however, that the critical period would be longer with irritants causing a more permanent tissue damage and with longer acting conditioning agents. Thus it is known that the more chronic inflammatory response to croton oil can be turned into necrosis at almost any time by heavy overdosage with glucocorticoids(6,7).

It was noted some time ago that in rats conditioned with 5-HT, an otherwise well-tolerated ligature of the abdominal aorta causes extensive necrosis in the skeletal musculature affected by the reduction of blood supply(8). Hitherto unpublished work revealed furthermore that subcutaneous injection in normally well-tolerated amounts of several tissue irritants other than hypertonic solutions (*e.g.*, ethanol, $ZnCl_2$) also causes massive skin necrosis in rats conditioned with 5-HT or mast-cell dischargers. Apparently, just as the development of inflammation is subject to regulation by pro- and anti-inflammatory hormones so the resistance of tissues to necrosis can be decisively influenced by humoral agents. Any future attempt at the further elucidation of this control mechanism will have to take the critical period into account since (especially with short-acting tissue irritants) reactivity largely depends upon the length of time elapsing between administration of the conditioner and challenger.

Summary. Normally well-tolerated doses of hypertonic NaCl produce extensive tissue necrosis in the rat if administered simultaneously with serotonin or such mast-cell dischargers as dextran, cpd. 48/80 or polymyxin. This predisposition for necrosis is demonstrable only if the conditioning agent is administered during a brief "critical period" simultaneously with or shortly before the hypertonic solution.

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Received April 20, 1966. P.S.E.B.M., 1966, v122.

Unique Specificity of Mouse Angiotensinogen to Homologous Renin. (31291)

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It is well known that renin of one species may release angiotensin I, the precursor of angiotensin II, from renin substrate (plasma) of other animals. The reaction, however, does not proceed at equal rates between species. Substrates of primate origin resist conversion by non-primate renin(1), but substrates from non-primates are thought to react readily with all mammalian renins studied(2). In the white mouse, we have found a unique resistance of mouse angiotensinogen (plasma) to conversion to pressor substances by renin from all other species tested. In contrast, mouse renin reacts promptly with heterologous substrate. In other respects (response to nephrectomy and salt-loading), the mouse renin-angiotensin system resembles those of other mammals.

Material and methods. Inbred male white mice (Charles River strain) with weights of 25-50 g were routinely fed commercial ("Nafag") pellets and tap water *ad libitum*. In sodium-loading experiments, the pellets were soaked in 0.9% NaCl (500 ml/2.5 kg of pellets), then dried, and the animals given 1.0%

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NaCl as drinking fluid. Sodium loading was produced in unilaterally nephrectomized mice by the high salt diet plus subcutaneous injection of desoxycorticosterone acetate (DCA) in microcrystalline suspension at a dose of 200 mg/kg weekly for 3 weeks.

To obtain mouse plasma with a high concentration of renin substrate, bilateral nephrectomy was performed under ether anesthesia and the animals bled 16-20 hours after operation. Blood was collected under ether anesthesia from the femoral artery in centrifuge tubes containing sodium ethylenediamine tetra-acetate (EDTA). The EDTA was used to inhibit angiotensinases and was added in sufficient quantities to give a final concentration of 0.38% in the resultant plasma. Following centrifugation for 20 minutes at 2,500 r.p.m., nonhemolyzed plasma samples were pooled. Generally, 0.5-0.75 ml of plasma could be obtained from each animal. The pooled plasma served as the source of substrate for studies of reactivity with various renins. This technique was also used to obtain pooled plasma from normal mice, nephrectomized rats, and nephrectomized rabbits.

For the preparation of renin, 300 mg of fresh renal tissues, obtained from slices of the distal end of kidneys, were homogenized in 2.0 ml of isotonic saline and treated as described(3). By this procedure renal extracts with a high concentration of renin were obtained from rabbits, rats, hamsters, and mice. Partially purified hog renin was obtained commercially.† Purified, angiotensinase-free human renin was kindly provided by Dr. John H. Laragh, New York.

