(11). It is, of course, possible that stress, in some undefined way, may induce a non-specific enhancement of R.E. function; but such a hypothesis will not explain the observation that a circulating toxin in the shocked animal, which can convert reversible to irreversible shock, and which can induce tolerance to shock, is absent in shocked animals rendered tolerant to shock by pre-treatment with antibiotics, or by repeated prior exposure to shock plasma or endotoxin. In support of their hypothesis that non-specific resistance is involved in shock they report data purporting to show that while endotoxin can induce cross tolerance to shock from drum trauma, the reverse is not the case; for they state that sublethal drum trauma fails to induce cross-tolerance to endotoxin(11). A statistical examination of their data, however, indicates that sub-lethal drum trauma *does* induce significant cross-tolerance to endotoxin. Since the cross-tolerance so demonstrated does not proceed via classic immunochemical mechanisms, one cannot regard it as constituting adequate proof of the *chemical* identity of the shock toxin and endotoxin. Nevertheless, the demonstration of cross-tolerance in both directions (*i.e.* trauma/endotoxin and endotoxin/trauma) is best explained on the theory that endotoxemia is a concomitant of shock.

Summary. It has been demonstrated that whole plasma and saline extracts of lung, liver, spleen, and kidney of the shocked animal, but not the normal animal, are capable of inducing the generalized Shwartzman reaction in endotoxin or Thorotrast-primed recipients with the same severity and frequency as purified bacterial endotoxin. The active material may be isolated by extraction with trichloroacetic acid and dialysis in the manner employed for bacterial polysaccharides. A number of similarities are noted in the biologic effects of shock toxin and bacterial endotoxin. The appearance of the toxin in the circulation within minutes of the onset of shock and the prevention of its appearance by antibiotic pretreatment are discussed with respect to the endogenous or exogenous source of the toxin.

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Received December 17, 1957. P.S.E.B.M., 1958, v97.

A Vulnerable and Rate-Limiting Step in Urea Synthesis in Patients with Hyperammoniaemia.* (23768)

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The role of portal-systemic shunts, either spontaneously developed or surgically constructed, in the production of hyperammoniaemia and ammonia intoxication is well established(1,2,3) but there has been no direct evidence regarding vulnerability in man of the mechanism of synthesis of accumulated ammonia to urea. Animal experiments, how-

* This investigation was supported by Research Grant from N.I.H., P.H.S.

ever, have suggested that there may be a rate-limiting step in the Krebs-Henseleit ornithine cycle. Gornall and Hunter(4) showed that during incubation of rat liver slices, citrulline accumulated in the medium. Archibald(5) found that when he produced hemorrhagic shock in dogs, there was a 2-5 fold increase in plasma citrulline, a simultaneous depletion of circulating arginine, and depression of urea formation. Both of these studies suggested that conversion of citrulline to arginine may be a rate-limiting and vulnerable point in urea synthesis.

This study was designed to demonstrate whether or not such a specific point of breakdown in urea synthesis exists in patients with liver disease and hyperammoniaemia. This was done by analyzing peripheral venous blood from patients on the wards, for citrulline, arginine and ammonia.

Methods. Citrulline was measured by modification of the method described by Archibald(5). 0.57 ml of 2% Sigma urease powder was added to 4 ml of plasma for digestion. The digest was dialyzed against 13.5 ml of 0.02 N H_2SO_4 to obtain a larger volume to pass through the resin column containing amberlite IR-120 (H), analytical grade.† Resin columns were washed with 15% NaCl solution. The resin was then converted to the hydrogen form with several 10 ml washes of 3 N HCl. The final dialysate represented a 5-fold dilution of plasma. The colorimetric values were obtained as described by Archibald(5). Arginine was determined by performing the Sakaguchi reaction as described by Sims(6) on 4 ml of Folin-Wu blood filtrate. It has been shown by Weber(7) that 95% of the color which results when the Sakaguchi reaction is performed on blood filtrates is due to arginine. A standard curve using citrulline and arginine solutions of 4 different concentrations was run simultaneously with every citrulline and arginine determination. Ammonia was determined by modification of the microdiffusion technic of Conway(1).

