JOURNAL OF NUCLEAR MEDICINE 7:594-602, 1966

Increased Iron-59 Absorption in Patients with Hepatic Cirrhosis[2,3]

Ben I. Friedman, M.D., John W. Schaefer, M.D.[1] and Leon Schiff, M.D.

INTRODUCTION

In 1957 at the Veterans Administration Hospital in Cincinnati, a patient was observed whose liver showed posthepatitic cirrhosis. At the time a portacaval shunt was performed for bleeding esophageal varices. Three and one-half years later he died of hepatic failure. Necropsy revealed the presence of hemochromatosis (1). As similar experiences had been previously reported by Tuttle (2) and Hoffbauer (3), the question immediately arose as to the role of the portacaval anastomosis in the production of the hemochromatosis.

While it is recognized that genetic abnormality (4, 5), excessive dietary intake of iron (6), alcoholism (7), pancreatic disease (8, 9), a low phosphate diet (10), and other dietary insufficiences (11) might influence iron absorption, it seemed advisable to determine the effects of liver disease, per se, on iron absorption in man.

MATERIAL AND METHODS

Eight control subjects were selected from the general medical wards of the Cincinnati General Hospital (Table I). They were nonanemic and convalescent without clinical or laboratory evidence of hepatic disease. Liver biopsies were not performed on the control subjects.

---

[1]University of Kentucky Medical Center, Department of Medicine, Lexington, Kentucky.

[2]From the Radioisotope Laboratory and the Gastric Laboratory, Departments of Radiology and Medicine, University of Cincinnati Medical Center, Cincinnati, Ohio, 45229.

[3]This investigation was supported by U.S.P.H.S. grants AM 06204 and TI AM 5143-08 from the National Institute of Arthritis and Metabolic Diseases, and U.S.P.H.S. grant FR 00010 from the Division of Research Facilities and Resources, Bethesda, Maryland.

Patients with hepatic disease ranged in age from 26 to 67 (Table II). Their hematocrits were from 27 to 51 per cent. Three patients (Case Nos. 4, 13, 22) were overtly iron deficient at the time of investigation. In none of the patients studied were pancreatic calcifications noted on abdominal x-ray examination. Twenty-one of the twenty-two patients in the hepatic disease group were biopsied by transthoracic needle or at the time of surgery. The one patient, not biopsied because of a clotting abnormality, was presented with the clinical picture of nutritional cirrhosis and alcoholism.

Two patients had a mixed pathologic picture of nutritional and postnecrotic cirrhosis. Two others had hepatitis superimposed on cirrhosis. Primary biliary cirrhosis was diagnosed in one patient. In the specimen in which cirrhosis was not diagnosed, hepatitis with submassive collapse was seen. No excess iron was found on any of the 18 liver biopsies which were studied with special stains.

Two of the cirrhotics were studied before and after portasystemic shunt. A total of 24 studies were performed in the 22 patients with hepatic disease. Patients were diagnosed as *decompensated* if jaundice, ascites, edema or precoma or a combination of these findings was present. At the time of study two cirrhotics had neither decompensation nor shunt; 17 were decompensated without shunt; two had shunt without decompensation; and three were both decompensated and postoperative.

Absorption of iron-59 ferrous citrate from the gastroenteric tract was determined by a modification of Bonnet's method (12). Initially one microcurie of

TABLE I

^{59}Fe ABSORPTION STUDY CONTROL GROUP

Study No.	*Patient*	*Diagnosis*	*HCT*	*Percentage of ^{59}Fe Absorbed in 7 days*
030	57 MW	ASHD c̄ MI[1]	47	66.5
011	60 MNW	ASHD c̄ MI	46	40.9
031	62 MW	ASHD c̄ MI Diabetes mellitus	47	12.5
010	53 MNW	ASHD c̄ MI	45	47.2
032	61 MW	ASHD c̄ MI Diabetes mellitus	41	65.4
009	45 MNW	Conversion reaction	42	58.2
024	63 MW	ASHD c̄ MI Chronic alcoholism	42	67.7
023	59 FNW	ASHD c̄ MI Diabetes mellitus	45	44.6

