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Effect of Removal of a Clipped Kidney or Cessation of Renin Administration on Hypertension.* (30613)

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Subcutaneous injections of renin into uninephrectomized rats cause hypertension and hypertensive vascular disease, as well as nephrosclerosis, stimulation of the zona glomerulosa and depletion of renal renin(1). The same changes are seen in rats with unilateral clipping of the renal artery(2). This strongly suggests that renin-induced hypertension is similar, if not identical, to experimental renal hypertension. Additional evidence of this similarity is provided by the present experiments in which we studied the changes in arterial pressure after removal of the "clipped kidney," after cessation of renin administration and the effect of renin injections during remission of renal hypertension.

Methods. Female Sprague-Dawley rats weighing about 150 g were fed Purina fox chow and given tap water to drink. Blood pressure was measured by tail sphygmography once or twice a day. In the first experimental series the effects of renin on blood pressure were studied following removal of a clipped kidney. After a control period, rats were made hypertensive by application of a silver clip on the left renal artery and were kept under observation for a period of 17 days at the end of which only those with pressures around 200 mm Hg were divided into 3 groups. The left kidney was removed in animals of Groups 1 and 2; animals of Group 3 were subjected only to a sham operation. Starting on the same day, animals of Group 2 received subcutaneous injections of crude hog renin 4 times per 24 hours in a daily dose of 45 Goldblatt units, while animals of the other 2 groups received saline injections according to the same schedule. The number of animals per group was 18, 12, and 12 respectively. The experiment was terminated 7 days later. Tissues were removed, weighed and processed for histologic examination.

In the second series we studied the effects of cessation of renin treatment on the course of renin-induced hypertension. Rats were uninephrectomized and divided into 3 groups of 12 animals each, which were placed in metabolism cages. Animals of Groups 1 and 2 were treated with crude hog renin which was administered subcutaneously every 8 hours at a daily dose of 75 Goldblatt units. On

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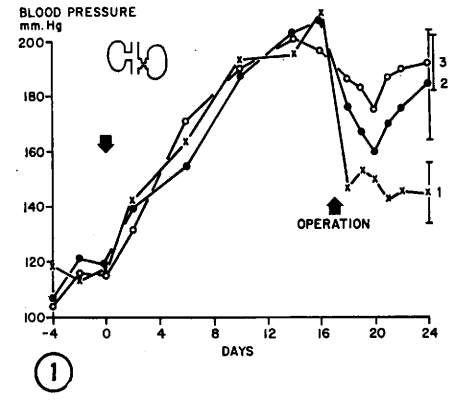

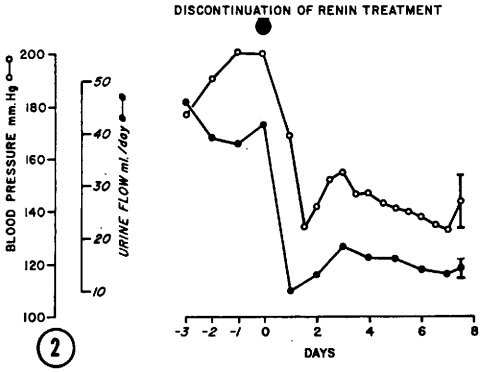

FIG. 1. Effects of removal of a clipped kidney on arterial pressure. At first arrow a clip was placed on left renal artery. At second arrow clipped kidney was removed and injections started. Group 1, left nephrectomy plus saline injections; Group 2, left nephrectomy plus renin injections; Group 3, sham operation plus saline injections. Cross bars represent standard deviations.

