method at 6, 9, 10, and 13 weeks after the implantation. Blood flow progressively decreased while the DES capsules were in place (normal values in ml/g/min, mean ± SD: 0.93 ± .12 laterally and 1.15 ± .11 medially, decreasing to 0.25 ± .07 and 0.24 ± .07, respectively, by 13 weeks). Histology confirmed nodular hyperplasia, development of large vascular lakes, and hemorrhages within the adenohypophysis. Total adenohypophysial blood flow was calculated as a product of mean blood flow as measured per unit weight and the weight of the gland. This figure progressively increased from 12.7 ± 2 to 22.2 ± 8 μl/min by 13 weeks. All these changes were significant at the $P < 0.001$ level. These blood flow measurements suggest that the hyperplastic adenohypophysis outgrows its blood supply which is additionally compromised by "useless" pooling of blood in the vascular lakes. However, there is an overall increase in the amount of blood flowing through the hyperplastic gland which may be explained by newly formed supplying vessels.

It has long been recognized that prolonged estrogen treatment causes hyperplasia and adenomatous changes in the adenohypophysis (1–3). The mechanism of this tumor induction is still not fully understood. One of the common explanations is the increased mitotic activity among prolactin (PRL)-secreting cells elicited by the estrogen (4, 5). A second mechanism would be an alteration in the hypothalamic regulation of PRL cells. Estrogen-uptake sites were shown in the tuberoinfundibular dopaminergic (TIDA) cells (6) and it has been suggested that estrogen suppresses the inhibitory action of these cells (7), possibly indirectly *via* increasing PRL production (8). Such suppression would lead to uncontrolled PRL production and proliferation of PRL cells. A third mechanism could be the development of an abnormal blood supply to the anterior lobe with effective circumvention of the portal system. This would provide the adenohypophysis with low-dopamine systemic blood and allow it to escape the tonic hypothalamic inhibition. Extensive vascularization of the dura covering the anterior pituitary after estrogen treatment was described (9) and it was demonstrated by microsphere technique to be arterial (10).

Previously it was shown that female Fisher 344 (F344) rats were especially susceptible to develop various types of cancer (11) and pituitary adenomas (12) on estrogen induction. It is this experimental model that was used in this study. The present paper describes the changes of the blood flow in the adenohypophysis of F344 rats after prolonged diethylstilbestrol treatment.

Material and Methods. *Animals.* Female Fisher 344/01a rats ($N = 79$) were housed in translucent cages in a thermostabilized room with a 12:12-hr photoperiod. Standard laboratory food and tap water were provided *ad libitum;* 30 rats comprised the control group. The remaining 49 rats were implanted at the age of 4 weeks under ether anesthesia with diethylstilbestrol (DES; Sigma Chemical Co., St. Louis, Mo.) containing Silastic capsules. These were prepared according to (13) and were estimated to provide 18–45 μg DES daily. The implantation was staggered to provide 2 rats daily for the measurements described below. The capsules were left in place until the measurements, i.e., 6, 9, 10, and 13 weeks in 13, 12, 8, and 11 rats, respectively. The remaining 5 rats had the capsule *in situ* for 9 weeks when it was removed. The measurements in this

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group were performed 13 weeks after the original implantation.

Measurements. On the day of the experiment the rats were weighed and anesthetized with intraperitoneal injection of 25% urethane solution (1.25 g/kg). Body temperature was maintained by a heated operating board. The femoral arteries and veins were cannulated; Hartmann solution was given intravenously (1.5 ml/hr) to maintain good hydration throughout the experiment. Blood pressure was monitored and recorded via the femoral artery. Tracheostomy was performed and spontaneous ventilation maintained through the tracheostomy tube. The pituitary gland was exposed using the parapharyngeal route and Teflon-coated platinum wire electrodes (0.125 mm in diam with 0.2-mm bare tips) were placed, one in the midline and another about 2 mm laterally, in the adenohypophysis. The operation was performed under an operating microscope (OPMI 6-SF, Zeiss, West Germany).

Blood flow was measured using the hydrogen clearance method. The approach and the experimental arrangements were described in detail elsewhere (14). When the clinical state was stable with arterial pH and blood gases within normal limits, multiple blood flow measurements were taken. The rats were then decapitated, and the anterior pituitary gland was dissected, weighed, and retained for histology.