[1]ASHD c̄ MI—Arteriosclerotic heart disease with myocardial infarction.

iron-59 citrate, 50 μg of carrier iron as 350 μg of ferrous ammonium sulfate, and 300 mg. of ascorbic acid were given orally to the subjects. Later the amount of ferrous ammonium sulfate was increased to 700 μg so that absorption of iron in control subjects would approximate 50% of the oral dose. Only studies with the larger dose of 100 μg of carrier iron (ferrous ammonium sulfate) are included in this report.

All subjects fasted for at least 12 hours before receiving the oral test dose. One microcurie of ferrous-59 citrate was added to a beaker containing the ferrous ammonium sulfate and ascorbic acid. Distilled water was added. The beaker was rinsed three times with distilled water; the patient drinking each rinse solution. No food was given for at least three hours after the iron-59 was administered. All stool was collected for seven days after the oral dose and dried in a metal container at 170° Fahrenheit as described by Cameron (13). All specimens were negative for blood when examined by the guaiac test.

The total weight of the seven day stool collection was measured. The entire stool was homogenized in an electric blender. At least two 10 g aliquots were counted in a well-type gamma scintillation counter. Each 10 g aliquot of dried stool has a volume of approximately twelve milliliters. Each aliquot was divided equally into 16 × 150 mm counting tubes maintaining exactly the same geometry as the standard. The sum of the net counts per minute of the tubes was equal to the net counts per minute of a 10 g aliquot. The average net counts per minute (net cpm) of the 10 g aliquots was calculated.

A 1:100 dilution of a standard solution was prepared at the time the oral dose was given. Three milliliter aliquots of this 1:100 standard were counted in triplicate. The average net cpm of these aliquots was the number of counts from $\frac{1}{33.3}$ of the oral dose. All specimens and standards from a study were counted on the same day so it was not necessary to account for physical decay.

The per cent of the ^{59}Fe dose excreted in a stool specimen was calculated from the formulae:

$$\text{Per cent of } ^{59}\text{Fe dose excreted} = \frac{\text{Avg total net cpm per 10 g aliquot} \times \frac{\text{Total wt of 7 day stool collection}}{\text{10 g}} \times 100\%}{\text{Avg. net cpm standard per counting tube} \times 33.3}$$

$$\text{Per cent of } ^{59}\text{Fe dose excreted} = 100\% - \text{the percentage of } ^{59}\text{Fe exceted in 7 day stool collection}$$

RESULTS

The mean value of iron-59 absorption in seven days of the control group was 50.4 ± 18.6 per cent (Fig. 1). Patients with hepatic disease have been divided into subgroups according to the presence of hepatic decompensation and portasystemic shunt. All subgroups of patients with hepatic disease had iron-59 absorption in the same range. The mean value of absorption for the patients with hepatic disease was 74.0 ± 17.4 per cent.

TABLE II

^{59}Fe ABSORPTION STUDY HEPATIC DISEASE GROUP

Group	*Study No.*	*Age*	*Sex*	*Biopsy Diagnosis*	*HCT*	*Serum Iron γ/100 ml*	*Percentage of ^{59}Fe Absorbed in 7 Days*
Without decompensation	006	50	FNW	P. Necr. C.[2]	40	72	80.5
Without portasystemic shunt	008	59	FW	P. Necr. C.	51	90	94.4
With decompensation	001	48	FW	Primary biliary cirrhosis	27	376	94.2
Without portasystemic shunt	003	45	MW	P. Necr. C.	32	50	85.9
	004	56	FW	Nutrit. C.[3]	37	68	93.7
	005	42	MNW	Nutrit. C. and Alc. Hep.[4]	39	45	46.8
	007	41	MW	P. Necr. C.	45	102	65.8
	012	37	FNW	P. Necr. C.	35	105	77.1
	013	57	FW	P. Necr. C.	38	78	83.6
	014	52	FNW	P. Necr. and Nutrit. C.	44	415	74.8
	015	55	FW	Nutrit. C.[1]	35	88	44.7
	016	50	FNW	P. Necr. C.	40	92	55.4
	018	46	MNW	P. Necr. C.	31	110	44.5
	019	43	NW	Nutrit. C.	42	112	70.4
	022	49	MW	P. Necr. C.	30	38	81.6
	025	26	FNW	P. Necr. C.	32	—	96.0
	026	53	MW	P. Necr. and Nutrit. C.	35	—	71.4

