Role of the Liver in the Regulation of Plasma Angiotensinogen and Renin Levels[1] (36131)

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The role of the liver in the regulation of plasma levels of angiotensinogen, the substrate of renin, is not clear. Page *et al.* (1) found that plasma angiotensinogen, then called renin activator, was decreased to undetectable levels by hepatectomy. On the other hand Leloir *et al.* (2) found that angiotensinogen levels were not significantly altered when hepatectomy was associated with nephrectomy. More recently it has been reported (3, 4) that reduction of liver mass or administration of carbon tetrachloride did not affect substrate levels nor prevent the rise in substrate normally seen after bilateral nephrectomy. In most of these studies, only angiotensinogen levels were measured.

The purpose of the present experiments was to reexamine the participation of the liver and kidneys in the regulation of substrate levels by studying the effects of hepatectomy alone or in combination with nephrectomy, of subtotal hepatectomy and of carbon tetrachloride poisoning on both angiotensinogen and renin concentration in plasma.

Methods. 1. Total hepatectomy and nephrectomy. Female Sprague-Dawley rats, weighing around 150 g, were hepatectomized according to the procedure of Bollman and Van Hook (5). About 2 months after the vena porta had been partially constricted and the vena cava was ligated, animals were divided into four groups. Animals of group 1 were hepatectomized; those of groups 2 and 3 were nephrectomized and then hepatectomized, and those of group 4 were hepatectomized and then nephrectomized. Removal of the liver was done immediately after nephrectomy in group 2 and 2 hr later in group 3. In group 4, kidneys were removed immediately after hepatectomy. Numbers of animals per group at the end of the experiments were respectively 6, 6, 10, and 10.

After hepatectomy, animals were kept in restraining cages at a room temperature of 20° and received intravenous infusion of dextrose at a rate of 15 mg/100 g/hr. Blood samples were taken under ether anesthesia from the jugular vein 1 week prior to hepatectomy and from the aorta shortly before death after hepatectomy. An additional sample was drawn from the animals of group 3 two hours after nephrectomy just before hepatectomy.

2. Partial hepatectomy. The following experiments were performed to see whether reduction of liver mass would interfere with the response to angiotensinogen-stimulating factors such as nephrectomy or diethylstilbestrol. Animals were divided into 9 groups of at least 6 animals each (Table II). Animals of group 1 were normal controls; those of groups 2 and 3 were sham operated; those of groups 4 to 7 were either partially hepactectomized, nephrectomized, or partially hepatectomized–nephrectomized; finally those of groups 8 and 9 were normal or partially hepatectomized animals treated with diethylstilbestrol. Animals of groups 6 and 7 were all partially hepatectomized, but nephrectomy was performed immediately after partial hepatectomy in group 6 and 16 hr later in group 7. Partial hepatectomy consisted in the removal of the medial, right and left lateral lobes which amounts to about 70% of the total organ. Diethylstilbestrol in oil solution was administered subcutaneously at the dose of 1 mg. The experiments were terminated 7 or 24 hr after the last surgical intervention. Blood samples were withdrawn from the aorta following anesthesia with sodium amobarbital (9 mg/100 g of body wt).

3. Carbon tetrachloride intoxication. Rats

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weighing 200 g were divided into 6 groups of at least 8 animals each (Table IV). Animals of group 1 were normal controls and those of group 4 were 24 hr nephrectomized controls. Carbon tetrachloride was administered subcutaneously as follows: one daily injection of 0.2 ml (groups 2 and 5) or 0.3 ml (groups 3 and 6) during 2 days. Animals of groups 5 and 6 were also nephrectomized at the time of the second injection. Animals were anesthetized with amobarbital 24 hr after the last injection and bled. Pieces of liver were removed for histologic examination.

Blood samples were collected in a syringe moistened with a 0.3 *M* solution of disodium EDTA as anticoagulant and were used for determination of angiotensinogen (6) and renin concentration (7). The angiotensin formed was bioassayed in pentolinium-treated rats using val-5-angiotensin II amide (Hypertensin, Ciba) as standard. Angiotensinogen concentration is expressed in nanograms of angiotensin II per milliliter of plasma and renin concentration in nanograms of angiotensin II per milliliter of plasma per hour of incubation. Values given are means with standard deviations.

