Urinary Excretion of Zinc in Alcoholism and Postalcoholic Cirrhosis

JAMES F. SULLIVAN, M.D.* AND HAL G. LANKFORD, PH.D.†

A NEW aspect to the still perplexing relationship between the ingestion of alcohol, dietary protein deficiency and liver disease has been presented by Vallee.[1,2] In a series of studies he and his associates have demonstrated that alcohol dehydrogenase[3] and glumatic dehydrogenase[4] are zinc metallo-enzymes. They have also found, as previously noted by Vidbladh,[5] a decrease in the serum zinc content of patients with postalcoholic cirrhosis; in addition, they have demonstrated an augmented urinary excretion of zinc in these patients.[1,2]

The alcohol dehydrogenase of horse liver shows increased activity in the presence of ethyl alcohol to a concentration of 0.8 to 0.9 mg./ml. Concentrations greater than this cause inhibition of the enzyme activity. Approximately a 50 per cent decrease from the maximal activity is noted at a concentration of 4.6 mg./ml. of alcohol, a blood level consistent with severe intoxication.[2] Demonstration of this inhibition of alcohol dehydrogenase by high concentrations of alcohol has enabled Vallee to form an hypothesis explaining the participation of alcohol in the development of chronic liver disease. In essence, this hypothesis considers the possibility that continued high blood alcohol concentration may lead to inactivation of the alcohol dehydrogenase, increased urinary excretion of zinc and low serum zinc levels with disruption of other zinc dependent enzyme systems, including those important in protein metabolism such as glutamic dehydrogenase. Postalcoholic cirrhosis might be considered a conditioned zinc deficiency in light of this hypothesis.[2]

A study of the urinary excretion of zinc in patients with postalcoholic cirrhosis and in patients with chronic alcoholism without clinical evidence of cirrhosis, which constitutes the subject matter of this paper, confirms the increased amounts of zinc in the urine of most patients with postalcoholic cirrhosis. A high level of zinc excretion was also found in patients with chronic alcoholism who had no evidence of cirrhosis and in certain cirrhotic patients in whom the ingestion of alcohol did not seem to be a contributory factor in the development of the liver disease.

METHOD OF STUDY

All patients described were hospitalized during the study. Particular attention was given to the evaluation of liver function, history of alcoholism and physical evidence of the presence of cirrhosis. The history in regard to the ingestion of alcohol was accepted only if confirmed by members of the patients' family or by record of previous hospital admission. Liver function was evaluated by a battery of tests, whose normal values in our laboratories are listed in Table I.

The zinc content of urine was determined by the method of Kagi and Vallee.[2] Twenty-four hour urine collections were made in polyethylene jugs. Zinc determinations were made in duplicate on aliquots of these specimens. Urine specimens containing protein were excluded from study except when otherwise noted. The normal zinc excretion in our laboratory as determined from evaluating thirty-nine twenty-four hour urine specimens from twenty-two house staff physicians in good health without evidence or history of liver disease, was 402 μg. of zinc/twenty-four hours, with a standard deviation of ±150 μg. of zinc.

From the Department of Medicine, St Louis University School of Medicine, St. Louis, Missouri.

* Associate Professor of Medicine; Present address: Creighton University School of Medicine, Omaha, Nebraska; † Biochemist, St. Louis City Hospital.

This study was supported in part by research grant of the Institute of Medical Education and Research, St. Louis City Hospital.

TABLE I
Tests Used to Evaluate Liver Function

Test	Normal Values
Bromsulphalein retention at thirty minutes (5 mg./kg. injected)	0–5%
Serum bilirubin	0.1 direct mg./100 cc.
	1.0 total mg./100 cc.
Serum alkaline phosphatase	2.3 μM/L.
Cephalin cholesterol flocculation	2 plus at 24 hr.
Serum albumin	4.5–5.5 gm./100 ml.
Serum globulin	1.4–3.0 gm./100 ml.
Prothrombin time	100%
Thymol turbidity	Less than 3 units
Diastase	80–160 Somogyi units

The accuracy of the procedure in our laboratory as determined from evaluations made in duplicate at various levels of zinc excretion indicated an error of 4 per cent in the twenty-four hour zinc determinations. The daily variation in zinc excretion as determined from two successive twenty-four hour specimens in seventeen subjects was 83 μg. of zinc with a mean deviation of ±73 μg. of zinc.