FIG. 2. Effect of discontinuation of renin treatment on arterial pressure and urine flow.

the 11th day when rats were hypertensive, those of Group 1 were killed; those of Group 2 were kept without treatment and killed 8 days later. Animals of Group 3 were untreated controls. At the end of the experiment, blood was withdrawn under ether anesthesia for determination of pressor activity in non-incubated plasma(1). Results are expressed in nanograms (ng) of angiotensin equivalents per ml. A piece from each kidney was used for determination of pressor activity; results are expressed in Goldblatt units per gram of tissue(2). The width of the zona glomerulosa in μ was measured with a contour projector from adrenal glands stained with hematoxylin-eosin. Juxtaglomerular index was determined in kidneys stained with Bowie's stain(3). All values given are means with standard deviations.

Results. First series (Fig. 1). On the 17th day following application of a renal clip, removal of the clipped kidney caused an abrupt fall in arterial pressure from 211 $\pm$ 10.3 to 139 $\pm$ 12.9 mm Hg around which it remained until the end, 7 days later (Group 1). When treatment with renin was initiated at the same time as removal of the clipped kidney (Group 2), there was a more gradual fall in arterial pressure from 209 $\pm$ 10.5 to a minimum of 160 $\pm$ 18 mm Hg, followed by a rapid return towards hypertensive levels. Similar but less accentuated changes as in Group 2 were seen in rats of the control Group 3 which were subjected to laparotomy without nephrectomy; arterial pressure fell from 197 $\pm$ 8.8 to 177 $\pm$ 22 mm Hg. On the last day of the experiment, blood pressure values in Groups 2 and 3 were significantly different from those in Group 1 ($P<0.01$). These results agree with those obtained from heart weights which averaged 395 $\pm$ 39, 462 $\pm$ 25 and 436 $\pm$ 13 mg per 100 g of body weight respectively. P value was less than 0.01 between Groups 1 and 2, and more than 0.05 between Groups 1 and 3. On histologic examination, rats from all groups showed vascular disease. There was no difference between groups as concerns degenerative vascular lesions in heart and kidney which were mostly associated with fibrinoid deposits. However, mesenteric arteritis characteristic of hypertensive vascular disease in rats showed signs of healing after removal of the clipped kidney; inflammatory cells had disappeared and were being replaced by scar tissue. This healing process was prevented by administration of renin; arteritis was equally severe in Groups 2 and 3. Thus these experiments indicate that injections of renin are able to replace the endocrine function of a clipped kidney.

Second series. (Table I, Fig. 2). Results from chronic treatment with renin confirmed those already reported(1); inhibition of growth, diuresis, thymus atrophy, decrease in renal renin and juxtaglomerular index, increase in width of the zona glomerulosa, in heart weight and in pressor activity of plas-

TABLE I. Effects of Discontinuation of Renin Treatment.*

Treatment	Body wt, g	Heart, mg/100 g body wt	Thymus, mg	Urine flow, ml/24 hr	Pressor activity in plasma in ng/ml			J.G.I.	Renal renin, u/g	Zona glomerulosa, μ
					Incubated	Non-incubated	Diff.			
Renin	151.5 ±11.3	405.7 ±22.4	166.7 ±51.6	31.7 ±9.3	1.35 ±.34	.55 ±.32	.80 ±.12	3 ±2	.69 ±.25	64.8 ±6.7
Renin—then no treatment	178.8 ±7.4	347.5 ±27.7	254.8 ±25.2	14.3 ±2.3	1.06 ±.27	.35 ±.24	.71 ±.15	16 ±8	1.65 ±1.16	54.7 ±11.8
0	168.8 ±4.1	316.3 ±12.2	346.7 ±18.5	13.8 ±5.3	.97 ±.09	.47 ±.07	.49 ±.11	33 ±6	5.7 ±1.94	44.2 ±1.9

* All values are means with standard deviations.

ma, and, finally, hypertension and vascular disease. All these effects reversed when renin injections were discontinued. There was a sudden and deep fall in arterial pressure, followed by a small rise and then a progressive decrease; at the end of the experiment on the 8th day, pressures averaged 144 ± 18 mm Hg which is significantly higher than the control value of 120 ± 5.5 mm Hg ($P<0.01$). Body weight which had not shown any increase during treatment rose by 30 grams. Rate of urine flow returned to normal within 24 hours.