Results. Fig. 1 shows scattergrams of

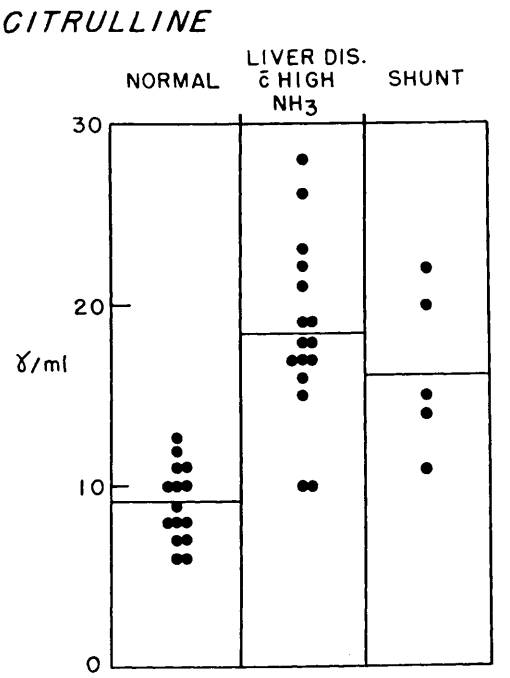

FIG. 1. Plasma citrulline levels in normal individuals and in patients with disorders of liver and portal circulation. Cross-bars represent calculated mean.

plasma citrulline concentrations in 3 groups of patients: (1) normal, (2) patients with liver disease and blood ammonia levels greater than 70 μg ammonia N/100 ml, (3) patients with surgically constructed portal-systemic shunts. Fifteen normal persons had an average plasma citrulline level of 9.2 μg/ml, range of 6.3-12.5 μg/ml. Sixteen determinations of citrulline on patients with various types of liver disease and blood ammonia-nitrogen levels greater than 70 μg/100 ml revealed an average plasma citrulline of 18.4 μg/ml, range 10.0-27.5 μg/ml. This difference is statistically highly significant, with a p value of less than 0.001. Five determinations of citrulline levels in patients with surgically constructed shunts showed average citrulline level of 16.2 μg/ml, range of 11.3-21.6 μg/ml.

Fig. 3 shows the relation between ammonia and citrulline levels in peripheral blood. The correlation coefficient of 0.66 with standard error of 0.18 confirms the relationship between the ammonia load and elevations in citrulline levels.

† Supplied through courtesy of Rohm & Haas Co., Washington Sq., Phila., Pa.

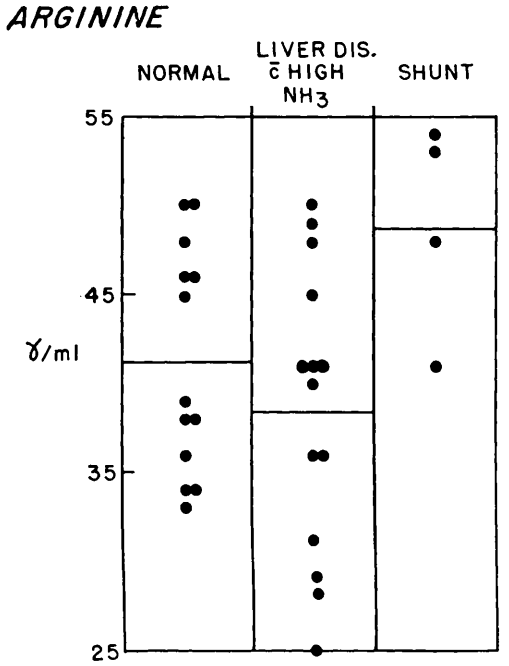

FIG. 2. Blood arginine levels in normal individuals and in patients with disorders of liver and portal circulation. Cross-bars represent calculated mean.

Fig. 2 is a scattergram of blood arginine concentrations in the same groups, although a few patients did not have both arginine and citrulline levels determined simultaneously. The average blood arginine level in 13 normal people was 41.2 μg/ml. range 32.5-50.0 μg/ml. Fourteen determinations on patients with liver disease showed an average blood arginine of 38.6 μg/ml, range of 25-50 μg/ml.

Four determinations on patients with surgical shunts gave an average value of 48.8 μg/ml, range of 41.3-53.7 μ/ml. This is considerably higher than the mean level found in normals and in unoperated cirrhotics. The number of cases is too small to calculate statistical probabilities but the possible reasons for elevated levels in patients with shunts will be discussed later.

All patients reported in this series and their citrulline, arginine and ammonia levels are recorded in Table I.

