

# Hypertension in the opossum *Didelphis virginiana*

ARTHUR GROLLMAN

*Department of Pathology, University of Texas Southwestern Medical School, Dallas, Texas 75235*

GROLLMAN, ARTHUR. *Hypertension in the opossum *Didelphis virginiana**. Am. J. Physiol. 218(1): 80-82. 1970.—The injection of deoxycorticosterone, cortisone and ethacrynic acid into the “pouch” opossum resulted in a gradual rise in arterial blood pressure which attained hypertensive levels when the animal reached maturity (6-12 months). The results are thus comparable to those previously attained by treating pregnant rats in the same manner at a critical phase of fetal development and demonstrate that the teratogenic induction of hypertension is not dependent on changes induced in the maternal organism. The adult opossum develops hypertension in response to the same procedures as does the mammal (e.g., bilateral nephrectomy, application of a figure-of-eight ligature, or freezing of the renal cortices) with the appearance of structural changes in the arterioles characteristic of hypertension in the mammal. The stress inherent in maintaining animals trapped in the wild under laboratory conditions was not associated with a rise in their blood pressure.

blood pressure in opossum; teratogenic induction of hypertension; freezing of kidney; deoxycorticosterone; cortisone; ethacrynic acid

---

ALTERATIONS in the electrolyte and water balance of the organism induced by dietary methods or by the injection of certain hormones and diuretics at a critical period of pregnancy in the rat results in the development of hypertension in the offspring on their attaining adulthood (4). The opossum is unique in that its early development occurs, in large part, after parturition and it appeared desirable therefore to study the effects of treatment of the “pouch” animal at a period of life corresponding to that of the fetus in the mammal. In this way one avoids the possibility of the experimental manipulations exerting their effects through their action on the maternal organism.

Procedures which induce hypertension in the mammal have also been applied to the adult marsupial with results comparable to those observed in other laboratory animals.

## MATERIALS AND METHODS

Opossums trapped in the wild during early spring were transferred to the laboratory and kept in metal rabbit cages. They were allowed tap water and a diet of meat (rat) supplemented with commercially available canned cat food, ad libitum. The young were injected intramuscularly or intraperitoneally, without removing them from the pouch, while restraining the mother. The first injections were made when the animals had reached a body length (exclusive of the tail) of 5-9 mm and were repeated once weekly for 3 weeks.

Arterial blood pressures were determined: 1) by a tail plethysmograph similar to that used in the rat (8), but enlarged to accommodate the larger tail of the opossum; and 2) by directly recording the pressure from the cannulated femoral artery. The former procedure can be carried out on the unanesthetized animal, but is applicable only in younger animals the tails of which have not become markedly cornified. The latter procedure requires anesthesia, for which purpose 60 mg of pentobarbital per kilogram of body weight was used, supplemented by small doses of ether when necessary. The femoral artery was cannulated and the blood pressure recorded on a Sanborn (model 152-100B) apparatus connected to the artery by a PE-10 polyethylene catheter through a Statham transducer (model p 23AA). Where applicable, both procedures used for determining the blood pressure gave comparable results and only measurements obtained by cannulating the femoral artery are given in the tables.

At 6-12 months of age the animals were anesthetized and their organs were weighed and fixed in 10% formalin. After imbedding in paraffin, the tissues were cut into sections 6  $\mu$  thick, and stained with hematoxylin and eosin, and phosphotungstic acid and hematoxylin.

Procedures which in other mammals (mouse, rat, rabbit, cat, and dog) induce hypertension were applied to adult opossums 8-12 months of age. The procedures used included bilateral nephrectomy (5); the application of a figure-of-eight ligature to the right kidney with contralateral nephrectomy (1); and freezing of the renal cortex with solid carbon dioxide (Dry Ice). This procedure, which has been found to be effective in the rat (unpublished observations), is carried out by exposing the kidneys and applying a piece of Dry Ice to the entire surface of the kidney until it is rendered white and remains so for 2-3 min. Care is taken to avoid freezing the structures in the renal pedicle.

