

## STUDIES ON EXPERIMENTAL HYPERTENSION

### XX. THE BIOASSAY OF RENIN\*

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To test for potency of renin in a renal extract known to contain this substance, the unanesthetized rabbit (1), the anesthetized cat (1), rat (2), dog (3, 4), the unanesthetized (5) or anesthetized (6) bilaterally nephrectomized dog and the brain-pithed nephrectomized cat (6) have been used. It has been claimed (6) that the bilaterally nephrectomized dog is unusually sensitive to the pressor effect of renin injected intravenously, but this claim has not been substantiated (7). The quantity of renin in an extract has usually been determined by injecting variable quantities of it intravenously into animals and finding the minimum amount required to produce an arbitrary elevation of blood pressure, or by determining the elevation of blood pressure produced by injecting an amount of extract known to represent a definite quantity of renal tissue. Up to the present, no standard method or unit has been adopted, so that it is difficult or impossible to compare the results obtained by various investigators in studies dealing with the potency and purification of renal extracts containing renin.

In the course of studies on the isolation of pure renin, it became necessary to have a method whereby as many as twenty or more assays for renin could be made in a single day. The phenomenon of tachyphylaxis to renin (diminished response to frequent injections, after short intervals) precludes the use of the same animal for many assays in 1 day. The following method was therefore developed which has the advantages of being simple and rapid, does not require anesthetization, bilateral nephrectomy, or sacrifice of the animals, and could well serve as a standard method which would permit comparison of results obtained by different investigators.

#### *Method*

A normal unanesthetized dog, with one hind leg left free, is placed on a board. Training of the dog consists in placing the animal in position for the assay and determining the direct mean femoral blood pressure. Instead of being strapped down,

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the front legs may be held lightly by an attendant who also helps to keep the animal quiet. Gentle handling soon results in a tractable animal. The determination of the blood pressure is made by inserting into the femoral artery a 20 gauge needle attached to the barrel of a tuberculin syringe, which is connected by means of rubber tubing to a U-tube mercury manometer. The rubber tubing, syringe, and needle are filled with 4 per cent sodium citrate by a suitable pressure system. The needle is inserted through the skin and fascia and into the vessel, without local anesthesia.

TABLE I

*Relationship between Weight of Animal and Response of the Blood Pressure to an Intravenous Injection of the Same Quantity (a Unit Dose) of Renin*

Dog No.	Weight	Initial pressure	Maximum pressure* in 3 min.	Maximum rise of pressure in 3 min.‡
	<i>kg.</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
8-35	9.1	120	150	30
4-98	9.5	130	165	35
9-55	10.9	120	150	30
9-41	12.7	130	160	30
8-70	13.6	100	130	30
5-41	14.6	130	160	30
5-45	15.5	120	155	35
9-33	17.3	110	140	30
8-66	17.7	115	145	30
5-58	25.0	110	145	35

\* In no instance did the pressure rise above this value after 3 minutes.

‡ The manometer is read to the nearest 5 mm. Hg, which is the best that can be done if the system is not damped and wide oscillations are permitted to prevent clotting. It has been our habit to test various quantities of a specimen of renin, until an amount is reached that gives a rise of 30 mm. Hg, and then to test the effect of this amount in at least three dogs. If these give a pressor effect of not less than 30 mm. and not more than 35 mm. Hg this amount is regarded as containing one arbitrary dog unit of renin. The results, in 10 dogs, from the injection of a unit dose show clearly that this unit is independent of the body weight of the dogs.

If the needle is sharp, and the operator skillful, no evident pain is elicited by this procedure and the animal remains undisturbed.

After the animal has been trained to lie quietly while the blood pressure is being taken, assay of the potency of renal extracts can be undertaken. The test is performed as follows: A 20 gauge needle is inserted into the femoral artery for the determination of the blood pressure and an assistant inserts the needle (23 gauge) of the syringe containing the test material, into the external or internal vein of the other hind limb, just above the ankle. If the amount of material for assay is less than 2 cc. it is brought to this volume with physiological saline. The injection of the test material is made quickly, after the blood pressure has settled down to a constant level. It has been found that animals with an initial blood pressure of from 100 to 135 mm. Hg give the most uniform results. The blood pressure readings are taken visually every half minute from the time the injection of the extract is completed. It has been found that the maxi-

imum rise of pressure, even from a large dose of renin, usually occurs in 2 minutes or less. The blood pressure may remain at the maximum for several minutes, does not usually return to the initial level in less than 15 minutes, and may remain significantly elevated for as long as 30 minutes. It has been found also that an amount of renin