To measure the release of pressor materials from renin substrate (plasma) by the various renins, 1 ml of plasma was incubated at pH 5.5 and 37°C with 0.1 ml of freshly prepared kidney extracts or 0.1-4.0 Goldblatt units of hog or human renin. Incubation times were varied between 10 and 120 minutes. In several experiments, mouse plasma was incubated with heterologous renins at either a pH of 7.3 or a temperature of 40°C. Following incubation, the enzymatic reaction was stopped by placing the samples in a boiling water bath for 5 minutes. The incubates were cooled in ice water, then centrifuged at 2,500 r.p.m. for 30 minutes. The supernatant fluids were analyzed for vasopressor activity by bio-assay in nephrectomized rats and responses compared with standard doses of Val^5-angiotensin II-amide (Hypertensin, CIBA)(4). In experiments in which Val^5-angiotensin II-amide was added to either mouse or rat plasma in the absence of renin, quantities between 12.5 and 50.0 ng of the synthetic angiotensin were recovered without measurable loss after incubations of 10-30 minutes.

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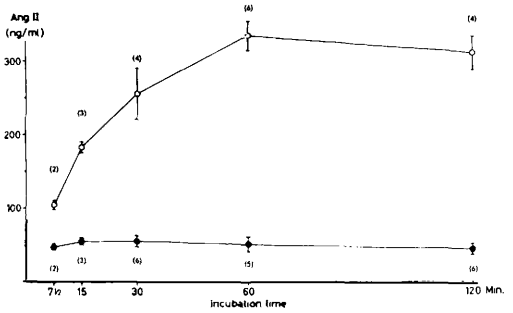

FIG. 1. Yield of angiotensin-like substance from plasma of normal (black dots) and nephrectomized (white dots) mice by incubation with mouse kidney extracts. *Ordinate:* Pressor activity expressed in ng/ml of the reference standard, Val^5-angiotensin II-amide. *Abscissa:* Incubation time in min. Means ± SEM. Numbers in parentheses indicate number of pools of mouse plasma used to derive the corresponding mean.

Results. 1. *Yield of angiotensin-like material from plasma of normal and nephrectomized mice by mouse kidney extracts.* In plasma from normal mice, the yields of angiotensin-like material after incubation with mouse kidney extracts were low (Fig. 1). Increase of incubation time caused no significant change in concentration. In contrast, the quantities of pressor substances released from nephrectomized mouse plasma were consistently greater (Fig. 1) and increased progressively with incubation periods up to 60 minutes, but fell slightly by 120 minutes. The pattern of concentrations of angiotensin-like substance released from mouse plasma following incubation with mouse kidney extract resembles those observed for plasma derived from rats(5), but the resultant concentrations were quantitatively less than those obtained in the latter species.

TABLE I. Production of Angiotensin-Like Substance by Incubation of Plasma from Nephrectomized Mice, Rats, and Rabbits with Kidney Extracts from Several Mammalian Species. + indicates production, — indicates absence of angiotensin-like substance.

Source of renin	Source of plasma substrate from nephrectomized animals		
	Mouse	Rat	Rabbit
Mouse kidney extract	+	+	+
Rat kidney extract	—	+	+
Hamster kidney extract	—	+	+
Rabbit kidney extract	—	+	+
Hog renin	—	+	+
Human renin	—	+	+

2. *Studies of cross-reactivity between mouse substrate and mouse renin with those derived from other species.* In Table I are summarized the results of incubating substrate (plasma from nephrectomized animals) from mouse, rat, and rabbit with renin derived from several species. For these experiments, the supernatant fluids obtained after incubation, boiling, and centrifugation were directly assayed in nephrectomized rats, as described. Renin derived from mouse kidney extract reacted with mouse plasma and with those from rat and rabbit. However, renin from 5 representative species did not react with nephrectomized mouse plasma. Neither increased pH (7.3), increased temperature (40°C), nor prolonged incubation time (120 minutes) led to release of angiotensin-like substances from mouse plasma by the heterologous renins. Mouse kidney extract, however, reacted with mouse plasma under both conditions. In fact, the only other renin-like material yielding pressor substances following incubation with mouse plasma was an extract of mouse submaxillary gland(6). Submaxillary extracts from rats, hamsters, and rabbits, which do not react with homologous substrate(7), gave no reaction with mouse plasma either.

