does react with a phenylhydrazine reagent developed in this laboratory. The substance shows absorption maxima at 285 and 390 mμ when mixed with concentrated sulfuric acid.

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Received June 23, 1953. P.S.E.B.M., 1953, v83.

Factors Influencing Renin Diuresis and Proteinuria.* (20441)

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Renin, injected subcutaneously, elicits a variety of abnormal phenomena associated with acute, widespread necrotizing vascular lesions in bilaterally nephrectomized dogs(1) and in uninephrectomized rats pretreated with adrenal cortical steroids(2-5). Administration of excess salt is an essential conditioning factor to the vascular disease elicited by renin. This association and the nature of the lesions suggested that renin might act deleteriously through its effects on electrolyte distribution and on permeability of vessel walls.

In this connection, renin is known to cause increased diuresis and proteinuria in rabbits (6-8) and rats(9-11); the diuresis is associated with changes in electrolyte output(7, 12); the proteinuria is attributed in part to increased permeability of glomerular capillaries(11). The association of renin diuresis with proteinuria was therefore studied as were also the factors which condition these two phenomena since it seemed that such a study might aid in further defining the pathogenetic mechanisms of renin-induced vascular disease.

The particular aspects investigated were: 1) effects of subcutaneous versus intraperitoneal administration; this also involved a comparison of two renin preparations and observation on the effects of subtotal hepatectomy; 2) effects of providing water, sodium chloride and potassium chloride as drinking fluids; 3) effects of adrenalectomy and replacement therapy; 4) effects of hypophysectomy and of ACTH and somatotrophin and 5) effects of large doses of angiotonin.

Procedure. Except as indicated below, the procedure was that male albino rats of uniform strain were placed in metabolism cages 48 hours before injection of renin and kept there for 48 hours after cessation of treatment; urine flow and proteinuria (determined by the Shevky-Stafford method) were measured daily. This general plan, in which each animal is his own control, was required because of the wide variability of urine flow and proteinuria found in these rats as in those studied by Addis *et al.*(9); in some experiments, observations were repeated several times before trends or differences could be established. Since antibodies might interfere with the responses to renin, all animals were discarded from the study after a course of renin treatment.

1. *Route of administration.* The route of administration and the nature of the renin preparation used were of particular interest because of the report(13) that renin given intraperitoneally is much more diuretic than when given subcutaneously. Preliminary experiments had indicated to us that the reverse was the case; the difference in these observations might result from differences in the non-renin content of the preparations used. Consequently, 2 porcine renin preparations were

* This investigation was supported by a grant from the National Heart Institute, National Institutes of Health, Bethesda, Md.

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TABLE I. Influence of Route of Administration, Nature of Renin Preparation and Salt on Renin Diuresis and Proteinuria.

Group		No. of animals	Avg body wt	Route of administration	Drinking fluid	Avg urine flow in cc/24 hr (a)	(b)	(c)	Avg proteinuria in mg/24 hr (b)
I	G	12	203	s.c.	NaCl	20 (18.8–21)	74 (28–158)	27 (20–57)	71.3 (19.6–210)
II	G	14	186	i.p.	"	14 (8–25)	47 (3–80)	21 (15–35)	25.5 (2.2–92)
III	A	4	205	s.c.	"	18 (9–29)	75 (27–145)	21 (16–25)	99.6 (35–189)
IV	A	6	187	i.p.	"	16 (12–25)	32 (10–100)	26 (18–43)	19.8 (2.2–75.5)
V	G	10	185	s.c.	Water	8 (5–18)	17 (5–37)	7 (3–10)	3 (1.4–8.6)
VI	G	10	183	i.p.	"	6 (5–8)	15 (10–30)	13 (5–16)	10.7 (1.8–30.2)
VII	A	6	187	s.c.	"	6 (2–9)	14 (2–30)	6 (5–10)	3.1 (1.7–4)
VIII	A	6	187	i.p.	"	6 (5–8)	12 (2–22)	8.8 (4–12)	11.2 (1.5–25)
IX		8	188		NaCl		16 (6–27)		2 (1.1–3.9)
X		9	200		Water		7 (2–12.5)		1.6 (0.6–3.5)

All treated rats (Group I to VIII) received 2 injections of renin (total daily dose 28 dog units). The source of renin is indicated by A (Armour) and G (Green). Values given are: (a) before, (b) during and (c) after, treatment.