Results. 1. Effects of hepatectomy and nephrectomy (Table I). *Survival time.* Survival time was variable, depending presumably on the extent of the collateral circulation around the constriction on the vena porta. Each group included animals which survived up to 6 or 7 hr. Data from animals with survival under 2 hr were discarded.

Measurement of arterial pressure by direct cannulation of the carotid artery showed that the operative procedures had little effect, with mean pressure varying between 80 and 105 mm Hg until about 0.5 hr before death when it fell around 50 mm Hg.

Angiotensinogen concentration. Partial constriction of the portal vein had no significant effect on substrate levels in plasma. Control values obtained about 2 months after constriction were not significantly different from normal; they averaged between 316 ± 58 and 397 ± 89 ng compared with the average value of 352 ± 45 ng of angiotensin/ml found in normal females. Following hepatec-

TABLE I. Effects of Hepatectomy Alone or in Combination with Nephrectomy on Angiotensinogen and Renin Concentration in Plasma.

Group and procedure	Survival time (min)	Plasma angiotensinogen (ng angiotensin/ml)			Plasma Renin (ng angiotensin/ml/h incubation)		
		A[a]	B[a]	C[a]	A[a]	B[a]	C[a]
1. Hepatectomy	338 ± 82	364 ± 128		Not detectable	94 ± 44		9282 ± 6864
2. Nephrectomy[b] + Hepatectomy	358 ± 96	378 ± 78		192 ± 74	39 ± 19		70 ± 80
3. Nephrectomy[b] + Hepatectomy	230 ± 105	397 ± 89	498 ± 122	349 ± 98	46 ± 23	3.4 ± 5.5	0.8 ± 0.4
4. Hepatectomy[b] + Nephrectomy	217 ± 102	316 ± 58		203 ± 74	38 ± 24		64 ± 44

[a] Values before surgery (A); and before death (C). Values in B were obtained 2 hr after nephrectomy just prior to hepatectomy.
[b] Both surgical operations were performed in succession except in group 3 where there was an interval of 2 hr.

tomy alone, angiotensinogen rapidly decreased to undetectable levels, whether animals survived for 3 or 7 hr. This effect was partially prevented by nephrectomy. When nephrectomy was performed immediately before (group 2) or immediately after (group 4) hepatectomy, substrate remained in plasma but at significantly lower levels ($p < .005$ in each case). When nephrectomy was performed 2 hr before hepatectomy, there was a small but significant increase ($p < .05$) after 2 hr, which was subsequently erased by hepatectomy (group 3).

Renin Concentration. Plasma renin concentration was not significantly affected by partial constriction of the portal vein. The averaged values of 39, 38, and 46 ng of angiotensin/ml/hr incubation in groups 2 to 4 are similar to the average value of 47 ng previously reported in normal plasma withdrawn under the same conditions (7). Three rats were responsible for the high average of 94 ng in group 1.

Hepatectomy alone caused a remarkable increase in renin concentration about 100 times above control levels. In one rat, plasma renin rose from 50 to 10,333 ng. The extent of the increase was not related to survival time. In one instance there was a 50 time increase after a survival of 7 hr, while in another, there was a 160 time increase after 3 hr. Such high increases in plasma renin were prevented by nephrectomy. When nephrectomy immediately preceded (group 2) or immediately followed (group 4) hepatectomy, there was a slight increase which was not significant ($p > .05$). In group 3, nephrectomy alone caused a rapid and marked decrease or disappearance of renin during the next 2 hr. The presence of large amounts of renin in the plasma of hepatectomized rats (group 1) was confirmed by the elevated pressor responses to the intravenous administration of unincubated plasma into 18 hr bilaterally nephrectomized rats. As shown in Fig. 1, injection of 0.2 ml of plasma from a hepatectomized rat with a plasma renin concentration of 8430 ng elicited a marked pressor response. The prolonged and sustained response is characteristic of renin. On the other hand, injection of 0.2 ml of plasma

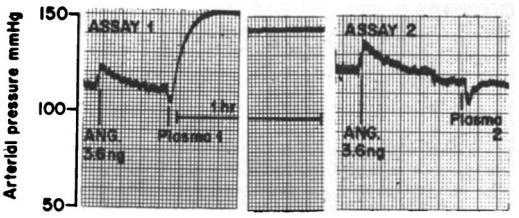

FIG. 1. Pressor responses to injection of plasma from an hepatectomized rat (assay 1) and from a nephrectomized–hepatectomized rat (assay 2).

from a nephrectomized–hepatectomized rat with a plasma renin concentration of 28 ng had no effect. Plasma from normal rats elicited no pressor response.

