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Resistance of W/Fu Rats to Adrenal Regeneration Hypertension[1] (38978)

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Skelton (1) described a severe cardiovascular disease in young female rats that were unilaterally nephrectomized and adrenalectomized with enucleation of the contralateral adrenal gland. This disease was accompanied by severe cardiovascular lesions in the heart, kidney, brain, and blood vessels. The pathogenesis of the hypertension is related to the increased secretion of 11-deoxycorticosterone (2, 3), a potent mineralocorticoid and hypertensinogenic agent (4).

The cardiovascular disease is inducible in Sprague–Dawley rats of the Holtzman strain (1) or of the Charles River strain (5) and has been attempted in Wistar animals as well (6). Previous studies have shown a difference in susceptibility of various strains of rats to the development of other forms of hypertension (7).

It was the purpose of the present investigation to examine the inducibility of hypertension in an inbred strain of rats (W/Fu) developed by Furth (8). This strain of rats has a high incidence of spontaneous pituitary and mammary tumors as well as leukemias; these neoplasms are readily transplantable in this strain of animals.

Materials and methods. Twenty-four rats weighing between 150 and 155 g were obtained from Holtzman Breeding Company, Madison, Wisconsin. Twenty-four female rats weighing 130–135 g of the W/Fu strain were obtained from Microbiological Associates, Walkersville, Maryland. All animals were accustomed to the laboratory for 1 wk before the experiment was begun.

Animals were divided equally into four groups. Groups 1 and 3 were controls of the Holtzman and W/Fu strains, respectively. Control animals were uninephrectomized and uniadrenalectomized on the right side. Groups 2 and 4 were uninephroadrenalectomized on the right side and received contralateral adrenal enucleation according to the procedure of Skelton (1). All animals received lab chow and 1% sodium chloride as drinking fluid *ad libitum*. Body weight and blood pressure were recorded at weekly intervals in lightly anesthesized animals. Animals were sacrificed 5 weeks after the operation. Organs were removed and placed in 10% neutral buffered formalin before being weighed. The adrenal gland was fixed in 3% purified glutaraldehyde (Ladd Research Industries, Burlington, Vermont) buffered to pH 7.3 with 0.1 *M* phosphate. After 4 hr, the various zones of the hypertrophic adrenal glands in Groups 1 and 3 were obtained as described previously (9). In the regenerating adrenals, the outer as well as inner portions of the regenerating adrenal gland were obtained. Tissues were processed as described previously (9).

Results. The body weight of all animals increased progressively (Table I). Systolic blood pressure of W/Fu animals (Groups 1 and 2) did not increase appreciably. With the Holtzman strain of animals, there was an increase in blood pressure to the hypertensive range by the end of 5 weeks (Table I). However, the basal blood pressure of Holtzman controls was significantly higher than that of W/Fu animals.

The relative weights of heart and kidneys in Groups 2 and 4 became significantly greater than that in controls. In W/Fu animals, the relative weight of the regenerated adrenal gland was significantly less than that of the corresponding control (Group 1). With Holtzman animals however, the weight of regenerated adrenal gland in Group 4 did not differ significantly from

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TABLE I. ADRENAL REGENERATION HYPERTENSION IN (ARH) HOLTZMAN AND W/FU RATS.

Group	Body weight		Systolic blood pressure		Heart[c]	Kidney	Spleen	Adrenal	Thymus
	Initial	Final	Initial	Final					
Holtzman controls	153±2[a]	210±4	100±3	120±4	410± 9	750±47	276±10	23±2	173±12
Holtzman ARH	154±3	208±5	103±2	150±5[b]	470±13[b]	935±67[b]	323±12[b]	25±5	180±10
W/Fu controls	132±2	173±2	80±3	85±3	379± 5	595±33	260± 6	26±2	133± 5
W/Fu (ARH)	130±2	186±2	80±3	95±3	422± 5[b]	782±27[b]	296± 7[b]	18±1[b]	162±10[b]

[a] Mean ± SEM.
[b] $P < 0.001$.
[c] Mg/100 g body wt.

that in Group 3. There was no significant difference in weights of the thyroid, ovary, pituitary, or liver (Table I). Splenic weight, however increased in animals bearing regenerating adrenal glands. There was no significant difference in the weight of the thymus in Holtzman animals (Groups 3 and 4), although the weight of the thymus in W/Fu animals (Group 2) was significantly greater than that of controls (Group 1).