The same surgical technique was used as has been described previously for the rat and dog (1) approaching the kidney through a lumbar incision under pentobarbital (30 mg/kg, ip), supplemented when necessary by light ether anesthesia.

## RESULTS

*The pouch opossum (Table 1 and Fig. 1).* A total of approximately 80 pouch animals were injected but only a small proportion of these survived, most succumbing usually within a few days after injection. Despite the relatively small number of surviving animals, it is apparent from Table 1 that injection of the pouch opossum results in the induction of hypertension on the animal's attaining adult-

TABLE 1. *Effect of injection of deoxycorticosterone, cortisone, and ethacrynic acid on blood pressure of opossum*

Experimental Procedure	Number of Animals	Body Weight at Time of Sacrifice, kg	Blood Pressure, mm Hg	
			Average	Range
Saline controls	6	2.33	111/77	100/65-118/82
Deoxycorticosterone, 0.25 mg	5	2.19	151/106	145/100-160/110
Cortisone acetate, 0.25 mg	3	1.17	168/112	160/105-170/115
Ethacrynic acid, 0.125 mg	2	2.47	145/100	140/100-150/100

Values are averages. Injections were made once weekly for 3 weeks while animals were in the mother's pouch, and sacrificed 6-12 months later. Controls received 0.9% normal saline.

hood. The observed differences in blood pressure between the animals treated with steroids and the controls receiving normal saline are statistically significant ( $P < 0.001$ ).

The heart and kidney weights of the treated animals were not significantly different from those of the controls and hence are not included in the table. The blood vessels, on the other hand, showed the arteriolar thickening characteristic of the hypertensive state (Fig. 1A). Similar thickening of the smaller arteries and arterioles were observed in all organs examined, including the afferent arterioles of the kidney (Fig. 1C), in all of the hypertensive animals. The kidneys of three of the five animals treated with deoxycorticosterone showed cystic degeneration (Fig. 1B).

As in the case of the rat (4), an elevation in blood pressure when induced teratogenically is not apparent at birth but only later in life. The blood pressure of the opossums taken by the tail plethysmograph (8), soon after leaving the pouch or at the age of 3 months, was essentially normal with systolic pressures of 100-130 mm and diastolic pressures of 80-95, which are not significantly different from the normal (Table 2). Only at the age of 6-12 months, as shown in Table 1, were hypertensive levels attained. In this respect, teratogenically induced hypertension resembles essential hypertension in the human being; in the latter also, the blood pressure is normal at birth but becomes increasingly elevated with age.

*Induction of hypertension in the adult opossum (Table 2).* Procedures which induce hypertension in other laboratory animals were equally effective in inducing this disorder in the marsupial (Table 2).

Arteriosclerosis observed in spontaneous hypertension in man and in the hypertensive dog is also observed in the opossum. Lesions similar to those shown in Fig. 1A were observed in animals rendered hypertensive by freezing the renal cortex or applying a figure-of-eight ligature to one kidney with removal of the contralateral organ. The cystic degeneration of the kidney (Fig. 1B), noted in some of the animals treated with deoxycorticosterone while in the pouch, however, was not observed when hypertension was induced in the mature animals.

The adult opossum trapped in the wild and maintained caged in the laboratory may be considered as being in a state of chronic stress, but the animals observed in the present study remained normotensive after being in the labora-

tory for as long as 10 months. The average arterial blood pressure of a series of 12 animals, as determined under pentobarbital anesthesia within 1 week of their capture, was 115/80  $\pm$  10/8 and 10 months later it was 118/80  $\pm$  10/7.

DISCUSSION

Chronic elevation of the arterial blood pressure occurs spontaneously with great frequency in man and may be induced in laboratory animals by various manipulations including nephrectomy, partial occlusion of the renal artery, application of a figure-of-eight ligature to the kidney, by dietary restrictions, nephrotoxic agents, and teratogenically (2). An elevation in blood pressure has also been induced in amphibia (6) by bilateral nephrectomy. The presently reported results add the marsupial to the list of animal species in which hypertension may be induced, thereby supporting the concept of the basic systemic nature of this pathologic disturbance (2). Of the procedures used to induce hypertension, the application of Dry Ice to the renal cortex, presumably by infarcting the kidney (7), has proven most effective in the adult opossum (Table 2).

