Angiotonin Induction of Vascular Lesions in Desoxycorticosterone-Treated Rats.* (20618)

G. M. C. MASSON, A. C. CORCORAN, IRVINE H. PAGE, AND FRANCESCO DEL GRECO.†

From the Research Division, Cleveland Clinic Foundation, and the Frank E. Bunts Educational Institute, Cleveland, Ohio.

Injection of renin in rats pretreated with desoxycorticosterone acetate (DCA)(1,2), cortisone(3), or hydrocortisone(4) elicits an eclampsia-like state associated with acute vascular lesions. Similar lesions are also elicited by administration of renin to bilaterally nephrectomized dogs maintained on saline(5). However, there was no conclusive evidence that the renal pressor system was integrally concerned in eliciting these lesions. The renin preparations used were relatively impure, and furthermore, orienting experiments with small amounts of very crude angiotonin then at our disposal were unsuccessful in provoking the eclampsia-like syndrome and vascular lesions in rats pretreated with DCA(1).

The present brief report shows that angiotonin can elicit fluid retention and acute vascular lesions in rats pretreated with DCA and NaCl which resemble those precipitated by renin; from this we conclude that the metabolic and morphologic changes induced by renin in sensitized animals can justifiably be ascribed to the action of renin as such.

Methods. Female rats of about 150 g body weight were uninephrectomized; 2 35-mg pellets of DCA were implants subcutaneously and 1% NaCl was given as drinking fluid. On the 20th day, they were divided into 3 groups. The schedules of treatments follow: Group I (7 rats) received hourly intraperitoneal injections of angiotonin over the course of 12 hours; Group II (6 rats) received 3 subcutaneous injections of 0.5 cc of a renin preparation over 24 hours; Group III (5 rats) was given 0.9% NaCl intraperitoneally, hourly, for 12 hours. The total volume administered (4.8 cc) corresponded to that of the angiotonin solution given Group I. The animals were placed in metabolism cages immediately after the first injections. The functions observed were fluid intake and urine output, proteinuria (Shevky-Stafford method), and rectal temperature. The animals were sacrificed at the end of 24 hours, autopsied and tissues selected for histologic examination; the fixative was Zenker's, and the stains used were hematoxylin-eosin-methylene blue (H and E), periodic acid-fuchsin (PAS), and phosphotungstic acid hematoxylin (PTH). The renin preparation contained 15 mg protein per ml and its pressor activity was 1.9 Goldblatt dog units per mg. The angiotonin preparation had a pressor activity of about 70 dog units per ml; it was some 30 times more potent than the material used in the preliminary experiments previously described (1); estimates by ultraviolet spectroscopy indicated that it was about 10% as pure as Edman's best samples(6). Both the renin and angiotonin preparations were furnished by Dr. Arda A. Green.

Results. The data are summarized in

TABLE I. Functional and Morphological Effects of Angiotonin and Renin in DCA-Treated Rats.

Group and treatment	Body wt changes in g after 12 hr	24 hr	Fluid intake minus fluid output, cc	Urinary protein, mg/24 hr	Rectal temp.	Vascular lesions — Incidence (%)	Severity*	Hemorrhages, incidence (%)
I Angiotonin	+35	+33	33	151	96	57	1.2	85
II Renin	+38	+52	72	185	87	100	2.6	100
III	— 5	+ 7	24	32	96	20	.2	0

* Graded from 0 to 4+.

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† Post-doctorate Fellow, Public Health Service.