To facilitate analysis the patients were divided into two groups: (1) those in whom a severe decompensated form of cirrhosis was present (thirty-three patients), and (2) those who had no evidence of liver dysfunction (nineteen patients). Patients in the second group did not have ascites or jaundice and the bromsulphalein retention, serum proteins and cephalin cholesterol flocculation were normal except as noted in Table II.

An attempt was made to study all alcoholic patients admitted to the wards, and this series is selective only in that the patients included were sufficiently cooperative to allow complete evaluation and sufficiently trustworthy to make the twenty-four hour urine collection valid.

For purposes of comparison three patients with cirrhosis but without alcoholic history and four patients with albuminuria are included.

RESULTS

The urinary excretion of zinc, liver function studies and clinical course of the patients with postalcoholic cirrhosis (group 1) appear in Table III. Twenty-four of the thirty-three patients in group 1 excreted more zinc in their urine than normal subjects. The degree of urinary excretion of zinc ranged up to 5,010 μg. in a twenty-four hour period. Fourteen of these twenty-four patients excreted more than 1,000 μg. in a twenty-four hour period; four died in hepatic coma. Of the remaining nine patients with postalcoholic cirrhosis in whom zinc excretion was normal, two were studied at the end of a period of hospitalization during which their condition had greatly improved. Four of the remaining seven patients were studied during terminal hepatic coma; two of these had elevations in the blood urea nitrogen. Positive correlation could not be established between the degree of zinc excretion and any of the liver function tests used.

The urinary excretion of zinc and volume of urine for a twenty-four hour period for nineteen

TABLE II
Urinary Excretion of Zinc in Patients with Chronic Alcoholism

Case No.	24-hr. Zinc Excretion (cc.)	24-hr. Urine Volume (cc.)	Biopsy Diagnosis	Abnormal Liver Function Study
	271		Fatty	
1	213	1,050	liver	
	1,046	3,050	...	...
2	1,158	3,180	...	...
	1,561	3,770	...	...
3	1,010	3,700		
4	521	1,580	...	...
5	708	3,755	...	...
	990	2,720		
6	672	2,715	...	...
7	1,190	165	...	...
8	1,089	730	...	...
9	1,161	3,465	...	...
	188	270	...	Obstructive
10	421	445		jaundice, fatty liver
11	227	1,050	...	...
12	185	880	...	...
13	266	1,735	Fatty liver	Bromsulfalein 40 per cent retention
14	339	1,740	Fatty liver	Cephalin cholesterol flocculation 3+
15	308	3,925	...	Obstructive jaundice, pancreatitis
16	366	2,350	...	...
17	228	1,120	...	...
18	1,805	2,685	...	...
19	791	2,375	...	...

TABLE III
Urinary Excretion of Zinc in Patients with Postalcoholic Cirrhosis (Group 1)