TABLE II

*Maximum Rise of Blood Pressure Caused by Two Successive Intravenous Injections of One Unit of Renin with an Interval of 2 Hours between Injections*

Dog No.	Weight	1st determination	2nd determination (2 hrs. later)
	<i>kg.</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
8-33	11.5	35	30
9-41	12.8	30	30
9-33	16.4	30	35
8-43	21.4	30	35

TABLE III

*Maximum Rise of Blood Pressure Caused by the Intravenous Injection of Increasing Quantities of Renin\* (One to Five Units), with an Interval of at Least 24 Hours between Successive Injections*

Dog No.	0.4 cc.	0.8 cc.	1.2 cc.	2.0 cc.
	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>	<i>mm. Hg</i>
8-70	35	40	45	60
8-72	30	45	55	70
9-33	30	45	55	65
9-67	30	40	50	60
9-42	30	40	45	65

\* The renin used in this study (Tables I to III) was prepared from hog kidney by a method which is soon to be published. In brief outline, the method consists of the following steps: Fresh hog kidneys are ground and extracted with distilled water and adjusted to pH 8 with NaOH. Trichloroacetic acid is added to the filtered extract to pH 2.9. The supernatant fluid is dialyzed free from acid and the renin is precipitated from it with acetone. The precipitate is dissolved in distilled water and dialyzed. The assay shows that the sample of renin used in this study had a *potency* of 2.5 dog units per cc. The *purity* was 15 units per mg. N and the *yield* was 0.8 unit per gm. (wet weight) of kidney.

which causes an elevation of 30 mm. Hg in a dog weighing 10 kilos, produces a similar rise, with remarkable uniformity, in dogs weighing up to 25 kilos. Table I shows that the response to a quantity of renin which will raise the blood pressure 30 mm. Hg or more is independent of the body weight of the test animal. Only one dog out of 40 has consistently given a much greater response to this quantity of renin. The arbitrary dog unit of renin has therefore been defined as "the minimum quantity which will raise the direct mean femoral blood pressure of three unanesthetized dogs weighing between 10 and 25 kilos at least 30 and not more than 35 mm. Hg in 3 minutes."

As a rule, the animals have been used for the assay of renin only once a day, but

reliable results can be obtained if the dogs are used more frequently, provided an interval of several hours is allowed between the injections. Table II shows the uniformity of the values obtained in four dogs as a result of two successive intravenous injections of a unit dose of renin, with an interval of 2 hours between injections. There is no indication of the development of tachyphylaxis from one dose of renin after an interval of 2 hours. To avoid injury to the vessels, however, it is better to have a large colony of test animals and to use them not more than once a day. Table II also shows that the effect on the blood pressure of a unit dose of renin is independent of the body weight of the test animals.

That the response of the blood pressure to the injection of two, three, and five times the unit dose of renin, is not directly proportional to the amount of renin but that the response does increase with larger doses is well indicated in Table III. There is a greater variation in the response of different dogs to an intravenous injection of more than one unit of renin.

#### *Determination of Purity of a Sample of Renin*

If an estimate of the purity of renin in a sample of renal extract is desired, both the number of units of renin per cubic centimeter of extract and the amount of nitrogen in milligrams per cubic centimeter of extract should be determined. Purity can then be expressed as the number of units of renin per milligram N. This affords the best method for the comparison of the purity of renal extracts prepared by different investigators.

#### SUMMARY

A simple, rapid method for the bioassay of renin has been presented. Reliable and consistent results are obtained by this method whereby an arbitrary dog unit of renin has been established.

The response of normal unanesthetized dogs to renin is independent of the body weight of dogs weighing between 10 and 25 kilos. An estimate of potency of renin per kilo of body weight of the test animals is therefore not only unnecessary but misleading.

A dog unit of renin has been defined as that amount which raises the blood pressure at least 30 and not more than 35 mm. Hg within 3 minutes in at least three unanesthetized dogs. The *potency* is expressed as dog units of renin per cubic centimeter.

The determination of the amount of renal substance represented by a cubic centimeter of extract and the number of dog units per cubic centimeter give an estimate of the *yield* of renin which can be expressed as dog units of renin per gram of original renal tissue.

By determination of the number of dog units per cubic centimeter and the amount of nitrogen in milligrams per cubic centimeter in any renal extract the *purity* of the renin can be determined and expressed as dog units of renin per milligram N.

The establishment of a standard method for the bioassay of renin is considered highly desirable and the method outlined in this paper is suggested for this purpose.

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