2. Effects of partial hepatectomy (Table II). Sham hepatectomy or nephrectomy had little effect on substrate. Substrate levels after partial hepatectomy in 18 rats were not significantly different from those in either sham operated or normal animals because of large variations. This variability was observed in three series of experiments included in group 4. As expected, nephrectomy increased substrate levels; this effect was not modified whether nephrectomy was performed at the time (group 6) or 16 hr after

TABLE II. Effects of Partial Hepatectomy as Modified by Nephrectomy and Stilbestrol Treatment.

Groups and procedures	Time[a] (hr)	Plasma angiotensinogen (ng of angiotensin/ml)
1. Normal controls	—	370 ± 63
2. Sham nephrectomy	7	375 ± 30
3. Sham partial hepatectomy	24	360 ± 73
4. Partial hepatectomy	24	320 ±190
5. Nephrectomy	7	1485 ± 98
6. Partial hepatectomy + nephrectomy[b]	7	1390 ± 44
7. Partial hepatectomy + nephrectomy[b]	7	1640 ±296
8. Stilbestrol[c]	24	577 ± 98
9. Partial hepatectomy + stilbestrol[c]	24	568 ±100

[a] Time indicates intervals after surgery at which blood was drawn.

[b] Nephrectomy was performed immediately after hepatectomy in group 6 and 16 hr later in group 7.

[c] One injection of 1 mg.

partial hepatectomy (group 7). Similarly the angiotensinogen-stimulating effects of stilbestrol were not altered by partial hepatectomy. Determination of plasma renin concentration in 6 partially hepatectomized animals of group 4 showed a wide range of values from 18 to 900 ng of angiotensin (Table III) giving

TABLE III. Plasma Angiotensinogen and Renin Concentration After Partial Hepatectomy.

	Rat nos.					
	1	2	3	4	5	6
Angiotensinogen[a]	140	180	240	280	360	720
Renin[b]	900	410	?34	148	144	18

[a] Angiotensin (ng/ml).
[b] Angiotensin (ng/ml/hr incubation).

an average which is statistically different from normal ($p < .001$). When these values are listed with those of substrate concentration, they show an inverse relationship.

3. Effects of carbon tetrachloride (Table IV). Carbon tetrachloride caused a decrease

TABLE IV. Effects of Carbon Tetrachloride and Nephrectomy on Plasma Angiotensinogen Concentration.

Group	Procedure	Plasma angiotensinogen (ng of angiotensin/ml)
1	—	362 ± 48
2	CCl_4, 0.2 ml	226 ± 38
3	0.3 ml	157 ± 23
4	Nephrectomy	2186 ± 515
5	Nephrectomy + CCl_4, 0.2 ml	2552 ± 703
6	0.3 ml	2325 ± 569

in plasma substrate which was dose dependent, with p values $< .005$ with the dose of 0.2 ml and $< .001$ with the dose of 0.3 ml. The effects of nephrectomy on substrate were not modified by carbon tetrachloride. On the other hand, carbon tetrachloride had little effect on plasma renin concentration, which averaged 27 ± 12 ng/ml/hr incubation with the dose of 0.2 ml and 37 ± 20 with the dose of 0.3 ml compared with a control value of 38 ± 19. The first experimental value is barely different from normal ($p = .05$). Pathologic examination of livers showed large hydropic cells and necrotic cells extending toward the middle of the lobule, while the liver took a yellowish coloration indicating some fatty degeneration. These lesions, which are characteristic of acute carbon tetrachloride poisoning were more severe with the higher dose.