Values for heart weight, thymus weight, renal renin, juxtaglomerular index and width of the zona glomerulosa were intermediate between those in the control group and those in the renin-treated group. The most informative data on pressor activity in peripheral plasma were those obtained following incubation. It was slightly but significantly elevated by renin treatment (P value <0.02) but returned to normal on cessation of the injections. As in the first series, all rats that received renin showed vascular disease. Degenerative hyaline lesions were present in animals of Groups 1 and 2. However, retrogression of mesenteric arteritis was seen only in rats of Group 2 in which treatment had been discontinued (Fig. 3 and 4).

Discussion. The observation that cessation of renin treatment like removal of a clipped kidney caused a fall in pressure which in renal hypertension was prevented by renin injections constitutes additional evidence for the view that renin-induced and renal hypertension may have the same pathogenesis. In both cases pressure fell rapidly and then stabilized at a level somewhat higher than the original base line. Similar findings after removal of a clipped kidney have been reported by Byrom and Wilson(4) who like Floyer(5) attributed this residual hypertension to the vascular disease in the contralateral kidney since removal of the clip in association with contralateral nephrectomy was followed by a complete return to control levels. In our experiments renal vascular lesions were also present. This cause-effect relationship is further supported by the observation that in the absence of lesions such as after continuous infusion of renin or angiotensin(6,7) pressure fell immediately to normal upon cessation of treatment.

The mechanism leading to this residual hypertension is not clear. The suggestion that renal vascular lesions would act as microscopic Goldblatt clamps to trigger renin re-

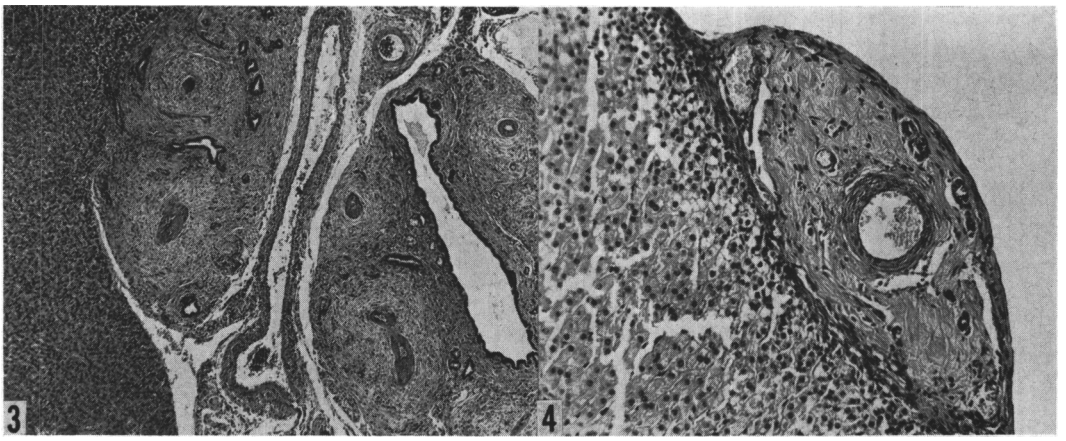

FIG. 3. Hepatic arteries in renin-treated rats showing intense inflammatory reaction. H and E. × 44.

FIG. 4. Small artery in the adrenal capsule showing after cessation of renin treatment complete disappearance of inflammatory cells and replacement of exudate by sclerotic tissue, while media and intima remain abnormal. H and E. × 120.

lease does not seem to be valid. Determinations of renin content and renin release showed that kidneys contralateral to clipped kidneys are almost completely devoid of renin (8,9). This renin depletion eliminates one important source of stimulation of the zona glomerulosa and therefore would greatly reduce aldosterone participation. As concerns the antihypertensive function of the kidney, too little is known to speculate about its role and the effects of renal damage upon this function.

