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## Substance in Kidneys and Muscle Eliciting Prolonged Reduction of Blood Pressure in Human and Experimental Hypertension.*

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Evidence published elsewhere[1-8] strongly suggests that an inhibitor to the pressor action of angiotonin and renin is contained in kidneys and probably muscle and lung.

*Methods.* Extracts prepared from fresh kidneys, lung and muscle were tested for their ability to reduce arterial pressure in dogs made hypertensive by the silk or cellophane perinephritis method.[8] Blood pressure was determined by direct femoral arterial puncture. Hypertensive rats (Drury method[9]) were also employed, the blood pressure being measured in the tail by the method of Williams, Harrison and Grollman.[10] The blood pressure of the animals was stabilized at the elevated pressure before extracts were assayed. Some of the dogs exhibited the syndrome of malignant hypertension. Extracts were given by injection subcutaneously, into muscles or by mouth.

The extracts have been tested also on patients with typical essential hypertension and malignant hypertension after the patients had been studied for long periods in the Lilly Clinic. The studies were conducted much as those described previously in evaluation of the various surgical treatments of hypertension (Page and Heuer[11]).

Several experiments have been performed to ascertain whether the

* A preliminary report of this work was made at the American Association for the Advancement of Science, Section N, Columbus, Ohio, December 27, 1939.

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10 Williams, J. R., Jr., Harrison, T. R., and Grollman, A., *J. Clin. Invest.*, 1939, **18**, 373.

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ability of plasma to activate renin has been reduced after administration of extracts. The method employed was that of Page and Helmer.[6]

*Extracts of Kidney and Muscle.* Extracts of minced tissues were prepared from pork kidney and from rat, rabbit and beef muscle by 6 general methods of extraction; (a) with saline, (b) with acetone, (c) with alcohol, (d) by fractional precipitation with $(NH_4)_2SO_4$, (e) by isoelectric precipitation of water and saline extracts, and (f) saline and acetic acid followed by heating to 58° C. The materials obtained were concentrated *in vacuo* at low temperatures, and, in most cases, dialyzed for 24 hours or more. The concentrated solutions were passed through Seitz filters before intramuscular administration to human beings. Details of the procedures used and results obtained are shown in the accompanying tables.

*Relative Potency of the Various Extracts in Dogs and Rats.* The filtrate from the isoelectric precipitation of saline extracts of kidney seemed most effective. The amount of extract equivalent to 300 g of original kidney when injected intramuscularly in dogs, over a period of 2 to 4 days, caused marked reduction in arterial pressure (Figs. 1 and 2). The isoelectric precipitate also contains some of the inhibitor.

TABLE I.
**Effect of Tissue Extracts on the Arterial Blood Pressure of Dogs with Hypertension.**

| Type of extract | Estimated* effectiveness | G of fresh kidney required to produce maximum fall in B.P. from hypertensive (>180 mm Hg) levels |
|---|---|---|
| Saline extract of whole kidney | +++ | 600 |
| Isoelectric precipitate of saline kidney extract | +++ | 400 |
| *Filtrate* of an isoelectric precipitate of a saline kidney extract | ++++ | 300 |
| 0.3 saturated $(NH_4)_2SO_4$ precipitate of saline kidney extract | ++ | — |
| 0.3 to 0.8 saturated $(NH_4)_2SO_4$ precipitate of kidney extract | 0 | — |
| 70% acetone extract of kidney | +++ | 2100 |
| 50 to 70% alcohol extract of kidney | ++ | 6000 |
| Extracts of lung | + | — |
| Filtrate of heated saline-acetic acid extract | ++++ | 960 |

*Results given in table are an estimate of the effectiveness of the extracts in reducing blood pressure based on the following grades:

+ Transient effect.
++ Some reduction in blood pressure but not reduced to normal level.
+++ Definite reduction in blood pressure but not sustained.
++++ Marked reduction (reduced to normal level), well sustained.


FIG. 1.
Aqueous extract equivalent to 120 grams of beef kidney given to a hypertensive dog.

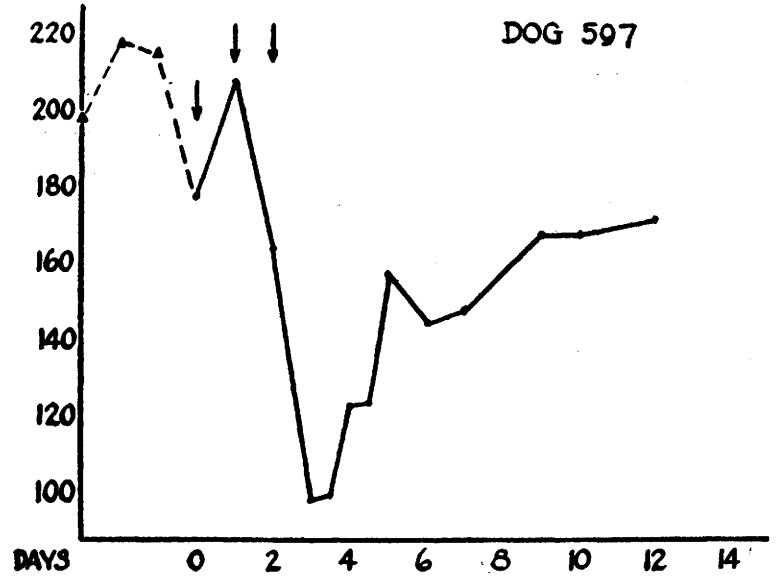


FIG. 2.
Filtrate after isoelectric precipitation equivalent to 100 grams of kidney daily.