No lesions were observed grossly or microscopically in the kidney or heart of W/Fu animals. With Holtzman animals, renal and cardiac hypertrophy with petechial hemorrhages and scars on the surface were seen (Group 3). Glomerular hyalinosis, tubular hyaline casts, and hypertrophy of the media of the arterioles were prominent in the kidneys. In contrast, only moderate renal hypertrophy with no significant gross or histologic changes was seen in the kidneys and hearts of W/Fu animals (Group 2).

Adrenal ultrastructure. The ultrastructure of the hypertrophic adrenal glands in Groups 1 and 3 was virtually identical. Therefore only the appearance of the adrenal gland in W/Fu animals will be described. Mitochondria were round and some of them appeared enlarged (Fig. 1). Virtually the entire matrix of the mitochondria was filled with vesicular cristae. Numerous lipid droplets and abundant tubules of smooth endoplasmic reticulum were observed in zona fasciculata cells (Fig. 1).

In Holtzman animals (Group 2), there was excellent restitution of the adrenal cortex. Mitochondria in zona fasciculata cells were almost indistinguishable from those in control animals. Smooth endoplasmic reticulum was especially prominent in these cells (Fig. 2).

In contrast, the regenerating adrenal gland in W/Fu animals (Group 4) showed mitochondria which were smaller than those in controls. Mitochondrial cristae were reduced in number and often were located in a peripheral position. Tubules of smooth endoplasmic reticulum were dispersed throughout the cytoplasm (Fig. 3).

Discussion. W/Fu strain of rats was resistant to the effects of adrenal regeneration hypertension. Dysfunction of the adrenal cortex is directly involved in the pathogenesis of ARH (2, 3). It is therefore of interest that regeneration of the adrenal gland is not complete in W/Fu rats at 5 wk in comparison to the Holtzman animals where regeneration was complete. The sequence of events occurring during regeneration of the adrenal gland in Sprague–Dawley, Holtzman strain has been described previously (9). Regeneration of the adrenal gland is largely complete by 35–42 days after operation. In the regenerating adrenal cortex, mitochondrial cristae of zona fasciculata cells in Holtzman animals appear virtually identical to those in adrenal glands of control, uninephro-adrenalectomized animals where the adrenal gland becomes hypertrophic (9). In a preliminary experiment (Unpublished) it is of interest that the W/Fu rats were also resistant to the hypertensive effects of methyl-androstenediol, a synthetic androgen (10, 11) which proved to be powerfully hypertensinogenic in other strains of rats. It is difficult to explain why the percentage of change in the weight of the heart and the kidneys of the rats in both strains is about the same while the changes in blood pressure are greater in the Holtzman than in the W/Fu strain. Previous studies from our laboratory have shown that an increase in