To determine whether renin of other species was inactivated by some factor in mouse plasma, hog renin was first incubated with nephrectomized mouse plasma; then nephrectomized rat plasma was added and the resultant mixture incubated for an additional period. Under these conditions, preservation of the enzyme activity of the hog renin was clearly demonstrable (Table II).

3. *Response to salt loading.* With DCA and salt loading in unilaterally nephrectomized mice, the concentration of renin in kidney extracts was unchanged after one week, but progressively decreased by the end of the third week of the experiment (Table III). In this respect, mice responded similarly to rats in which, however, pressor activity disappears completely from the kidneys after 3-weeks treatment with comparable or even lower doses of DCA and 1% saline as drinking fluid(3). The difference from control animals was further accentuated by the age-dependent, increased renin activity of kidney extracts from the control animals(8).

TABLE III. Renin Activity of Kidney Extracts (Expressed in ng Angiotensin II/g Kidney/Min) from Unilaterally Nephrectomized Control Mice and from DCA-Sodium Loaded Mice. Renin activity was measured by incubation of mouse kidney extract with pooled nephrectomized rat plasma.

	Duration of experiment		
	1 wk	2 wk	3 wk
Control	35.3 ± 2.8	72 ± 2.6	70.5 ± 2.2
DCA-sodium loaded	35.1 ± 1.5	28.8 ± 3.2	19.5 ± 1.5

Discussion. The resistance of mouse renin

TABLE II. Effect of Mouse Plasma upon Enzyme Activity of Hog Renin.

First incubation		Second incubation		Yield angiotensin II (ng/ml)
Material	Incubation time (min)	Addition to incubate	Incubation time (min)	
4 U hog renin in 0.1 ml				
+ normal mouse plasma, 1.0 ml	30	Nx rat plasma, 1.0 ml	30	2,450
+ Nx mouse plasma, 1.0 ml	30	*Idem*	30	2,331
+ 0.9% NaCl, 1.0 ml	30	"	30	2,275
+ normal mouse plasma, 1.0 ml	30	0.9% NaCl, 1.0 ml	30	0
+ Nx mouse plasma, 1.0 ml	30	*Idem*	30	0

Nx = nephrectomized.

substrate to renin obtained from other animals was an unexpected finding in the present study. In agreement with a previous report(8), mouse renin (kidney extracts) was observed to release angiotensin-like materials readily from heterologous renin substrate. Following nephrectomy or sodium-loading, the renin-angiotensin system in the white mouse gave responses which were qualitatively similar to those found in other species(3).

The specificity of mouse renin substrate for homologous renin was not due to inactivation of the heterologous renin, for hog renin retained its enzyme activity for other substrates after incubation with mouse plasma (Table II). Although the methods used in this study could detect small quantities of pressor substances (1.25 ng of angiotensin II/0.1 ml), it is possible that the resistance of mouse renin substrate is not absolute. Application of a more sensitive technique for detection of angiotensin-like materials(9), as illustrated by the studies of human substrate with non-primate renin(1), likely would demonstrate a slow release of pressor substances from mouse renin substrate by heterologous renins. Nevertheless, the resistance of mouse renin substrate to heterologous renin appears unique among non-primates.

It is unlikely that the enzymatic reaction between mouse renin and mouse renin substrate differs significantly from those occurring in other animals, for mouse renin reacts promptly to split heterologous renin substrate. Mouse renin substrate may be somehow protected from the reaction with heterologous renin or may differ in its basic structure from the substrate of other animals. Since the structure of mouse renin substrate is unknown, it can only be concluded that, under the conditions we used, the bond of mouse substrate is not split by heterologous renin enzymes.

Summary. Angiotensinogen contained in mouse plasma reacts exclusively with mouse kidney extract containing renin, to release angiotensin. Renin or kidney extracts from other species (pig, rat, hamster, rabbit) as well as from man do not liberate angiotensin if incubated with mouse plasma substrate. Plasma from nephrectomized mice, when incubated with mouse kidney extract, gave about 6 to 7 times higher yields of angiotensin-like substance. Administration of DCA together with salt loading for a period of 3 weeks reduced renin activity in mouse kidneys, but not as markedly as in rats. The resistance of mouse renin substrate to heterologous renin appears unique among non-primates.

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