TABLE II (Cont.)

^{59}Fe ABSORPTION STUDY HEPATIC DISEASE GROUP

Group	*Study No.*	*Age*	*Sex*	*Biopsy Diagnosis*	*HCT*	*Serum Iron γ/100 ml*	*Percentage of ^{59}Fe Absorbed in 7 Days*
	027	38	MW	Nutrit. C. with Alc. Hep.	43	68	86.9
	028	45	MW	Alc. Hep. with Submass. collapse	35	58	91.5
Without decompensation	017	47	FW	P. Necr. C.	38	88	41.2
With portasystemic shunt	021	47	MW	P. Necr. C.	42	115	73.6
With decompensation	002	47	MW	P. Necr. C.	28	—	76.4
With portasystemic shunt	020	52	MW	P. Necr. C.	37	142	88.0
	029	67	MW	P. Necr. C.	35	202	56.9

[1]No biopsy because of clotting abnormality.
[2]P. Necr. C.—postnecrotic cirrhosis
[3]Nutrit. C.—nutritional cirrhosis
[4]Alc. Hep.—alcoholic hepatitis

Student's "t" test was performed between the mean of the eight control subjects and the 24 studies of patients with hepatic disease (Fig. 2). The value of "t" is 3.265, which with 30 degrees of freedom, is significant at beyond the 0.01 level of confidence. This suggests there is a significant difference in iron absorption over seven days between the control group and the patients with hepatic disease.

An analysis of variance was performed, investigating the effects of hepatic decompensation and portasystemic shunt in patients with hepatic disease (Fig. 3). Studies were separated by the presence or absence of hepatic decompensation and the presence or absence of portasystemic shunt. An analysis of variance suggests, that among the patients with hepatic disease, there is no significant effect on iron absorption associated with the presence or absence of portasystemic shunt or hepatic decompensation.

The analysis of variance, combined with the "t" test showing a significant difference of iron absorption between patients and controls, suggests that the effect on iron absorption depends on the presence or absence of *hepatic disease alone.*

PERCENTAGE OF ^{59}Fe ABSORBED IN SUBJECTS STUDIED

	No. of Subjects	*Percentage of ^{59}Fe Absorbed (7 days)*	
Control	8		50.4 ± 18.6 (1 SD) (12.5 to 67.7)
Hepatic Disease s decomp.[1] s P.S. shunt[2]	2	(80.5 to 94.4)	
Hepatic Disease c̄ decomp. s P.S. shunt	17	74.4 ± 17.6 (1 SD) (44.5 to 96.0)	74.0 ± 17.4 (1 SD)
Hepatic Disease s decomp. c̄ P.S. shunt	2	(41.2 to 73.6)	
Hepatic Disease c̄ decomp. c̄ P.S. shunt	3	(56.9 to 88.0)	

[1]decomp.—hepatic decompensation
[2]P.S. shunt—portasystemic shunt

Figure 1.

STUDENT'S t-TEST IRON ABSORPTION (SEVEN DAYS) CONTROLS VS PATIENTS

	Controls	*Patients*
No. of observations	8	24
Mean	50.4	74.0
Standard deviation	18.6	17.4
Standard error of the mean	6.6	3.6
Variance	345.0	303.7

Degrees of freedom 30
t = 3.265
Significant at better than 0.01 level of confidence

Figure 2.