Histological methods. The pituitary glands were fixed in buffered Formalin and processed for paraffin sections. Sections (5 μ) of the glands were stained with hematoxylin and eosin, PAS–orange G, Gomori's stain for reticulin, and a Perl's stain for iron. Small pieces of tissue were fixed in 2% glutaraldehyde in cacodylate buffer and processed for electron microscopy.

Statistics. The statistical significance of differences observed among various groups was determined with one-way analysis of variance (ANOVA) and two-sample Student's t test.

Results. *Body weight.* The growth of the rats slowed down after the implantation and their weight leveled with that of the normal 6-week-old rats (Fig. 1). The weight of the anterior pituitary progressively increased up to eight times the control by 13 weeks (Table I).

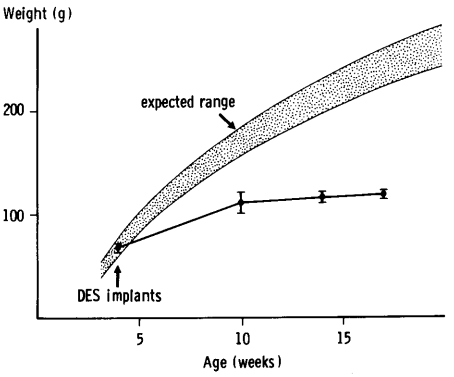

FIG. 1. Body weight of normal and diethylstilbestrol (DES)-treated Fisher 344 rats. The bars represent means and SD for each age group. Growth is retarded after the DES implantation.

Blood pressure. Mean arterial pressure was 102.6 ± 16 mm Hg (1 mm Hg = 133.322 Pa) in control rats. A slight hypertension was noted in the estrogen-treated rats which was not progressive (about 18% increase by 13 weeks, Table I).

Blood flow in the adenohypophysis. Significant reduction was noted in both areas by 70% (Fig. 2). The normally registered difference between blood flow of the medial and lateral parts disappeared with estrogen treatment. Total blood flow (the product of the blood flow as measured per unit weight and the weight of the adenohypophysis) represents the amount of blood flowing through the adenohypophysis. This parameter significantly increased under DES treatment (Table I).

Histology. Compared with normal controls all the glands examined showed diffuse chromophobic cellular hyperplasia with increased mitotic activity. At each stage apparently half the glands showed well-demarcated nodules consisting of prominent vascular spaces separated by cords of epithelial cells. At electron microscopy the majority of cells were identified as prolactin cells with their characteristic pleomorphic granules, abundant rough endoplasmic reticulum, and granule secretion by exocytosis. Reticulin staining at 6 weeks after implantation showed compression of the sinusoids but at later stages open sinuses were present in addition to increasing numbers of vascular lakes associated with hemorrhage

TABLE I. THE EFFECT OF DIETHYLSTILBESTROL (DES)-CONTAINING CAPSULES ON MEAN ARTERIAL BLOOD PRESSURE (MABP), TOTAL ADENOHYPOPHYSIAL BLOOD FLOW (TPBF), AND THE WEIGHT OF THE ANTERIOR PITUITARY LOBE

	Time after implantation (weeks)							
	Control	6	9	10	13	*F* ratio	*df*	*P*
MABP (mm Hg)	102.6 ± 16	—	120.8 ± 18	120.6 ± 23	121.1 ± 14	5.27	57	<0.01
TPBF (μl/min)	12.7 ± 2	17.9 ± 5	18.5 ± 9	18.1 ± 6	22.2 ± 8	10.77	66	<0.0001
Pituitary weight (mg)	11.5 ± 3	34.4 ± 8	51.8 ± 22	80.0 ± 34	92.5 ± 32	47.97	67	<0.0001
Number	30	13	12	8	11			

Note. Values are means ± SD.

and increasing hemosiderin deposition. At 10 and 13 weeks after implantation occasional glands showed small areas of infarction.