studied. One, "G", similar to the one used in previous studies, was prepared by Dr. A. A. Green by isoelectric fractionation with ammonium sulfate, contained 15 mg of protein per cc and had a pressor activity of 1.9 Goldblatt dog units per milligram protein. The other renin, "A", was furnished by Dr. R. J. Seidel. Armour Laboratories, in the form of a powder which had an activity of 0.6 Goldblatt units per mg; this material was suspended in 1% NaCl prior to injections. The effects of subcutaneous and intraperitoneal injections of equipressor doses of both were compared; the dose used was 28 pressor units daily divided in 2 doses and continued over a period of 3 days. The reported difference between intraperitoneal and subcutaneously injected renin might also have been due to some effect of the liver on renin given intraperitoneally. To test this point, observations were made in subtotally hepatectomized animals. The operation consists in ablation of medial, right and left lateral hepatic lobes which amount to about ⅔ of the total organ. The hepatic insufficiency which results from this loss and from the fatty infiltration of the hepatic residue is maximum at about 24 hours postoperatively(14). In this study the animals were placed in metabolism cages immediately after operation and renin injections of the preparation "G" were made both intraperitoneally and subcutaneously.

2. *Effects of salts.* The effects of 1% NaCl and of 1.5% KCl solutions as drinking fluids were compared with those of water in animals given renin "G" and "A" intraperitoneally and subcutaneously.

3. *Adrenalectomy.* Adrenalectomy has been noted to impair renin proteinuria and inhibit the proteinuria of normal rats; adrenal cortical extract partly and cortisone and desoxycorticosterone wholly restore these functions (15). The present experiments were done in order to determine whether the association between renin diuresis and proteinuria persists after adrenalectomy and the effect on both of replacement therapy with hydrocortisone acetate (Hydrocortone, Merck), desoxycorticosterone acetate (DCA) and Lipoadrenal extract (Upjohn). Adrenalectomy was performed in

TABLE II. Influence of Subtotal Hepatectomy and Route of Administration on Renin Diuresis and Proteinuria.

Group	No. of animals	Avg body wt	Route of administration	Avg urine flow in cc/24 hr (b)	Avg urine flow in cc/24 hr (c)	Avg proteinuria in mg/24 hr (b)	Avg proteinuria in mg/24 hr (c)
I	3	459	i.p.	64 (27–115)	21 (12–33)	30.3 (12.2–48.7)	5 (3–7.1)
II	4	443	i.p.	93.8 (16–154)	22 (14–30)	106 (34.5–213)	8.7 (3.5–21.6)
III	4	405	s.c.	56 (7.5–121)	27 (17–31.5)	16.7 (2.5–45)	5.3 (4.5–6.7)
IV	4	425	s.c.	142.5 (99.6–209)	28.8 (17.5–52)	69.5 (29–120)	4.8 (3–9.4)

Rats in Groups I and III were subtotally hepatectomized. Total daily dose of renin: 28 dog units in 2 injections. Drinking fluid: 1% NaCl. (b) and (c) as in Table I.

TABLE III. Influence of Potassium and Sodium Chlorides on Renin Diuresis and Proteinuria.

Group	No. of animals	Avg body wt	Drinking fluid	Avg urine flow in cc/24 hr (a)	Avg urine flow in cc/24 hr (b)	Avg urine flow in cc/24 hr (c)	Avg proteinuria in mg/24 hr (b)
I	6	134	1% NaCl		11 (4–22)		4.5 (1.5–7.9)
II	6	134	1.5% KCl		11 (4–20)		3.1 (1.7–5.8)
III R	6	134	1% NaCl	11 (8–20)	59 (20–100)	18 (17–20)	40 (10.1–86.4)
IV R	6	141	1.5% KCl	11 (8–15)	16 (8–23)	12 (10–15)	4.5 (1.8–9.7)

Renin treated rats (R) received 2 daily subcut. injections totalling 28 dog units. (a), (b) and (c) as in Table I.

two stages, and the animals were maintained on 1% NaCl as drinking fluid during the experimental period. The steroids were given subcutaneously in daily doses of 0.5 or 1.0 mg; hydrocortisone was in saline suspension and DCA in oil. The Lipoadrenal extract was administered subcutaneously in a daily dose of 0.5 cc; this amount had the glycogenic activity of 0.5 mg of cortisone.

4. *Hypophysectomy.* Hypophysectomized rats were obtained from Hormone Assay Laboratories. These were maintained on a high-carbohydrate diet. The hormones tested were ACTH (Armour) and somatotrophin (Antuitrin Growth Hormone, Parke Davis), both in a powder form. ACTH had an activity of 1 LA-1-A unit per mg and Somatotrophin, an activity of 2.5 units per mg. Both were dissolved in 1% NaCl in a concentration of 10 mg per cc and injected intraperitoneally in 5 daily doses of 0.2 cc each.

5. *Angiotonin.* The angiotonin used was prepared by Dr. A. A. Green. Preliminary experiments with a cruder product and with mixtures of incubated renin and renin-substrate indicated that 35 pressor doses did not elicit diuresis or proteinuria.* The preparation used in final experiments was injected intraperitoneally every 30 minutes for 12 hours in a total dose of 247 units. Control animals were given similarly spaced equal volume of fluid (1% NaCl).