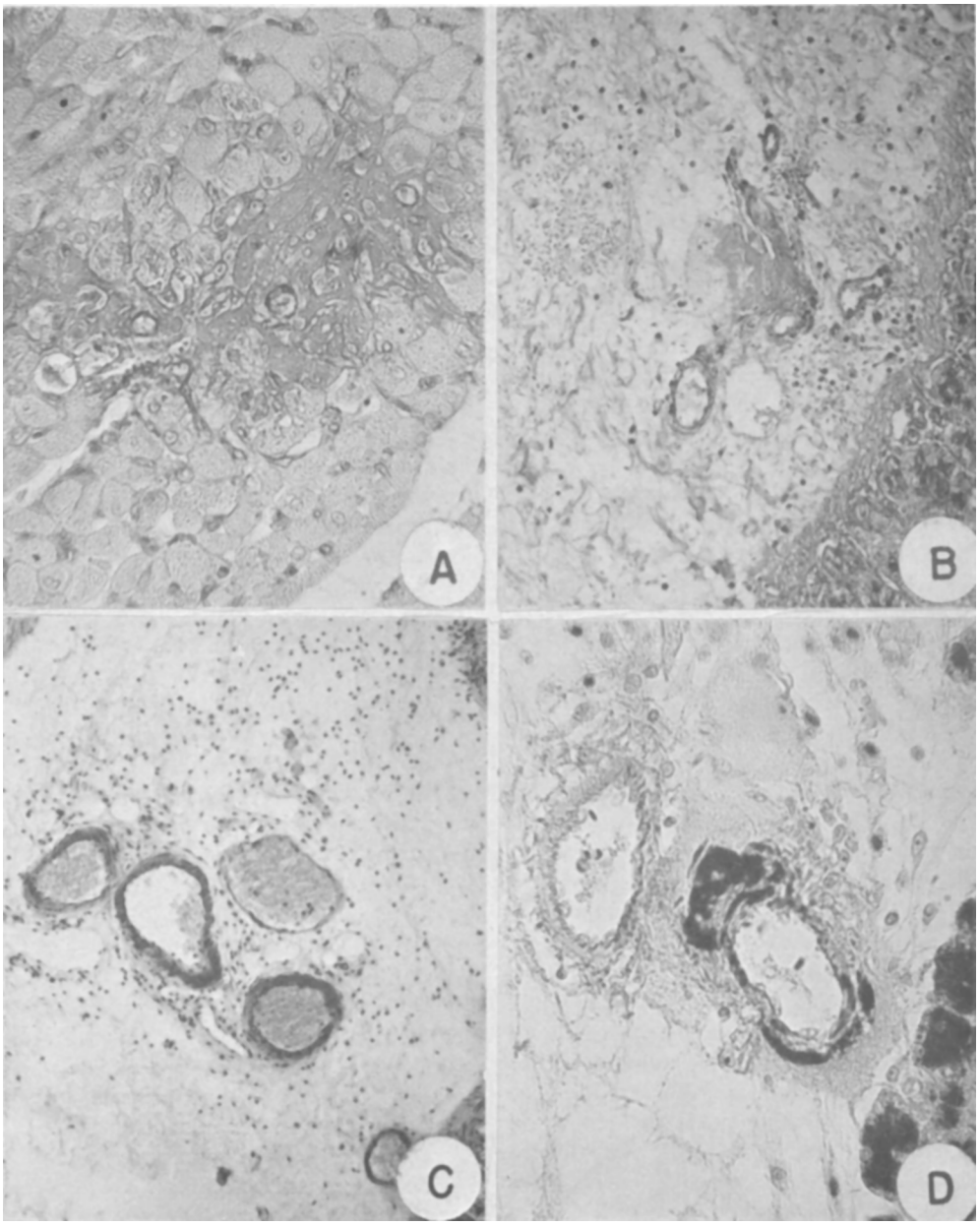

FIG. 1. Tissues of rats treated with DCA and angiotonin. (A) myocardium showing dilatation of small vessels and perivascular hyaline deposits which infiltrate between degenerating muscle fibers (×400, PAS); (B) stomach wall, showing edema, extravasation of erythrocytes and formation of hyaline deposit around a small vessel, portions of which seem dilated and necrotic (×200, PAS); (C) duodenal submucosa showing dilatation of hyalinized arterioles with stasis and edema (×120, PAS); (D) pancreatic arteriole with dilatation, perivascular exudate and intramural fibrinoid deposits (×400, PTH).

Table I. Animals of both Groups I and II demonstrated weight gain, large excesses of fluid intake over output, and proteinuria; rectal temperature decreased in the animals given renin. The prostration caused by the crude angiotonin formerly used was entirely absent in animals treated with the purer preparation.

The visceral hemorrhages characteristic of the gross appearance of the DCA-renin disease were found at autopsy in 6 of the 7 rats of the angiotonin group and in all the rats given renin. Two of the angiotonin-treated rats had convulsions; cerebral hemorrhages, to which these might be attributed, were not demonstrated at autopsy.

In the angiotonin group, 5 animals showed only subcutaneous edema; the other 2 showed also anasarca with fluid accumulations in serous cavities. Among the animals given renin, one failed to show edema, while the remaining 5 were in frank anasarca. Neither hemorrhages nor edema were demonstrable in the controls (Group III).

The severe renal vascular lesions previously described in DCA-renin disease were not demonstrable in animals of either Groups I and II. However, both groups did show hyalinization of large arterioles and necrosis of the wall of small arterioles with the smudged perivascular appearance of a PAS-positive "transudate". The myocardial and intestinal small arterioles showed the dilatation, fibrinoid necrosis, perivascular deposits and the associated necrosis of myocardial and smooth muscle tissues previously described. The relative severity of these changes was graded from one to 4+ and averaged for purposes of summary in Table I. Only minor glomerular, tubular and vascular lesions were found in animals of the control group.

