100-182 mg of marrow were found to be contained within femurs of 260-280 g rats; 55% of this marrow was located within the diaphysis whereas the remainder was in epiphyseal regions. Using the cell enumeration procedure, approximately 140-325 million marrow cells were found within femurs of 260-280 g rats with 65% being located within the diaphyses.

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Inhibition of Aldosterone-Stimulating Activity of Hog and Dog Renin by the Plasma of Dogs Immunized with Hog Renin.* (28251)

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The pressor activity of hog renin, and also that of dog renin, is inhibited if these renins are incubated with the plasma of dogs that have received multiple injections of renin. The inhibition is believed to be due to anti-hog renin antibodies which also neutralize dog renin(1,2). Recently, it has been shown that hog and dog renin stimulate the secretion of aldosterone in dogs(3,4,5). The present experiments demonstrate that the aldosterone-stimulating as well as the pressor activity of hog and dog renin is inhibited by the plasma of dogs immunized with hog renin.

Methods. Male mongrel dogs weighing approximately 10 kg were treated with hog renin prepared by the technique of Haas and Goldblatt(6). The amount of this renin preparation which raised the systolic blood pressure of pentobarbital anesthetized normal dogs 30 mm Hg was designated as one "unit." The injected dogs received approximately 25 "units" per day subcutaneously 5 times a week for 5 to 36 weeks. The plasma of the dogs was assayed for its effect on the pressor and adrenal stimulating activity of renin. As a control, frozen plasma which had been obtained from the dogs before they were immunized was also assayed. Two "units" of hog or dog renin were mixed with 2 ml of plasma. The mixture was incubated at room temperature for one-half hour, then injected intravenously into nephrectomized hypophysectomized assay dogs. The assay dogs were prepared as described previously(5) with adrenal venous cannulae, and with arterial cannulae for measuring blood pressure continuously, using a Statham strain gauge and Grass model 5 polygraph. Two control adrenal venous blood samples were collected before the first renin-plasma mixture was injected. Each as-

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TABLE I. Effects of Renin Incubated with Control or Immune Plasma in Nephrectomized Hypophysectomized Assay Dogs. Values are means of each value 4 minutes after injection minus value before injection.

	No. of experiments	Mean change (± standard error) in Aldosterone secretion (mμg/min)	17-hydroxycorticoid secretion (μg/min)	Systolic blood pressure (mm Hg)
Hog renin & immune plasma	8	1.5 ± .6*	−.2 ± .7	4 ± 2.8*
Hog renin & control plasma	8	8.8 ± 2.4	1.2 ± .4	51 ± 7.4
Dog renin & immune plasma	9	0 ± 1.3†	.3 ± .3	2 ± 1.7†
Dog renin & control plasma	4	19.8 ± 4.1	2.0 ±1.8	40 ± 3.5

* Significantly different from hog renin and control plasma, $p < .02$.
† Significantly different from dog renin and control plasma, $p < .01$.

say dog received renin and control plasma, and renin and immune plasma, but the sequence of injections was intentionally varied from dog to dog. The interval between injections was 60-120 minutes. Adrenal venous samples were collected just before and from the 4th to the 14th minute after each injection. They were also collected at ½-hour intervals between injections. After each collection, the assay dogs were transfused with a volume of blood from nephrectomized hypophysectomized donor dogs equal to the amount collected. In 10 of the 11 assay dogs, one I.U. of ACTH was injected intravenously at the end of the experiment, and an adrenal venous blood sample collected starting 4 minutes later. All adrenal venous blood samples were centrifuged promptly and the plasma stored in the frozen state for analysis of aldosterone by the double isotope derivative method of Kliman and Peterson(7) and 17-hydroxycorticoids by the method of Silber and Porter(8).

Results and discussion. Control aldosterone and 17-hydroxycorticoid output values and blood pressure in the assay dogs were in the same range as the values previously reported from this laboratory(3,5). In Table I the effects of hog and dog renin after inactivation with immune plasma are compared to their effects after incubation with control plasma. The plasma of the immunized dogs inhibited the aldosterone and 17-hydroxycorticoid-stimulating, as well as the pressor activity of both hog and dog renin.

In the experiments in which renin and immune plasma was injected after renin and control plasma, it could be argued that the inhibition was due to tachyphylaxis to renin(1) rather than antirenin antibodies, even though the interval between injections was 60-120 minutes. Conversely, in the experiments in which immune plasma and renin were given before control plasma and renin, the response to the control renin might be diminished by excess antirenin antibodies remaining in the blood of the assay dogs. To circumvent these difficulties, the effects of the *first* injections given to each of the 11 assay dogs were analyzed separately. The results are summarized in Table II. The number of animals in each group is small, but the inhibition of the renin effects by incubation with immune plasma is still clear. The effects of injections of renin and control plasma *after* injections of renin and immune plasma are also shown in Table II. The effects of these subsequent control injections are less than those of the first injections, and although the differences are not statistically significant, they suggest that there was some passive transfer of antirenin antibodies.

In 2 instances, aldosterone output rose 60 minutes after injection of hog renin and immune plasma. It increased from low values to 13 mμg/min in one instance, and to 32 mμg/min in the other. This suggests that the blocking effect of the plasma may wear off after a period of time. However, in the other 6 hog renin and immune plasma experiments, no significant late rise occurred. This was so even though specimens were taken every half hour for 2 hours in one dog. Similarly, in none of the 9 instances in which dog renin and immune plasma was injected was a delayed response observed.

TABLE II. Response to the *First* Incubated Sample Injected into Each of the 11 Assay Dogs, And the Effect of Injecting Renin that had been Incubated with Control Plasma into Assay Dogs that had Received Renin plus Immune Plasma 60-120 Minutes Before.

	No. of experiments	Mean change (± standard error) in Aldosterone secretion (mμg/min)	17-hydroxycorticoid secretion (μg/min)	Systolic blood pressure (mm Hg)
Hog renin & control plasma (1st inj)	2	14.5	.4	63
Hog renin & immune plasma (1st inj)	5	1.0 ± 1.0*	−.5 ± .2	6 ± 4 *
Hog renin & control plasma after immune plasma	6	6.8 ± 2.8	1.5 ± .5	48 ± 9.5
Dog renin & control plasma (1st inj)	2	25.0	4.1	45
Dog renin & immune plasma (1st inj)	2	1.0†	.3	3†
Dog renin & control plasma after immune plasma	2	14.5	−.1	35

* Significantly different from hog renin and control plasma, $p < .01$.
† Significantly different from dog renin and control plasma, $p < .01$.

The adrenals of the assay dogs remained responsive to ACTH at the end of the experiment in the 10 dogs tested. The mean aldosterone output in these dogs after ACTH was 32.3 ± 4.3 mμg/min, and the mean 17-hydroxycorticoid output was 8.2 ± 0.8 μg/min.

Summary. The aldosterone-stimulating and 17-hydroxycorticoid-stimulating activity, as well as the pressor activity, of both hog and dog renin were inhibited when prior to injection into nephrectomized hypophysectomized assay dogs, the renin preparations were incubated with the plasma of dogs immunized with hog renin. Incubation with normal dog plasma had no effect on their activity.

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Auto-Antigenicity of Connective Tissue Extracts.* (28252)

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We have demonstrated(1,2) that injection of saline extracts of rabbit tendons into young guinea pigs elicits the production of antibodies to these extracts and to similarly prepared extracts of guinea pig tendons. The antigenic component of these connective tissue extracts (CTE) could not be isolated; therefore these antibodies were designated with the general term "connective tissue antibodies" (CTA). The growth of the injected animals was stunted, but following cessation

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