Case No.	Cephalin-Cholesterol Flocculation	Bromsulfalein (% + 30 min.)	Albumin (gm. %)	Globulin (gm. %)	Bilirubin (mg./100 cc.)	Urine Volume (cc./24 hr.)	Zinc Excretion (μg.)	Alkaline Phosphatase (μM/L.)	Course
1	0	23	4.5	3.4	2.2	3,410	787	...	Biopsy, cirrhosis, improved
2	4+	...	2.4	3.8	4.3	3,170	3,137	2.9	Autopsy, cirrhosis
3	3+	22	3.0	3.6	3.5	737	736	3.1	Improved
4	4+	..	3.0	5.0	7.0	1,210	2,643	3.8	Biopsy, cirrhosis; shunt chronic
5	2+	50	2.5	3.4	2.2	545	1,315	3.3	Autopsy, cirrhosis
6	3+	41	3.7	4.1	2.2	2,817	794	...	Autopsy
7	4+	...	2.6	4.4	5.4	480	2,851	3.6	Autopsy, cirrhosis
8	3+	20	4.0	2.5	1.1	2,380	1,366	...	Biopsy, cirrhosis; shunt, ascites
9	4+	...	...	...	9.0	660	1,109	10.1	Coma, improved
10	3+	18	...	...	0.6	3,840	1,640	3.3	Improved
11	3+	...	4.4	3.1	28.8	1,560	1,218	4.3	Improved
						2,630	295		Improved
12	3+	21	3.9	2.7	1.2	2,520	318	11.0	
						966	788		Improved
13	2+	34	3.9	2.3	1.0	690	978	6.09	
14	3+	...	...	...	9.8	1,370	1,228	1.4	Improved
						3,960	5,156		Improved
15	4+	28	2.9	3.5	1.6	3,810	1,747	2.1	
16	3+	40	3.0	4.2	3.7	1,680	2,811	4.0	Improved
17	3+	41	1.8	2.6	1.6	2,220	5,500	2.6	Autopsy, cirrhosis

patients with chronic alcoholism (group 2) appear in Table II. On the basis of a liver biopsy, a diagnosis of fatty liver without hepatic necrosis was made in three of these. All liver function studies were within normal limits except as noted. Eight patients had an abnormally high excretion of zinc. In three of these the increased excretion of zinc was demonstrable on a second urine determination. In one a second determination was within normal limits. Repeat observations were not possible in the remaining three. Two patients who were hospitalized with episodes of obstructive jaundice associated with a nonfunctioning gallbladder and diastase elevations consistent with a diagnosis of pancreatitis, had normal urinary excretion of zinc.

Definite increases in the urinary excretion of zinc were found in two of three patients with nonalcoholic postnecrotic cirrhosis and in four patients with albuminuria. As indicated in Table IV, two of these four had nephrosis; the remaining two were diagnosed as having orthostatic proteinuria. There was no clinical or laboratory evidence of liver disease in these patients with albuminuria.

COMMENTS

The pattern of zinc excretion in the patients

TABLE IV
Urinary Excretion of Zinc in Nonalcoholic Patients

Case No.	Condition	24-hr. Zinc Excretion (cc.)	24-hr. Urine Volume (cc.)
1	Nephrotic syndrome	1,797	3,300
2	Nephrotic syndrome	3,580	780
3	Post-necrotic cirrhosis	830	2,775
4	Post-necrotic cirrhosis	454	3,800
5	Wilson's disease	1,000	2,210
6	Orthostatic albuminuria	1,125	1,610
7	Orthostatic albuminuria	1,003	990

with postalcoholic cirrhosis (group 1) was quite similar to that reported by Vallee et al.[2] A marked increase in urinary excretion of zinc accompanied active severe cirrhosis except in certain patients who were studied during terminal hepatic coma. Of the nine patients who died in hepatic coma, six were studied during the terminal phase; four of these had normal zinc excretion. Oliguria and/or blood urea nitrogen elevation was present in three of these four when zinc excretion was studied, suggesting that in some cases impaired renal function may limit the excretion of zinc.

The zincuria noted in eight of the nineteen patients with chronic alcoholism (group 2) is of great interest. All were admitted during or shortly following acute bouts of alcoholism and the circumstances for the suggested inactivation of alcohol dehydrogenase were optimal.

The presence of fatty metamorphosis of the liver in the absence, or with minimal evidence, of physical and laboratory characteristics of cirrhosis as noted in these patients (Cases 1, 13, and 14) demonstrated the inability of currently used liver tests to discern early hepatic dysfunction. It would be desirable to know whether or not the observed zincuria in alcoholics is associated with at present undetected aberrations in liver function, or whether it may occur without hepatic dysfunction. In preliminary experiments in our laboratory, the ingestion of alcohol by normal volunteer subjects has not influenced the urinary excretion of zinc.