Effects of removal of estrogen induction. From five rats the capsules were removed on the ninth week and the measurements were performed 4 weeks later. These rats remained hypertensive (132.5 ± 16 mm Hg, $P < 0.001$ against control) but resumed growth (body wt: 157 ± 8 g, $P < 0.001$, compared with those receiving DES for the full 13 weeks). The weight of the pituitary gland was reduced (53 ± 19 mg, $P < 0.05$, compared with those with continuous DES treatment). Pituitary blood flow increased (0.55 ± .19 ml/g/min laterally and 0.60 ± .27 ml/g/min medially; significantly different from both control ($P < 0.001$) and the continuous DES treatment group ($P < 0.01$)). Total pituitary blood flow was more than twice the control (28 ± 9 μl/min, $P < 0.001$). Histological examination of these glands showed some return to the normal sinusoidal vascular pattern. There was less cellular hyperplasia with no mitotic figures. Hemorrhages were, however, still apparent with many siderophils and in some areas secondary fibrosis had occurred.

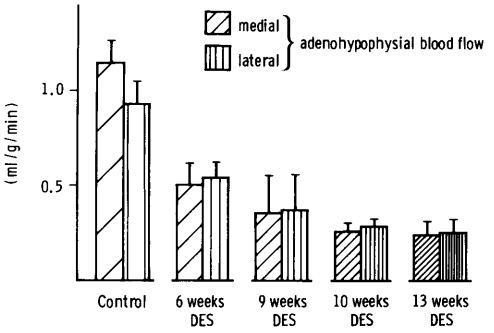

FIG. 2. Blood flow in the adenohypophysis of F344 rats with different lengths of diethylstilbestrol (DES) treatment. The columns represent means ± SD of a total of 79 rats. DES reduced blood flow both medially and laterally. There was a larger reduction in the medial region, thus abolishing the normally measured difference between these two regions. The changes were significant in both regions (lateral: $F(72) = 111.18$, $P < 0.0001$; medial: $F(73) = 204.16$, $P < 0.0001$).

Discussion. In concert with earlier results the implantation of diethylstilbestrol in slow-release Silastic capsules led to nodular hyperplasia and in the anterior lobe of the pituitary gland in F344 rats (13). Large "vascular lakes" were caused by hemorrhages and cavernous change in the capillaries. It was shown previously in human pituitary adenomas that there is a breakdown of the pericapillary–parenchymal interface (15) which causes extravasations. The development of vascular lakes led to stagnation of the blood which may contribute to the increased volume of the gland.

Blood flow in these glands progressively decreased with estrogen treatment which is in good correlation with earlier reports in a less susceptible rat strain (16). This results in relative ischaemia in the tissue as the tumor outgrows its blood supply which may explain the degenerative changes and occasional necrosis seen in our material.

As it was shown by other authors, dural vessels enter the adenohypophysis of long-term estrogen-treated rats (10, 9). The arteriogenesis provides extra supply to the compromised adenohypophysial circulation. This blood may

protect the outer parts of the tumor from ischaemia but being of systemic origin it lacks the high dopamine concentration of the portal blood. This mechanism may accelerate the growth of the gland. Our figures of increased total blood flow in the anterior lobe would be consistent with this theory, though the source of this additional blood cannot be determined by our method. The microsphere technique applied by other authors proved that it did not come through the portal vessels (10). This aberrant lateral blood supply may be reflected in the smaller fall of the lateral than the medial PBF under the influence of estrogen.

Withdrawal of the estrogen induction resulted in partial involution of the enlarged pituitary as was noted by previous authors (17). Histological examination of our material demonstrated partial restoration of the normal sinusoidal vascular pattern. The reduction of size and number of the vascular lakes may explain the improved blood flow as measured by the hydrogen clearance method. The recovery is, however, not complete, at least not so by 4 weeks following the removal of the pellets. It was shown in earlier studies (17) that plasma PRL level does not return to normal following removal of the estrogen induction. It is possible that this is due to persistent damage to the TIDA neurons inflicted by the estrogen. It is also possible, however, that the aberrant low dopamine-containing blood still reaches the anterior pituitary, allowing the lactotroph cells to maintain high PRL production (9). This would be supported by the high total pituitary blood flow found despite removal of the DES capsule in this study.

These results draw further attention to the possible role of vascular changes in the natural history of pituitary adenomas.

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