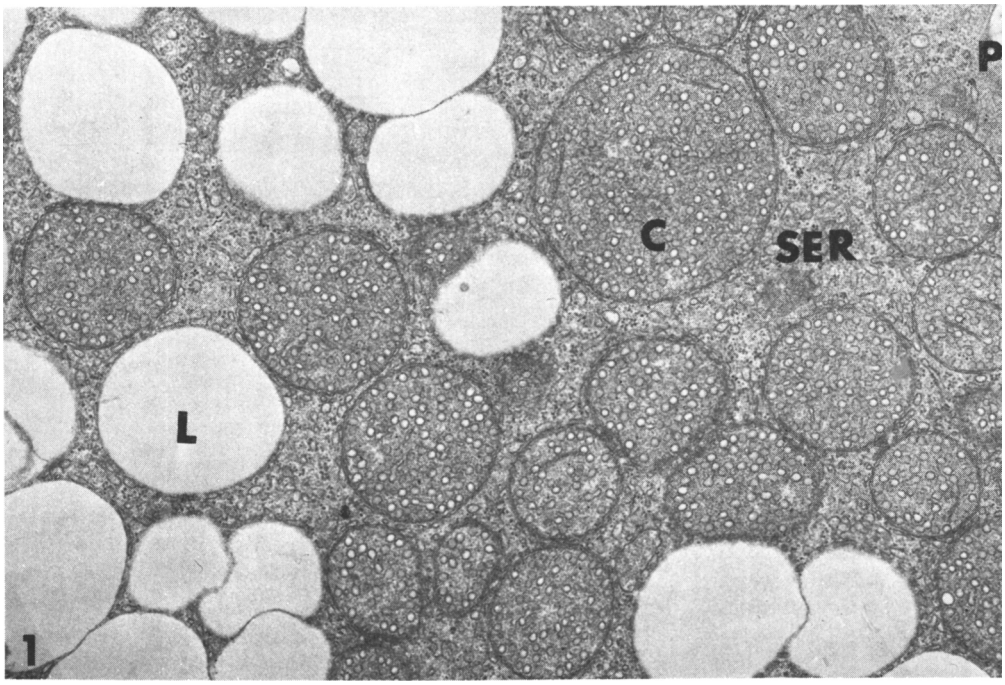

FIG. 1. Zona fasciculata cell from W/Fu control adrenal gland. Mitochondria are enlarged and round in shape. Cristae (C) are vesicular. Lipid droplets (L) and smooth endoplasmic reticulum (SER) are dispersed throughout the cytoplasm. Plasma membrane (P). (× 20,000.)

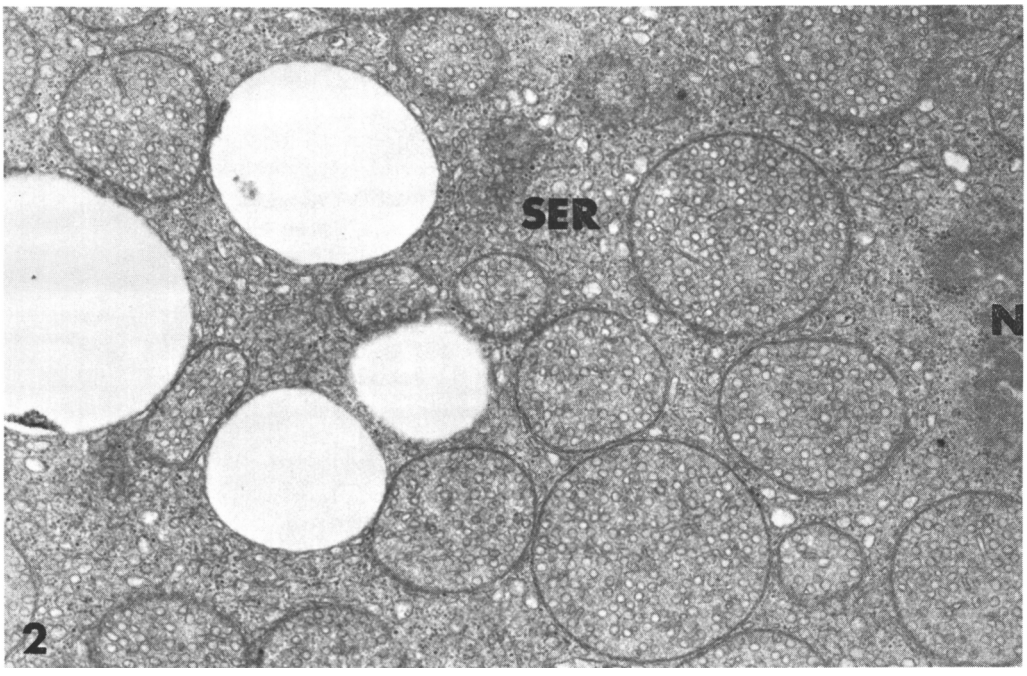

FIG. 2. Zona fasciculata cell from regenerated adrenal in Holtzman animal. Mitochondrial cristae are vesicular and virtually fill the entire mitochondrial matrix. Numerous tubules of smooth endoplasmic reticulum (SER) are observed in the cytoplasm. A portion of the edge of the nucleus (N) can be seen. (× 23,200.)