Correlation coefficients were calculated for iron-59 absorption and the listed studies (Fig. 4). The only coefficient greater than 0.5 was that with alpha 2 globulin. It was 0.6 and was significant at the five per cent level. No correlation with the degree of anemia, serum iron, unsaturated iron binding capacity, bone marrow iron, nor listed liver function tests was found.

DISCUSSION

Increased absorption of ferrous-59 citrate from the gastroenteric tract has been demonstrated in patients with hepatic cirrhosis. The data are in agreement with those of Greenberg (14) who has studied the absorption of iron in patients with liver disease by determining body radioactivity. He demonstrated that increased absorption of iron in patients with portacaval anastomosis paralleled that

ANALYSIS OF VARIANCE PATIENTS WITH HEPATIC DISEASE MEAN VALUES

		State of Hepatic Compensation				*Row Mean*
		Compensated		Decompensated		
Presence of Porta-systemic Shunt	No	87.5	(2)	74.4	(17)	75.7
	Yes	57.4	(2)	73.8	(3)	67.2
Column Mean		72.4		74.3		

( ) = No. of subjects in each group
For presence of portasystemic shunt
F = 2.224 p = 0.15
For state of hepatic compensation
F = 0.026 p = 0.87

Figure 3.

of his entire group of cirrhotics. Conrad *et al* (15) also have found increased absorption in patients with cirrhosis in the presence of normal or increased plasma iron levels. Mendel (16) has cited experimental and clinical evidence that some types of liver dysfunction cause increased iron absorption.

Deller (17) reported that iron absorption was increased in patients with iron deficiency, iron overload, cirrhosis, hemochromatosis and pancreatic disease. In our study we found increased absorption of iron in patients with hepatic disease whether the serum iron was low, normal or high. Increased absorption in the patients we investigated appears to be related to the presence of liver disease alone.

Alcohol (7) does not seem to influence absorption of ferrous iron. Its effect seems to be short-lived and these studies were carried on in a hospital environment after several days of observation.

There was no evidence of pancreatic disease in these patients as determined by the presence of diabetes or pancreatic calcification on x-ray. All of the patients David (9) reported had increased iron absorption with pancreatic disease and pancreatic calcification.

It is interesting to observe the correlation of alpha 2 globulin with the absorption of iron. Since transferrin is a beta globulin, it would not seem involved. However, haptoglobin and ceruloplasmin are alpha 2 globulins. Haptoglobin is decreased in chronic hepatocellular disease (18) and in hemolytic disease (19). The increased iron absorption seen in patients, who have hypercellular marrows with hemolytic disease and patients with hepatic disease, may be related to decreased haptoglobin. In addition, an increase in the alpha 2 globulin, ceruloplasmin, which is increased in iron deficiency and cirrhosis (20), may be important.

Such speculation may be unwarranted but introduces interesting concepts regarding the increased absorption of iron which occurs in patients with cirrhosis of the liver. Investigation of the possible role on iron absorption of qualitative and quantitative changes in alpha 2 globulin is indicated. Further studies of the absorption and kinetics of iron in patients with cirrhosis would be best performed utilizing whole-body counting and iron-59 turnover techniques.

CORRELATION COEFFICIENT VARIABLES RELATED TO ^{59}Fe ABSORBED IN SEVEN DAYS

Cephalin Flocculation	Serum Albumin
Thymol Turbidity	Serum Globulin
Thymol Flocculation	Alpha 1 Globulin
Zinc Sulfate Turbidity	Alpha 2 Globulin
Total Lipids	Beta Globulin
Direct Bilirubin	Gamma Globulin
Total Bilirubin	Plasma Prothrombin Time
Alkaline Phosphatase	Total Protein
SGOT	Serum Iron
SGPT	Total Iron Binding Capacity
BSP	

Figure 4.

CONCLUSIONS

1. Iron absorption is increased in patients with hepatic cirrhosis.
2. Neither the state of hepatic compensation nor the presence of a porta-systemic shunt influences the absorption of ferrous-59 citrate.