Discussion. The results of this study show that plasma citrulline levels are increased in patients with hyperammoniaemia and liver disease, the average level in the pathologic state being double that in normal individuals. Since citrulline is a small, readily diffusible molecule, does not enter into protein structure, is not a part of dietary intake and within limits of our present knowledge. is synthesized in the body only by enzymatic action on ornithine in the liver, it may be assumed that this citrulline measured in the peripheral blood is a direct reflection of citrulline metabolism in the liver. The fact that citrulline accumulates suggests that, the rate of conversion of citrulline to arginine has slowed relative to rate of production of citrulline from ornithine. Conversion of citrulline to arginine is therefore, the site of a relative block in urea synthesis in the abnormal liver in man.

Blood arginine values are somewhat more difficult to evaluate than the plasma citrulline levels because substantial amounts of arginine are absorbed from dietary protein and because arginine, unlike citrulline. is an active participant in many facets of body metabolism. The level of arginine in peripheral venous blood is then, a balance between (1) arginine absorbed by the gut from dietary protein, (2) liver synthesis of arginine, (3) peripheral utilization of arginine for synthesis of protein tissue structure, (4) degradation of tissue protein releasing arginine into the amino acid pool and (5) degree of shunting of portal blood around the liver.

Despite the elevations in citrulline (Table

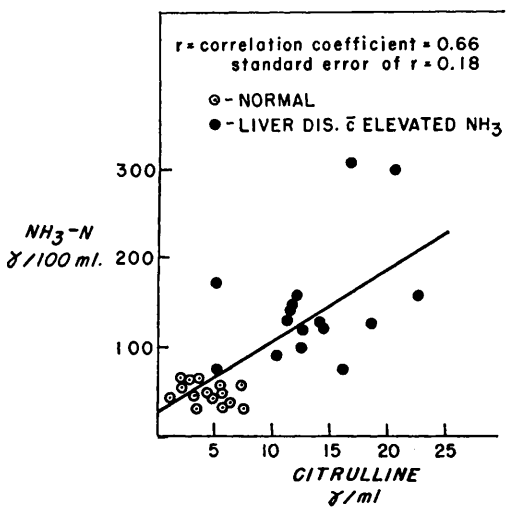

FIG. 3. Correlation between blood ammonia and plasma citrulline levels.

TABLE I. Blood Ammonia, Blood Arginine and Plasma Citrulline Levels in Patients with Disorders of the Liver and Portal Circulation.

Patient No.	NH_3, mg/100 ml	Citrulline, mg/ml	Arginine, mg/ml	Diagnosis
1	309	21.6		Infectious hepatitis
2	129	19.1		Carcinoma metastatic to liver
2	170	10.0	41.2	*Idem*
3	156	16.9		Laennec's cirrhosis
4	142	16.6	41.2	*Idem*
5	91	15.3	36.2	"
5	74	10.3	27.5	"
5	121	17.5	31.2	"
6	133	16.3	40.0	"
7	122	19.4	45.0	"
8	160	27.5	50.0	"
9	143	16.6	28.8	"
10	96		25.0	"
11	75	21.0	36.2	"
12	101	17.5	48.8	Biliary cirrhosis
12	136	23.4	41.3	*Idem*
12	299	25.6	47.5	"
13	182	13.7		Porta-caval shunt
13	121		53.7	*Idem*
13	115	21.6	47.5	"
14	134	19.7		Spleno-renal shunt
15	151	11.3	52.5	Porta-caval "
15	114	14.7	41.3	*Idem*

I, Fig. 1), there is no significant change in arginine levels in the unoperated patients with cirrhosis. These observations do not of necessity negate the possibility of a rate-limiting step in the Krebs-Henseleit cycle with decreased formation of arginine from this source. As mentioned in the above paragraph, other sources, particularly absorption of arginine from the gastro-intestinal tract by-passing the liver via portal-systemic collaterals, would tend to maintain the level of arginine in the peripheral blood. This possibility is supported by the high levels of arginine found in patients with surgically constructed portal-systemic shunts.

Further studies on this defect in urea synthesis are in progress.

Summary. 1. Elevations of citrulline in blood of patients with hyperammoniaemia and cirrhosis of the liver have been observed. 2. On the basis of the reported observations, a rate-limiting and vulnerable step in urea synthesis in man is suggested.

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