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Plasma Angiotensinase Activity in Cirrhosis.* (29455)

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Plasma angiotensinase activity is of theoretic interest in cirrhosis for several reasons. If such enzymatic activity reflects production of angiotensin(1) and thus aldosterone(2), increased activity would be expected in association with secondary hyperaldosteronism, as manifested by ascites and edema. Moreover, as activation of the renin-angiotensin-aldosterone system may result from decreased renal perfusion(2,3), plasma angiotensinase activity could be augmented by renal circulatory failure(4) in hepatic disease. An alternative suggestion is that, like other enzymes, angiotensinases might be released from the liver in abnormally great amounts as a result of hepatic injury(5), regardless of changes in production of angiotensin. Under such circumstances, the resulting acceleration of angiotensin degradation could contribute to the infrequency with which hypertension is said to occur in patients with cirrhosis(6,7) and might explain the reduced pressor responsiveness to angiotensin described in this disease(8).

The degradation of angiotensin has been studied in hypertension(1,9,10), edema(1), pregnancy(1,11), and toxemia(1,12). The conflicting results could come in part from use of assay methods based on different principles, namely, removal of radioactive iodine from the angiotensin molecule(10), destruction of its pressor activity(1), and release of valine measured by colorimetry(12). When the last method was applied to patients with various diseases(11), particularly high values were found in those with hepatobiliary disorders. Since the authors did not equate the rate of liberation of valine with the rate of pressor degradation, a biologic assay of angiotensinase activity in patients with cirrhosis seemed more desirable from the physiologic point of view.

Angiotensinase activity appears to have some substrate specificity, since plasma does not destroy the pressor activity of β-aspartyl angiotensin(13) nor does it inactivate arginyl[1] angiotensin, deaminoangiotensin or poly-O-acetylseryl angiotensin(14). The optimal pH and some ionic requirements are known. Klaus and associates(12) considered an aspartic acidaminopeptidase system responsible but conceded the participation of other aminopeptidases. However, a specific enzyme has not yet been identified. It is necessary, therefore, to refer to "plasma angiotensinase activity" until further purification reveals the peptidase system(s) responsible.

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The purposes of this investigation are, therefore, (a) to measure the degradation of the pressor activity of angiotensin by plasma from patients with cirrhosis; (b) to evaluate certain factors associated with hepatic disease which may influence plasma angiotensinase activity; and (c) to consider the possible consequences of alterations in plasma angiotensinase activity in patients with cirrhosis.

Material and methods. Observations were made on 29 patients with cirrhosis in a hospital (11 female and 18 male; average age, 52.9 years). In 17, the cirrhosis was associated with chronic alcoholism, whereas the cause of the disease was uncertain in the remaining 12. Diagnosis of cirrhosis was made on clinical examination supported by tests of hepatic function and, when necessary (16 patients), it was confirmed by histologic examination of hepatic tissue. Tests of hepatic function are those in standard use at the Mayo Clinic.

Angiotensinase activity was determined by a method(15) based on the degradation of pressor activity of synthetic valine-5 angiotensin II aspartic-β-amide by untreated plasma. Venous blood from fasting patients was placed in heparinized tubes and centrifuged. Blood samples showing evidence of hemolysis were discarded. Plasma was then frozen in 2 tubes and kept at —18°C for as long as one month. Plasma, 0.25 ml, from one tube was added to 0.75 mg of a fresh solution containing 2.66 μg of angiotensin per milliliter in 0.1 M phosphate buffer at pH 7.4 and incubated at 37°C for 20 minutes. The reaction was stopped by boiling for 2 minutes, and the resulting mixture was administered by jugular vein to a 200 to 250-g rat anesthetized with pentobarbital sodium. Tracheotomy, vagotomy and heparinization had been carried out previously and, pentapyrrolidinium bitartrate, 25 mg/kg, had been injected subcutaneously(16). Blood pressure was recorded by means of a mercury manometer connected to the carotid artery. An absolute control was obtained by boiling the patient's plasma immediately after mixing it with the angiotensin solution, whereas a "normal" sample was obtained by incubating with angiotensin pooled plasma from 25 normal subjects. Thus, each assay compared the angiotensinase activity of normal plasma with that of a patient. The 3 samples were injected alternately until each one had been given at least 6 times. The difference between the average pressor response of the normal sample and that from the absolute-control sample was considered to represent 100% angiotensinase activity. The difference between the pressor response of the patient's sample and that of the absolute control represented the patient's angiotensinase activity and was expressed according to the formula:

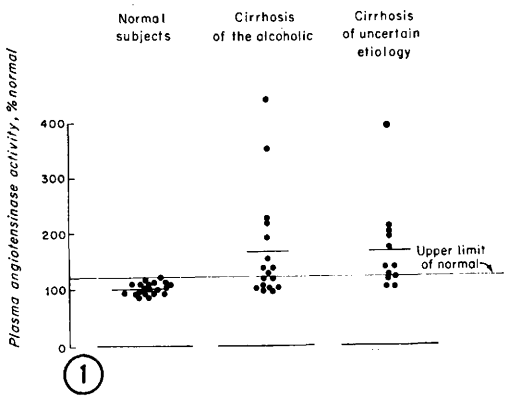
$$\frac{\dfrac{\text{mm Hg control} - \text{mm Hg patient}}{\text{mm Hg control}}}{\dfrac{\text{mm Hg control} - \text{mm Hg normal}}{\text{mm Hg control}}} \times 100.$$

All assays were done in duplicate, the second tube of plasma being incubated separately and assayed on a different rat. Reproducibility was within $\pm$ 15%. The normal range, as determined from a large series of healthy subjects tested against a pool of normals, was 80 to 120%. When plasma from 20 healthy subjects was tested individually against a pool of their own plasma, they yielded 99 $\pm$ 2% (mean and S.E. of mean) of normal angiotensinase activity.

Results. The mean plasma angiotensinase activity was significantly greater in patients with cirrhosis (164 $\pm$ 17% of normal; mean and S.E. of mean) than in normal subjects ($p<0.01$); 18 of 29 values were above or at the upper limit of normal (Fig. 1). Three patients exhibited striking elevations of plasma angiotensinase activity. Exclusion of these 3 values reduced the mean value from 164 to 138%; but did not alter the significance of the results.

Mean values of plasma angiotensinase activity were similar in alcoholics and nonalcoholics with cirrhosis (Fig. 1) but were greater in patients with more severe impairment of hepatic function. The latter was reflected by the presence of ascites, the depth of jaundice, or degree of derangement of tests of hepatic function (Table I).

Significant relationships were demonstrated



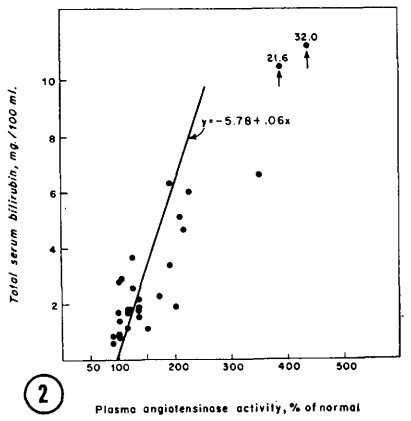


FIG. 1. Plasma angiotensinase activity in normal subjects and patients with cirrhosis. Mean values are indicated by horizontal lines.

FIG. 2. Relationship between plasma angiotensinase activity and total serum bilirubin concentrations. Correlation coefficient (r) = 0.83.

between increased plasma angiotensinase activity and the increased serum bilirubin concentrations (correlation coefficient = 0.83, $p<0.01$ (Fig. 2)) and, to a lesser extent, with the degree of Bromsulphalein (sulfobromophthalein) retention (correlation coefficient = 0.54, $p<0.05$). No other significant relationships were demonstrated between plasma angiotensinase activity and tests of hepatic function (table). Furthermore, plasma angiotensinase activity did not correlate significantly with height of the blood pressure or presence of ascites or azotemia (Table I).