Discussion. The data show that angiotonin can duplicate the essential phenomena of DCA-renin disease when a preparation of reasonable purity is administered in large doses at frequent intervals to rats pretreated with DCA and salt. The DCA-renin disease is widely variable in its severity between individuals; thus, none of the rats in this series developed its most severe and rapidly lethal form with anuria and thrombosis or necrosis of glomerular capillaries. This defect is perhaps fortunate for this demonstration, since the lesions caused by angiotonin and renin were more recognizably similar.

We would have preferred to elicit the condition in its severe form by administration of angiotonin. Unfortunately, there is no way of equating dosages of renin and angiotonin and it is very difficult to administer angiotonin in such a way as to simulate all the effects of the angiotonin liberated after subcutaneous injection of renin. The pressor effect could be reproduced by intravenous infusion of angiotonin; for this, the animal would require restraint and possibly anesthesia, both of which would interfere with the gratification of the thirst elicited by renin and the excess intake of saline which is one of the characteristics of the syndrome.

These experiments add to the similarities which have been shown to exist between the properties of renin and angiotonin; these include their pressor effects and their effects on fluid balance and proteinuria(7) and on electrolyte excretion(8). From the aspect of pathogenesis of the DCA-renin syndrome, salt intake and steroid treatment condition the production of vascular lesions by renin; this suggests that the lesions are somehow related to the effects of angiotonin on electrolyte distribution; the smudged perivascular deposits around small vessels seen in the DCA-renin and DCA-angiotonin treated rats seem to reflect an increased vascular permeability which is presumably the same as that which underlies the proteinuria induced by renal pressor agents.

Summary. 1. Hourly intraperitoneal administration of angiotonin to rats pretreated with DCA and salt elicited edema, convulsions, a positive fluid balance and increased fluid intake, together with proteinuria, visceral hemorrhages and acute necrotizing lesions in small arteries and arterioles. 2. The same series of changes occurred in similarly pretreated rats given renin subcutaneously. 3. We conclude that the vascular lesions elicited by renin in sensitized animals are very likely due to the action of renin as such, effected by liberation of angiotonin and are not, as hitherto seemed possible, attributable to vasculotoxic factors in renin preparations or to an action of renin independent of formation of angiotonin.

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A Closed-Vacuum System for Harvesting Infectious Infant Mouse-Brain Material. (20619)

C. P. A. STROME.* (Introduced by J. Casals.)

From U. S. Naval Medical Research Unit No. 3, Cairo, Egypt, Department of Virology; operated jointly by the U. S. Navy and the Division of Medicine and Public Health of Rockefeller Foundation.

The method developed by Casals, Olitsky, and Anslow(1) for obtaining high-titer complement-fixation antigen from infected suckling mouse brain has demonstrated the advantages of fewer anti-complementary and anti-mouse brain reactions in the serological study of certain neurotropic viruses. The method of antigen production, using a benzene extraction, has been adapted in this laboratory for the serological screening of human and vertebrate animal blood collected in Egypt and the Sudan (Taylor(2)) for immunity to West Nile and Ntaya viruses (Smithburn *et al.* (3); Smithburn and Haddow(4)).

The procedure, however, requires the tedious, time-consuming, and exposed harvesting of infectious mouse-brain material by laboratory personnel in order to obtain a sufficient quantity of material for extraction. These disadvantages have been partially overcome by a closed-vacuum, infant mouse-brain harvester developed in this laboratory.

Apparatus. The apparatus consists of a bluntly beveled 15-gauge needle attached by rubber tubing to a specimen vial and vacuum system. The needle is inserted into one end of a 6-inch section of catheter tubing; the other end of the tubing is attached to a right-angle glass tube that passes through one hole of a 2-hole rubber stopper placed in a specimen vial. The other hole of the stopper is connected to a vacuum system with an intervening trap flask containing chlorine solution. The needle and the rubber attachments are sterilized separately, and are connected with the specimen vial and vacuum system immediately before use. To assure instantaneous chilling and freezing of the brain material, the specimen vial is immersed in a bath of alcohol and CO_2 ice.

Technic. The mice whose brains are to be harvested are placed under deep ether or chloroform anesthesia and, if desired, exsanguinated. The head is cleansed with alcohol and the needle passed through the occiput of the skull. The vacuum, which is controlled by pressing the catheter tubing over the end of the needle attached to the tubing, is then applied by releasing the pressure, and the brain matter is sucked through the needle and tubing and deposited in the specimen vial. The aspiration of the brain is evidenced by the collapse of the skull. The vacuum is then closed until the next brain is harvested. A hundred mouse brains can be harvested completely in less than an hour by this method. Not only does it serve quickly and efficiently to collect all brain material, but it prevents open exposure of virulent material to persons harvesting highly infectious virus. The above technic has been used successfully in this laboratory to harvest brain material from infant mice up to 12 days of age.

* U. S. Naval Medical Research Institute, Bethesda, Md.