Discussion. The present experiments confirm the early observation (1) that total hepatectomy causes a rapid decline in plasma angiotensinogen to undetectable levels. The conclusion that this effect was due to the removal of the site of production was contested by Leloir *et al.* (2), who showed that hepatectomy when associated with nephrectomy did not alter angiotensinogen levels. This, plus the fact that injection of renin caused a rapid and sustained disappearance of the substrate in hepatectomized–nephrectomized animals and only a slight and transient decrease in eviscerated but not hepatectomized animals, then led to the suggestion that the disappearance of substrate after hepatectomy might be due to absence of synthesis as well as to an increase in destruction caused by the large amounts of circulating renin presumably released during the operative shock (2). The present results not only confirm the effects of nephrectomy and hepatectomy but are also in accord with this interpretation by showing a 100-fold increase in plasma renin after hepatectomy alone. All these experiments strongly suggested but did not prove, that the liver was the site of substrate formation. Direct evidence for such a location has recently been presented (8).

Hepatectomy affects both the production and the destruction of renin. The state of shock resulting from the operative procedures is a strong stimulus of renin release. However, this factor alone does not explain the extraordinary high levels of circulating renin: during hemorrhagic hypotension, plasma renin concentration does not increase more than 10 times (9). This stimulus is complemented, however, by a decrease in destruction, since the liver is considered to be the major, if not sole, site of renin inactivation (10).

From these observations, it is becoming clear that the liver plays a dual role in the

regulation of angiotensinogen levels in plasma: (i) by synthesizing substrate; and (ii) by regulating the destruction of substrate through its control of renin metabolism. Thus disappearance of angiotensinogen from plasma after hepatectomy can be explained by an absence of synthesis together with an increased destruction of what remains in the circulation. This situation is similar to that seen clinically in cirrhosis and ascites, where plasma angiotensinogen is undetectable and plasma renin remarkably elevated (11). The fact that substrate levels still decreased significantly in nephrectomized–hepatectomized rats (group 3) indicates a mode of destruction other than by renin. It is not known whether substrate is destroyed by other circulating proteolytic enzymes or removed from the circulation for storage in vascular tissues where renin is present (12).

Partial hepatectomy has been widely used for the study of hepatic inactivation of hormones (13). This operative procedure, however, had no effect on plasma renin substrate, but increased plasma renin concentration. This would suggest that the various hepatic functions are diversely affected. Detoxifying and inactivating mechanisms would be impaired while some of those related to synthesis would remain intact, or even stimulated, to compensate for the reduction of cell mass. This is supported by the observation that partial hepatectomy specifically causes an increase in the relative synthetic rates of some protein fractions (14). It is not known, however, whether the substrate participates in this increase. Nevertheless, if this interpretation is correct, reduction of liver mass would impair renin inactivation and therefore increase plasma renin concentration as shown here, which in turn would tend to increase substrate destruction. The latter effect would, however, be compensated by an increase in synthesis, thus resulting in normal substrate levels. The fact that partially hepatectomized animals responded normally to stilbestrol and to nephrectomy suggests that the remaining liver tissue can be overstimulated.

Although carbon tetrachloride has also been used in studies on hormone inactivation by the liver, it differed from partial hepatectomy by decreasing substrate levels without significantly altering plasma renin. One reason for this difference may be the complexity of the effects of carbon tetrachloride, which involve the liver and to some extent the kidney. The decrease in plasma substrate may be due to a decrease in synthesis, which may be part of the marked inhibitory effect of carbon tetrachloride on protein synthesis (15). The inhibition of substrate synthesis appears to be overcome by nephrectomy. We have, on the other hand, no explanation for the maintenance of normal renin levels. Since one would expect that renin inactivation be reduced, one has then to assume that renin secretion is decreased to the same extent. We have, however, no information on this point. In contrast with our results, the lack of effect on substrate previously reported (4) was obtained during chronic experiments.

Summary. Hepatectomy in the rat caused the disappearance of plasma renin substrate and a 100-fold increase in plasma renin. The effects were partially inhibited by nephrectomy. Partial hepatectomy caused an increase in renin and no change in substrate, while carbon tetrachloride caused a decrease in substrate and no change in renin. The last two procedures did not prevent the rise in substrate seen after nephrectomy or stilbestrol treatment.

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