Results. 1. *Route of administration.* The data in general indicate a close association between the diuretic and proteinuretic effects of renin (Table I). In confirmation of orienting studies, both responses were greater when renin was given subcutaneously than when it was given intraperitoneally; this was true of both preparations used, in spite of the fact

* Since there is no generally accepted unit of angiotonin, a pressor dose is the amount which causes a rise of 15 mm Hg in blood pressure in an anesthetized dog.

that the greater inactive protein content of renin "A" might have altered its absorption and activity by one route or the other.

Subtotal hepatectomy impairs the diuretic and proteinuretic responses to renin administered either subcutaneously or intraperitoneally (Table II). The degree to which the operation impairs the response is greater than it would seem to be from the tabulated data; this is because the operation as such (unlisted observations) increases urine flow to a mean of 33 cc and proteinuria to a mean of 9.5 mg per 24 hours during the first postoperative day; these abnormalities receded over the next 48-72 hours.

2. *Water, NaCl and KCl.* The effects of water, 1% NaCl and equimolar KCl on basal diuresis and proteinuria are listed in Table I and III. NaCl alone as compared with water or KCl causes a slight increase in urine flow but no proteinuria. Both renin preparations, whether given intraperitoneally or subcutaneously, elicit greatly enhanced diuresis and proteinuria in rats given NaCl as compared with rats given water or KCl; provision of NaCl causes a greater proportionate increase in renin proteinuria than it does in urine flow; responses to renin in rats given KCl are roughly equal to those observed in animals maintained on water.

3. *Adrenalectomy.* The observations (Table IV) confirm the fact(15) that adrenalectomy inhibits renin proteinuria; supplemental observations are that adrenalectomy similarly inhibits the diuretic response and that these inhibitory effects are not restored by provision of 1% NaCl. Lipoadrenal extract alone, in the dose used, did not affect urine flow or proteinuria; however, replacement therapy with this extract, or with DCA or hydrocortisone all restored diuretic and proteinuretic responses to renin. The spread of the data is such that no decision can be reached as to the relative effects of the 2 steroids or of the 2 dose levels used.

4. *Hypophysectomy.* This operation, like adrenalectomy impairs diuretic and proteinuretic responses to renin, (Table V). Because somatotrophin has been observed to restore renal function and water diuresis in hypophysectomized dogs(16,17), it seemed likely that

TABLE IV. Influence of Adrenalectomy and Replacement Therapy on Renin Diuresis and Proteinuria.

Group and renin treatment	No. of animals	Avg body wt	Adrenal steroid and dose/day	Avg urine flow in cc/24 hr (a)	(b)	(c)	Avg proteinuria in mg/24 hr (a)	(b)	(c)
I 0	6	156	0		15			3.6	
II 0	5	159	L.A.E.	10.1 (5.5-14.5)	14 (8–29)	17 (11–21)	2.4 (0.9–4)	3.1 (0.6–9.1)	3 (2.1–4.5)
III R	9	160	0	11.6 (7.5–22)	26 (9–44)	22 (9–42)	3.5 (1.4–6.3)	7 (1.3–15)	4.1 (1.2–14.4)
IV R	6	162	L.A.E., .5 cc	12.9 (8–25)	62 (27–106)	20 (12–30)	2.3 (0.6–5.4)	34.2 (8.4–93.6)	6.1 (1.2–8.6)
V R	6	157	Hydrocortisone, 1 mg	16.5 (9–21)	39.1 (13–81)	22 (17–45)	3 (.14–6)	102 (20.6–233)	3.6 (1.7–4.5)
VI R	6	154	Hydrocortisone, .5 mg	8.1 (6–11)	31 (14–82)	32.7 (2.8–43)	2.7 (1.8–4.4)	18 (2.4–92)	5.1 (2.3–10.8)
VII R	5	157	DCA, 1 mg	23 (18.5–26)	31.4 (19–55)	23 (9–49)	4 (1.5–5.8)	17.6 (15–19.4)	3.8 (1.6–14.7)
VIII R	2	168	DCA, .5 mg	8.5 (5–12)	64 (25–103)	40 (39–40)	2.4 (1.5–3.5)	14.2 (2.6–48.2)	7.1 (2.8–11.5)

Dose of renin (R) was 28 dog units in 2 subcut. injections. Drinking fluid: 1% NaCl. (a), (b) and (c) as in Table I.

TABLE V. Influence of Hypophysectomy and Pituitary Hormones on Renin Diuresis and Proteinuria.