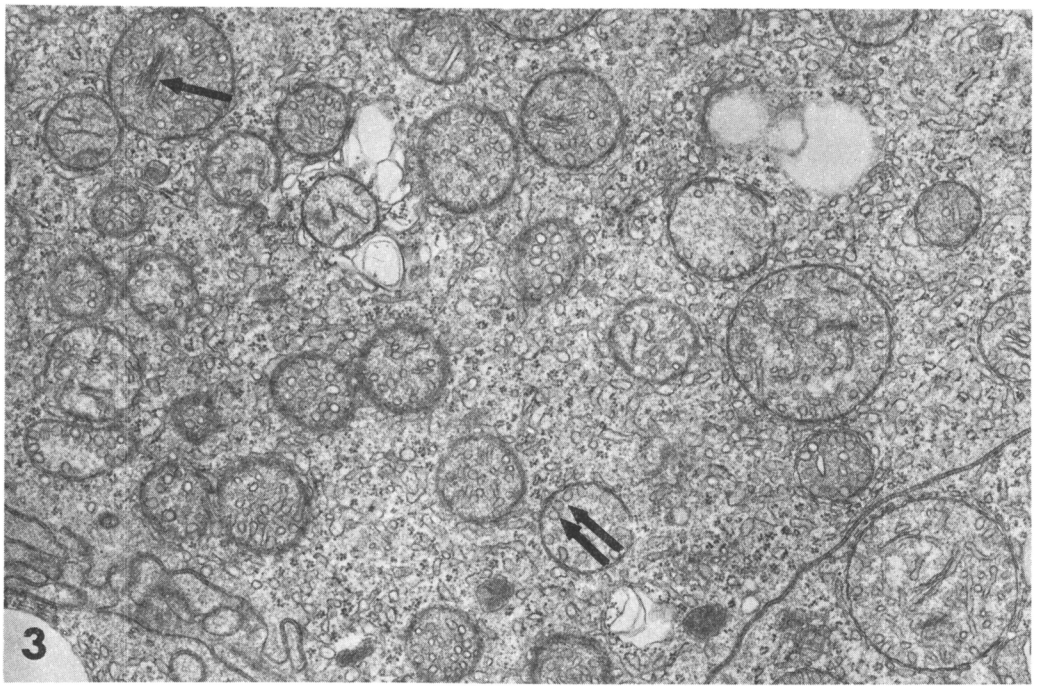

FIG. 3. Zona fasciculata cell from regenerating adrenal gland in W/Fu animal. Mitochondria are round and small in size. Cristae are reduced in number and many are tubulovesicular (arrow). In some mitochondria (double arrow) cristae are observed in a peripheral position. (× 20,000).

heart and kidney's weight does not always correlate to a proportional increase of the blood pressure. Rats of Charles River CD and Long Evans strains had heavier heart and kidneys than rats of Fischer F344 strain although their blood pressure was significantly lower (7). It has also been reported that cardiac left ventricular hypertrophy precedes the onset of hypertension in the spontaneously hypertensive Okamoto Aoki rats (12). It may be possible that a more prolonged treatment could eventually have produced hypertension in the W/Fu rats also. From the present study, therefore, it appears that the highly inbred W/Fu strain of rats has been selected inadvertently for traits which produce a resistance to hypertension, although at present the factors responsible for the resistance are unknown.

Summary. The procedure for producing adrenal regeneration hypertension did not cause an increase in the systolic blood pressure of W/Fu animals. The regenerating adrenal gland in W/Fu animals was not restored to normal; reduced numbers of mitochondrial cristae were seen and the mitochondria were smaller in size; regeneration was complete in Sprague–Dawley rats of the Holtzman strain and there was a severe form of hypertensive, cardiovascular disease.

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1. Skelton, F. R., Proc. Soc. Exp. Biol. Med. **90,** 342 (1955).
2. Skelton, F. R., Brownie, A. C., Nickerson, P. A., Molteni, A., Gallant, S., and Colby, H., Circ. Res. **24** (Suppl. 1), 1–35 (1969).
3. Rapp, J. P., Endocrinology **84,** 1409 (1969).
4. Selye, H., Hall, C. E., and Rowley, E. M., Can. Med. Ass. J. **49,** 88 (1943).
5. Bernardis, L. L., and Skelton, F. R., Proc. Soc. Exp. Biol. Med. **113,** 955 (1963).
6. Gardner, D. L., and Brooks, P. W., Brit. J. Exp. Pathol. **43,** 276 (1962).

7. Molteni, A., and Brownie, A. C., J. Med. **3,** 193 (1972).
8. Kim, U., Clifton, K. H., and Furth, J., J. Nat. Cancer Inst. **24,** 1031 (1960).
9. Nickerson, P. A., Brownie, A. C., and Skelton, F. R., Amer. J. Pathol. **57,** 335 (1969).
10. Skelton, F. R., Endocrinology **53,** 492 (1953).
11. Molteni, A., Brownie, A. C., and Skelton, R., Lab. Invest. **21,** 129, (1969).
12. Berte, L., Cutilletta, A. F., Sodt, P., and Arcilla, R. A., Ped. Res. **263,** 9 (1975) (abst.).

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