The acetone extracts of the kidneys were effective. In only one instance did a 70% acetone extract fail to reduce the dog's pressure

when injected intramuscularly. The alcohol extracts were the least effective.

It will be noted from Table II that 70% acetone extracts of muscle, 50% alcohol extracts, and the isoelectric filtrates and redissolved precipitates at pH 4.2 are most active. Poor results were noted with 70% acetone extracts of rabbit muscle, in which an average dose of

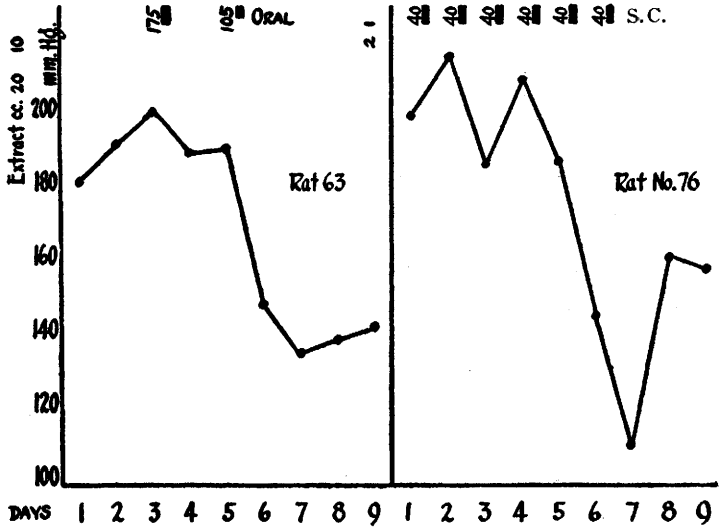


FIG. 3a.

Filtrate given orally after isoelectric precipitation equivalent to 380 grams of beef muscle.

FIG. 3b.

Filtrate given subcutaneously. Same extract as 3a.

TABLE II.

Effect of Muscle Extracts on Arterial Blood Pressure of Rats with Hypertension.

| Type of extract | Estimated effectiveness | G of fresh muscle required—Avg |
|---|---|---|
| 70% acetone | ++++ | 162 (105*) |
| 90% acetone precipitate from 70% extract | +++ | 190 |
| 90% " filtrate " " " | ++ | 220 |
| 50% " extract of 70% precipitate | + | 380 |
| 50% acetone extract | — | 360 |
| 50% alcohol " | ++++ | 190 |
| 70% " " | +++ | 520 |
| 50% alcohol in presence of 10% sodium chloride | — | 250 |
| 0.3 saturated ammonium sulphate | ++ | — |
| 0.3-0.5 saturated ammonium sulphate | + | — |
| 0.5-full saturated ammonium sulphate | ++ | — |
| Isoelectric precipitate | +++ | 119 |
| Filtrate of an isoelectric precipitate | ++ | 149 |
| Filtrate of heated saline-acetic acid extract | ++++ | 126 |

105* Average dose of rat muscle extract. 162 g includes both rat and rabbit.

250 g muscle had little effect, while 105 g of rat muscle seemed quite effective. The data at hand do not establish a similar difference between beef and rat muscle.

*Effect of Kidney and Muscle Extracts on Patients.* Eight patients have been treated with extracts. In all a sharp prolonged fall in arterial pressure occurred whether the hypertension was of the malignant or essential variety. Symptomatic improvement has been marked. After discontinuing the extracts for 3 to 5 days the blood pressure tends to rise again. What we consider to be a typical curve is illustrated in Fig. 4.

*Effect of Kidney Extracts on Renin- and Angiotonin-Activator.* Plasma from hypertensive dogs exhibits greater than normal ability to cause vasoconstriction when renin or angiotonin is added to it and the mixture perfused through an isolated rabbit's ear (Table III). When the arterial pressure is reduced by means of kidney extracts, this enhanced ability is reduced or lost.