The injection of hormones, diuretics, and manipulations of the diet which alter the electrolyte metabolism in the pregnant rat results in the development of hypertension in the offspring when the latter attain adulthood (4). Since these experiments require treatment of the mother, it can be argued that the observed results are secondary to changes induced in the maternal organism rather than a direct effect on the fetus. The presently described experiments utilizing the pouch opossum permitted treatment of the animal at an age comparable to that of the rat in utero and the results support the concept of the teratogenic nature of this disorder as induced in the rat (4).

The term teratogenic has been applied generally in its literal sense to the induction of physical defects during fetal life. Objection may be raised, therefore, to the use of the term to the induction of hypertension, a disorder which is not associated with gross structural abnormalities. Such a distinction, however, is purely arbitrary since it is impossible to exclude the presence of structural disturbances not apparent by superficial methods of examination.

TABLE 2. *Effect of various procedures on blood pressure of adult opossum*

Procedure Used	Arterial Blood Pressure, mm Hg		Time Elapsing Between Observations, days
	Pretreatment	Posttreatment	
Bilateral nephrectomy	115/90 $\pm$ 6/5	200/140 $\pm$ 25/20	6
Application of figure-of-eight ligature with contralateral nephrectomy	120/85 $\pm$ 8/6	190/115 $\pm$ 20/12	60
Freezing of renal cortices with Dry Ice	110/75 $\pm$ 12/10	185/116 $\pm$ 15/10	60

Values are averages  $\pm$  SE of observations on 6 animals.