ACKNOWLEDGMENT

The help of Dr. Donald Schumsky of the Medical Computer Center, Department of Environmental Health, with the statistical analyses is appreciated.

REFERENCES

1. SCHAEFER, J. W., AMICK, C. J., OIKAWA, Y., AND SCHIFF, L: The development of hemochromatosis following portacaval anastomosis. *Gastroenterology.* **42**:181, 1962.

2. TUTTLE, G. S., FIGUEROA, W. G., AND GROSSMAN, M. I.: Development of hemochromatosis in a patient with Laennec's cirrhosis. *Amer. J. Med.* **26**:655, 1959.

3. HOFFBAUER, F. W.: Primary biliary cirrhosis; observations on the natural course of the disease in 25 women. *Amer. J. Dig. Dis.* **5**:348, 1960.

4. WILLIAMS, R., SCHEVER, P. J., AND SHERLOCK, S.: The inheritance of idiopathic hemochromatosis. *Quart. J. Med.* **31**:249, 1962.

5. BOTHWELL, T. H., COHEN, I., ABRAHAMS, O. L., AND PEROLD, S. M.: A familial study in idiopathic hemochromatosis. *Amer. J. Med.* **27**:730, 1959.

6. MACDONALD, R. A.: *Hemochromatosis and Hemosiderosis.* Springfield: Charles C Thomas, 1964.

7. CHARLTON, R. W., JACOBS, P., SEFTEL, H., AND BOTHWELL, T. H.: Effect of alcohol on iron absorption. *Brit. Med. J.* **2**:1427, 1964.

8. CALLENDER, S. T., AND MALPAS, J. S.: Absorption of iron in cirrhosis of liver. *Brit. Med. J.* **2**:1516, 1963.

9. DAVIS, A. E., AND BADENOCH, J.: Iron absorption in pancreatic disease. Lancet. **2**:6, 1962.

10. HEGSTED, D. M., FINCH, C. A., AND KINNEY, T. D.: The influence of diet on iron absorption. II. The interrelation of iron and phosphorous. *J. Exp. Med.* **90**:147, 1949.

11. MACDONALD, R. A.: Experimental pigment cirrhosis. *Am. J. Path.* **36**:499, 1960.

12. BONNET, J. D., HAGEDORN, W. B., AND OWEN, C. A. JR.: A quantitative method for measuring the gastrointestinal absorption of iron. *Blood.* **15**:36, 1960.

13. CAMERON, A. D.,: Gastro-intestinal blood loss measured by radioactive chromium. *Gut.* **1**:177, 1960.

14. GREENBERG, M. S., STROHMEYER, G., HINE, G. J., KEENE, W. R., CURTIS, G., AND CHALMERS, T. C.: Studies in iron absorption: III. Body radioactivity measurements of patients with liver disease. *Gastroenterology.* **46**:651, 1964.

15. CONRAD, M. D., JR., BERMAN, A., AND CROSBY, W. H.: Iron kinetics in Laennic's cirrhosis. *Gastroenterology.* **43**:385, 1962.

16. MENDEL, G. A.: Iron metabolism and etiology of iron-storage diseases, *J.A.M.A.* **189**:45, 1964.

17. DELLER, D. J.: $Iron^{59}$ absorption measurements by whole-body counting: studies in alcoholic cirrhosis, hemochromatosis, and pancreatitis. *Amer. J. Dig. Dis.* **10**:249, 1965.

18. NYMAN, M.: Serum haptoglobin. *Scand. J. Clin. Lab. Invest.* 2, Suppl. 39, 1959.

19. ALLISON, A. C.: The genetical and clinical significance of the heptoglobin. *Proc. Royal Soc. Med. London.* **51**:641, 1958.

20. KAZAL, L. N.: Chemical and physiological aspects of plasma protein fractions with especial consideration of albumin and metal-binding proteins. In *Progress in Hematology.* L. M. TOCANTINS, Editor. New York: Grune and Stratton, 1962. Vol. III, p. 294.