*Discussion.* There seems little possibility that the observed prolonged fall in arterial pressure could be due to any of known depressor substances for 3 reasons. First, the fall occurs very slowly—from 12 to 48 hours—depending on dosage. Second, all crystalloidal

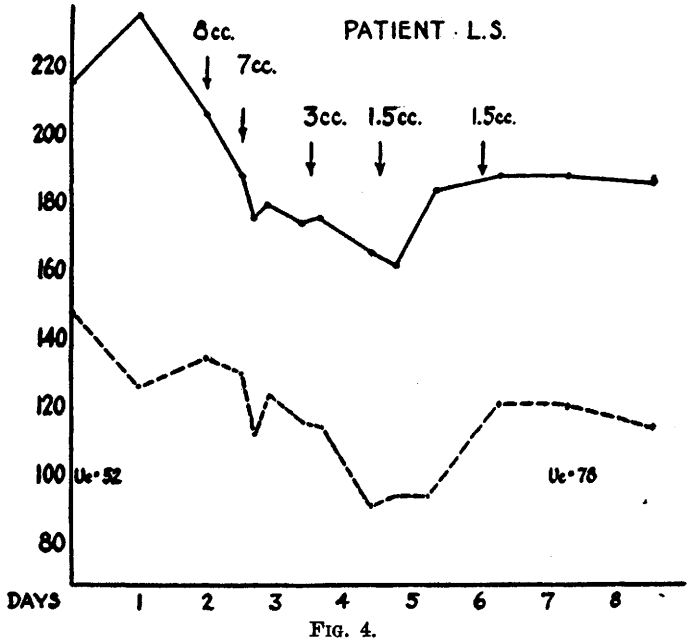


FIG. 4.

Administration subcutaneously of isoelectric filtrate equivalent to 800 grams of kidney to a patient with malignant hypertension. Urea clearance before extract was 52 percent of normal and 7 days later 78 percent of normal.

TABLE III.
Effect of Injection of Kidney Extracts on Activator Content of Hypertensive Dog's Blood.
Dog No. 391—Uninephrectomy plus cellophane on remaining kidney.

| Date | Initial drop rate per min. | Substance perfused | Reduction of flow time in min | % reduction of flow | B.P. in mm Hg. | Extract |
|---|---|---|---|---|---|---|
| | | cc | | | | |
| 11-20-39 | 146 | .2 plasma of dog No. 391 | 0 | 0 | 184 | None |
| | 141 | .2 " normal dog | 0 | 0 | | |
| | 140 | .2 normal dog plasma + renin | 1 | 39 | | |
| | 145 | .2 No. 391 + renin | 4 | 68 | | |
| 12-6 | 131 | .2 No. 391 | 0 | 0 | 112 | Saline extract of kidney, concentrated |
| | 135 | .2 normal plasma | 0 | 0 | | |
| | 132 | .3 No. 391 + renin | ½ | 20 | | |
| | 135 | .3 normal plasma + renin | 3 | 42 | | |
| | 132 | .2 angiotonin + 0.2 cc No. 391 | 1½ | 31 | | |
| | 130 | .2 " + 0.2 " normal plasma | 4 | 61 | | |
| 12-20 | 147 | .2 normal plasma | 0 | 0 | 176 | None |
| | 146 | .2 No. 391 | 0 | 0 | | |
| | 150 | .3 normal plasma + renin | 1½ | 42 | | |
| | 147 | .3 No. 391 + renin | 5½ | 81 | | |
| | 144 | .2 angiotonin + 0.2 cc normal plasma | 1½ | 29 | | |
| | 148 | .2 " + 0.2 " No. 391 | 4½ | 64 | | |
| 1-29-40 | 95 | .1 normal plasma | 0 | 0 | 52 | 50% alcohol oral extract |
| | 94 | .1 No. 391 | 0 | 0 | | |
| | 91 | .1 normal plasma + renin | 2 | 52 | | |
| | 94 | .1 No. 391 + renin | 1 | 20 | | |
| | 92 | .2 No. 391 + 0.2 cc angiotonin | ½ | 12 | | |
| | 93 | .2 normal plasma + 0.2 cc angiotonin | 2 | 39 | | |

substances would have been lost during dialysis, for the inhibitor is non-dialyzable. Third, the reduction in pressure remains for several days in contradistinction to the transient reduction resulting from injection of histamine, choline, adenylic acid, etc.

Injection of kidney and muscle extracts reduce or abolish the ability of the plasma to activate renin and angiotonin. This may be interpreted as either reduction in the amount of activators or increase in the amount of inhibitors.

It has seemed of especial interest that in 4 dogs suffering from the syndrome of malignant hypertension that injection of extracts has reduced their blood pressure and caused improvement in the signs in the eyegrounds. Two human beings with malignant hypertension have also been treated. The fall in arterial pressure has been striking (Fig. 4). Urea clearance in these patients also exhibited a change for the better. It is too early to assert that over prolonged periods these extracts will have therapeutic value. Animal tests suggest that they may have value.

Most of our extracts have been given by injection, but the few that we have tried by mouth in rats have proven effective. Harrison, Grollman and Williams were the first to show that kidney extracts were effective by mouth.†

*Summary.* Extracts of kidney and muscle effectively reduce arterial pressure in hypertensive human beings, dogs and rats for prolonged periods and simultaneously reduce the ability of plasma to activate renin and angiotonin.

† The present results on extracts of kidneys, although arrived at by an entirely independent route of investigation, are in accordance with those of Harrison, Grollman and Williams which are now in press.