Discussion. Plasma angiotensinase activity was significantly increased in our patients with cirrhosis, the degree of elevation being greater and more consistent than in other conditions hitherto reported(15). These results are in agreement with those of Klaus and associates(11), and the similarity is striking when the difference in methodology is considered. Activity was high also in the single case of cirrhosis reported by Hickler and associates(1). Failure to correlate angiotensinase activity with mean arterial pressure or with the presence of ascites argues against the increase in enzymatic activity reflecting an increased production of angiotensin in these patients. Angiotensinase elevations in our patients were associated primarily with deterioration of hepatic function, the latter being reflected both by clinical findings and by the appropriate tests. In particular, a re-

TABLE I. Plasma Angiotensinase Activity in Patients with Cirrhosis.

	Patients	Plasma angiotensinase activity, % of normal†
Cirrhosis		
Alcoholic	17	163.5 ± 23.8
Uncertain cause	12	165.8 ± 22.9
Arterial blood pressure, mean mm Hg		
<95	13	190.1 ± 29.9
95 or more	15	145.6 ± 17.9
Ascites		
Absent	11	156.3 ± 22.6
Present	18	169.4 ± 23.6
Blood urea, mg/100 ml		
<50	22	160.5 ± 17.0
50 or more	5	205.0 ± 62.3
Total serum bilirubin, mg/100 ml		
<1.5	7	106.4 ± 8.1
1.5 or more	22	182.9* ± 20.2
B.S.P. retention, %		
0-22	8	106.3 ± 7.1
23-40	10	131.0* ± 6.6
Serum albumin, g/100 ml		
>3.0	11	159.5 ± 26.2
3.0 or less	18	167.5 ± 22.2
Serum alkaline phosphatase, K.A. units		
<15	7	160.7 ± 36.8
15 or more	17	176.4 ± 23.4
Serum transaminase (G.O.T.), μmoles/hr/ml		
<5	16	144.1 ± 10.3
5 or more	8	192.5 ± 47.1

* Difference between mean values is significant ($p<.05$).
† Mean ± S.E.

lationship was established between height of serum bilirubin concentration and degree of Bromsulphalein retention and elevation of angiotensinase activity, thus suggesting a more specific association with cholestasis. Angiotensinases have been isolated from both liver tissue and bile(11), and liberation of these enzymes from damaged hepatic cells, a decrease in their biliary excretion associated with cholestasis, or an increase in production of the enzymes by the liver are all consistent with our results. Klaus and associates(11) found very high values in patients with hepatitis, metastatic disease of the liver, or biliary obstruction, as well as in those with active cirrhosis; in some instances, the observed fluctuations coincided with the degree of jaundice.

The present findings might account for the decreased pressor responsiveness of patients with cirrhosis to infusions of angiotensin(8), but they do not readily explain the paradoxic effect of the drug on excretion of sodium. The more rapid utilization of angiotensin resulting from plasma angiotensinase hyperactivity could contribute to the hypotension of hepatic failure(17) and, in a less extreme form, might explain the relative freedom from arterial hypertension reported by some authors (6,7) in association with cirrhosis.

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Effect of Acetoacetate on Adrenocortical Activity. (29456)

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Administration of acetoacetate in gradually increasing amounts for 21 days, to guinea pigs fed a diet completely lacking in vitamin C, was found to cause hyperglycemia and a rapid depletion of liver and muscle glycogen(1). Earlier studies to evaluate the role of the adrenals in mediating the deleterious effects of acetoacetate showed that acetoacetate administration for 21 days had no appreciable effect on adrenal weights and adrenal cholesterol and ascorbic acid contents of normal and scorbutic guinea pigs(2). However, acetoacetate increased greatly the adrenaline content of the adrenals of normal and scorbutic guinea pigs(3). As adrenaline enhances ACTH secretion, it was thought necessary to