Group and renin treatment	No. of animals	Avg body wt	Pituitary hormone and daily dose	Avg urine flow in cc/24 hr	Avg proteinuria in mg/24 hr
I 0	6	145	0	13.3 (1–37.5)	1.3 (.1–2.7)
II R	6	147	0	17 (5.2–47.5)	2.1 (.4–30.3)
III R	4	138	ACTH 5 × 2 mg	38.2 (5–83.6)	13.9 (3.7–33)
IV R	4	145	STH 5 × 2 mg	12 (1–39.5)	2.7 (.4–6.9)
V 0	5	137	STH 5 × 2 mg	7 (1–12)	1.3 (.2–2.6)
VI 0	10	138	ACTH 5 × 2 mg	32 (11–72)	28.7 (4–67)

Daily dose of renin (R) was 28 dog units in 2 subcut. injections. Drinking fluid: 1% NaCl.

TABLE VI. Effects of Angiotonin on Diuresis and Proteinuria.

Group and treatment	No. of animals	Avg body wt	Avg urine flow in cc/24 hr (a)	Avg urine flow in cc/24 hr (b)	Avg proteinuria in mg/24 hr (a)	Avg proteinuria in mg/24 hr (b)
I Angiotonin	3	203	18.3 (11.6–27)	45 (40–49)	2.3 (1–3.5)	49.6 (14.4–102)
II 0	3	233	22.9 (19–30)	24 (15.2–32)	5.5 (2.2–8.1)	8 (3.3–17.3)

Total dose of angiotonin: 247 pressor doses in 24 intraperitoneal injections. Drinking fluid: 1% NaCl. (a) and (b) as in Table I.

it might restore responses to renin; this, under our experimental conditions, was not the case. The observations in adrenalectomized rats given replacement therapy suggested that ACTH would restore the renin responses of hypophysectomized animals; however, in the dose used, ACTH was diuretic and proteinuretic of itself, and additive responses to renin were negligible.

5. *Angiotonin.* The observations are summarized in Table VI; these demonstrate that, in the dose used, intraperitoneally injected angiotonin increases both urine flow and proteinuria.

Discussion. The observations demonstrate the close association of diuresis and proteinuria as responses to injection of renin and, since these responses also follow on injection of angiotonin, reaffirm Addis' conclusion(9) that these urinary changes are integral responses to the activity of the renal pressor system. Both responses to renin are impaired by adrenalectomy and restored by replacement therapy; both are similarly impaired by hypophysectomy, and somatotrophin does not restore them, while, in the doses used, ACTH caused diuresis and proteinuria which obscured any additive effect of renin.

In contrast to the observations of Croxatto (13) the diuretic and also the proteinuretic effect of renin was found to be greater when the renin was injected subcutaneously than when it was given intraperitoneally. This was true of two quite different renin preparations tested in equipressor doses, so that the difference between our results and theirs remains unexplained.

Subtotal hepatectomy was found to impair diuretic and proteinuretic responses to renin; this may reflect defective formation of renin-substrate or, as has been suggested, liberation of endogenous renin in response to operative trauma(18). The diuresis and proteinuria observed during the first 24 hours after operation are consistent with the latter view.

Potentiation of renin diuresis by adminis-

tration of NaCl solution as drinking fluid had been previously observed(13). This potentiation is shown to apply also to postrenin proteinuria; KCl is shown to be ineffective. Thus, the sensitization to the diuretic and proteinuretic effects of renin is dependent on provision of sodium as such; renin-induced acute vascular diseases are similarly dependent on administration of sodium and of steroids which affect sodium distribution; thus, it may be that renin diuresis and proteinuria are in normal animals, aspects of general phenomena which under specific conditions precipitate acute vascular lesions.

Summary. 1. Administration of renin elicits increases in urine flow and urine protein in rats; these phenomena are closely associated; both are impaired by adrenalectomy or hypophysectomy and restored, after adrenalectomy, by replacement therapy; under the experimental conditions neither somatotrophin nor ACTH could be shown to restore the impaired renin responses of hypophysectomized rats. 2. The two different renin preparations used were more effective in stimulating urine flow and proteinuria when given subcutaneously than when given intraperitoneally. 3. Subtotal hepatectomy impaired these responses to injected renin. 4. The administration of sodium potentiates the diuretic and proteinuretic effects of injected renin. 5. These responses can be paralleled by appropriate intraperitoneal administration of angiotonin.

The authors are indebted to Dr. E. Alpert, Merck and Co., for supplies of cortisone and hydrocortisone; Dr. H. Hailman, Upjohn Co., for Lipoadrenal extracts; Dr. K. Thompson, Organon Inc. for desoxycorticosterone; Dr. R. J. Seider, Armour Laboratories, for a renin preparation and Dr. E. C. Vonder Heide, Parke, Davis and Co., for antuitrin growth hormone.

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Received June 24, 1953. P.S.E.B.M., 1953, v83.