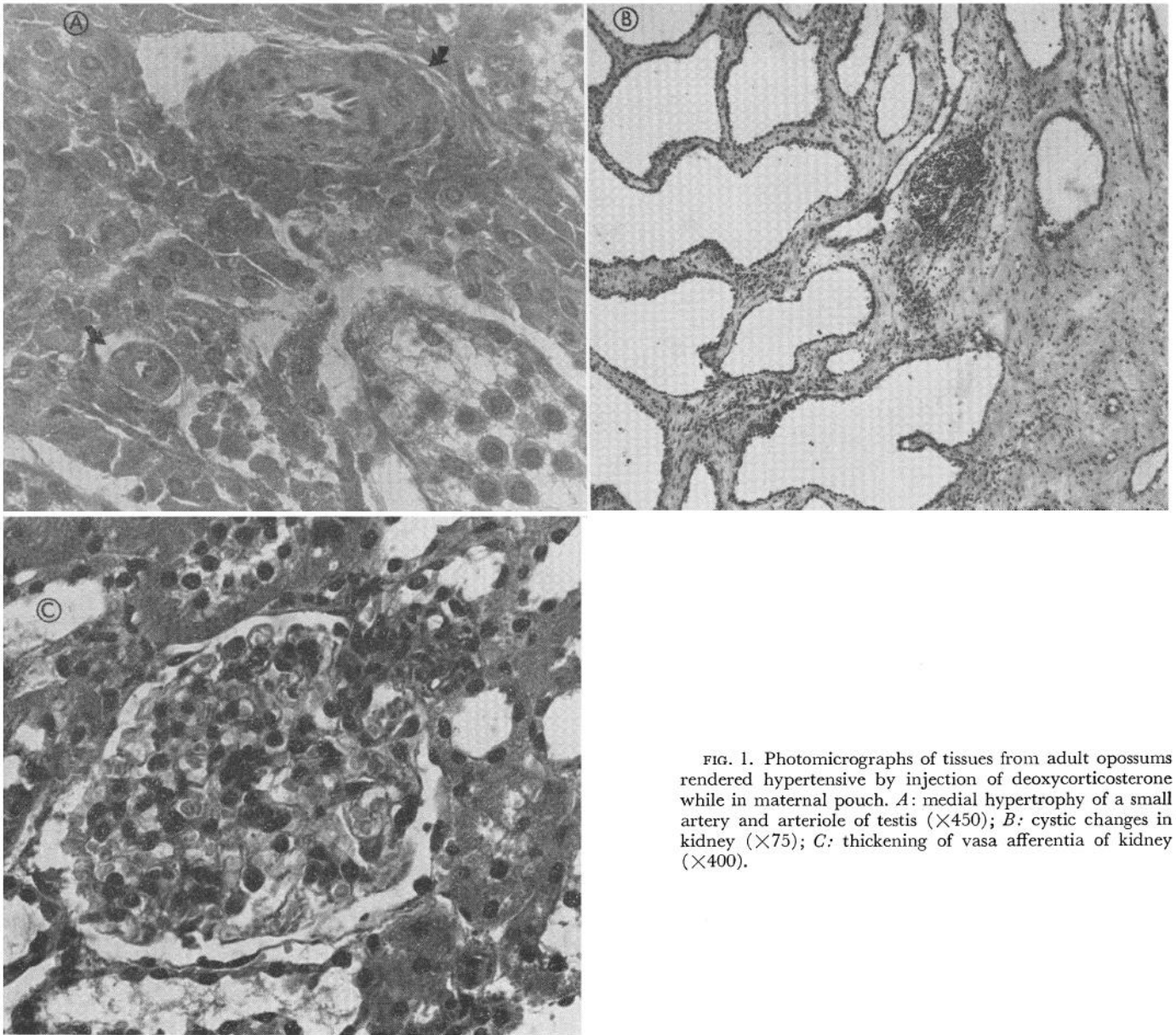


FIG. 1. Photomicrographs of tissues from adult opossums rendered hypertensive by injection of deoxycorticosterone while in maternal pouch. *A*: medial hypertrophy of a small artery and arteriole of testis ( $\times 450$ ); *B*: cystic changes in kidney ( $\times 75$ ); *C*: thickening of vasa afferentia of kidney ( $\times 400$ ).

I am indebted to Dr. Akio Ebihara and Dr. Toshio Takeda who, as Visiting Fellows in the Department of Experimental Medicine, participated in many of the studies reported in the present paper.

This investigation was supported by Public Health Service Grant HE 07916-07 from the National Heart Institute.

Received for publication 9 May 1969.

#### REFERENCES

- GROLLMAN, A. A simplified procedure for inducing chronic renal hypertension in the mammal. *Proc. Soc. Exptl. Biol. Med.* 57: 102-104, 1944.
- GROLLMAN, A. A unitary concept of experimental and clinical hypertensive cardiovascular disease. *Perspectives Biol. Med.* 2: 208-220, 1959.
- GROLLMAN, A. The natural history of essential hypertension. *Cardiovascular Clin.* 1: 1-11, 1969.
- GROLLMAN, A., AND E. F. GROLLMAN. The teratogenic induction of hypertension. *J. Clin. Invest.* 41: 710-714, 1962.
- GROLLMAN, A., E. E. MUIRHEAD, AND J. VANATTA. Role of the kidney in pathogenesis of hypertension as determined by a study of the effects of bilateral nephrectomy and other experimental procedures on the blood pressure of the dog. *Am. J. Physiol.* 157: 21-30, 1949.
- GROLLMAN, E. F., AND A. GROLLMAN. Induction of hypertension in the frog by bilateral nephrectomy. *Am. J. Physiol.* 199: 19-21, 1960.
- SOKABE, H., AND A. GROLLMAN. A study of hypertension in the rat induced by infarction of the kidney. *Texas Rept. Biol. Med.* 21: 93-100, 1963.
- WILLIAMS, J. R., JR., T. R. HARRISON, AND A. GROLLMAN. A simple method for determining the systolic blood pressure of the unanesthetized rat. *J. Clin. Invest.* 18: 373-376, 1939.