The zincuria observed in patients with cirrhosis but no history of alcoholism in this study, as well as the zincuria observed in patients with porphyria,[6] and with neurologic and psychiatric diseases,[7] indicates that the mechanism or mechanisms responsible for zincuria may, in some cases, be independent of alcohol. The demonstration of zincuria with impaired trytophan metabolism is of interest because of the dependence of trytophan metabolism on the presence of niacin and pyridozine. In alcoholism specific syndromes of the central nervous system have been attributed to the lack of these vitamins. It is possible that zincuria is a manifestation of a vitamin deficiency in alcoholics which occurs concommitantly with the development of cirrhosis.

The abnormal excretion in two of the alcoholics without cirrhosis but with well documented recurrent pancreatitis suggests that pancreatic disease may interfere with the utilization of zinc. Localization of $zinc^{65}$ in the pancreas and subsequent excretion through the pancreatic ducts demonstrate the active utilization of zinc by the pancreas.[8] The presence of zinc as a metallic component of a pancreatic enzyme carboxypeptidase[9,10] lends further support to this possibility.

SUMMARY

Increased urinary excretion of zinc is present in most patients with postalcoholic cirrhosis except in the terminal phase of the disease. Approximately 50 per cent of chronic alcoholics without clinical or laboratory evidence sufficient to warrant a diagnosis of cirrhosis and some nonalcoholic subjects with cirrhosis also show excess zincuria. The mechanism and significance of the excess zinc excretion is uncertain.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Mrs. Carol Banks and Mrs. Marie Imai.

REFERENCES

1. Vallee, B. L., Wacker, W. E., Bartholomay, A. F. and Robin, E. D. Zinc metabolism in hepatic dysfunction I. Serum zinc concentrations in Laennec's cirrhosis and their validation by sequential analysis. *New England J. Med.*, 255: 403, 1956.

2. Vallee, B. L., Wacker, W. E., Bartholomay, A. F. and Hoch, F. L. Zinc metabolism in hepatic dysfunction. II. Correlation of metabolic patterns with biochemical findings. *New England J. Med.*, 257: 1055, 1957.

3. Vallee, B. L. and Hoch, F. L. Zinc component of alcohol dehydrogenase of horse liver. *Fed. Proc.*, 15: 619, 1956.

4. Vallee, B. L., Adelstein, S. J. and Olson, J. A. Glutamic dehydrogenase of beef liver, zinc metalloenzyme. *J. Am. Chem. Soc.*, 77: 5196, 1955.

5. Vidbladh, I. Studies on zinc in blood. *Scandinav. J. Clin. & Lab. Invest.*, 3 (supp. 2): 1, 74, 1951.

6. WATSON, C. J. and SCHWARTZ, S. The excretion of zinc uroporphyrin in idiopathic porphyria. *J. Clin. Invest.*, 20: 440, 1941.
7. PRICE, J. M., BROWN, R. R. and PETERS, H. A. Tryptophan metabolism in porphyria, schizophrenia and a variety of neurological and psychiatric diseases. *Neurology*, 9: 456, 1959.
8. MONTGOMERY, L. M., SHELINE, G. E. and CHAIKOFF, I. L. The elimination of administered zinc in pancreatic juice, duodenal juice and bile of the dog as measured by its radioactive isotope Zn^{65}. *J. Exper. Med.*, 78: 151, 1943.
9. VALLEE, B. L. and NEURATH, H. Carboxypeptidase, zinc metalloenzyme. *J. Biol. Chem.*, 217: 253, 1955.
10. VALLEE, B. L. and NEURATH, H. Carboxypeptidase, zinc metalloprotein. *Am. J. Chem. Soc.*, 76: 5006, 1954.