Healing of some of the vascular lesions is probably the result of a fall in arterial pressure. Such effects have been observed following treatment with hypotensive drugs in rats with renal, desoxycorticosterone or salt hypertension(10). In all these instances there was disappearance of the inflammatory reaction from scarred or fibrous vessels, while degenerative lesions in the renal vasculature continued to progress. Although there is no evidence that adrenal secretions are involved, administration of cortisone or cortisol in DCA-hypertensive rats also caused resolution of the inflammatory exudate of mesenteric arteritis, but did not remedy nephrosclerosis (11).

More significance would have been attached to the present results if it had been possible to extend similar experiments to the chronic phase of hypertension period during which removal of a clipped kidney does not generally affect the course of hypertension. Unfortunately the effectiveness of heterologous renin is limited by the formation of antirenin which on the third week of treatment is present in sufficient amounts to inactivate exogenous renin(12).

Summary. In rats made hypertensive by clipping a renal artery or subcutaneous renin injections, removal of the clipped kidney or cessation of renin administration were followed by an abrupt fall in arterial pressure, which stabilized at a level higher than the control value. This was associated with healing of mesenteric arteritis, while hyaline lesions remained unaffected. The functional and pathologic changes caused by removal of the clip were prevented by administration of renin. These experiments provide additional evidence that renal and renin-induced hypertension may have the same pathogenesis.

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Identification of a Cytopathogenic Agent Infectious for Puppies as a Canine Herpesvirus.* (30614)

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A fatal disease of infant puppies caused by cytopathogenic organisms which initially were believed to be mycoplasma has been reported(1). Further study revealed, however, that certain tissues from puppies that died contained 2 organisms, a virus and mycoplasma. After attempts failed to relate the mycoplasma isolates to cytopathology in dog kidney cell (DKC) cultures or to the fatal illness in young puppies, it became apparent that the mycoplasma were not causally related to the disease. Moreover, cytopathic effects (CPE) in DKC cultures and typical pathologic changes in young puppies were produced consistently by inoculation with bacteriologically sterile materials that did not contain mycoplasma. A detailed clinical and pathologic description of the disease in puppies has been reported(2). A communication from Dr. L. N. Binn (Walter Reed Army Inst. for Research, Washington, D. C.) indicated that an agent (S-463) isolated from puppy tissues by Capt. R. O. Spertzel, and which is similar to our isolates(1), is viral and has characteristics of herpesviruses. Also, Dr. S. E. Stewart (Nat. Inst. Health, Bethesda, Md.) has reported to us that a herpes-like virus has been isolated from young puppies that died of a hemorrhagic disease.

This paper reports some of the properties of this newly recognized canine virus and compares characteristics of the virus with properties of the *Herpesvirus Group*.

Materials and methods. Tissue culture. Primary DKC cultures were prepared according to methods reported previously(1). For certain studies, secondary cell cultures were grown in glass rings on microscope slides according to Cairn's method(3). Tissue culture medium (TCM) consisted of medium 199 (GIBCO), 0.5% lactalbumin hydrolysate, 4% heated lamb serum, and antibiotics. Cultures used for testing effects of halogenated uridine compounds (see below) on viral growth were maintained in a similar medium, with the exception that Earle's saline solution was substituted for medium 199 in the formula.

Virus. Our prototype strain, designated F-205, was used in all experiments. The source of this strain was reported previously (1). Because both mycoplasma and a virus were present initially in our stock cultures, virus employed in these studies was prepared from stock in the following manner: Infected DKC cultures with advanced CPE were ultrasonicated for 1 minute at maximum frequency with a Biosonik probe (Bronwell) and emulsified for 5 minutes at 0°C with trifluorotrichloroethane (Genetron 113, Allied Chemical and Dye Corp.). Genetron-treated culture fluid was centrifuged at 30,000 rpm for 45 minutes in a #40 rotor of an ultracentrifuge (Spingo, Model L). Supernatant fluid portions were discarded and the pellets were

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