11. Stinebring, W. R., Youngner, J. S., Nature, 1964, v204, 712.
12. Wagner, H. N., Jr., Iio, M., Hornick, R. B., J. Clin. Invest., 1963, v42, 990.
13. Mannini, A., Medearis, D. N., Jr., Am. J. Hyg., 1961, v73, 329.
14. Brunner, K. T., Hurez, D., McCluskey, R. T., Benacerraf, B., J. Immunol., 1960, v85, 99.
15. Wheelock, E. F., Proc. Nat. Acad. Sci. U.S., 1966, v55, 774.
16. Wagner, R. R., Huang, A. S., Virology, 1966, v28, 1.
17. Maeno, K., Yoshii, S., Nagata, I., Matsumoto, T., ibid., 1966, v29, 255.
18. Postic, B., Thesis for D.Sc., Univ. of Pittsburgh Graduate School of Public Health, 1965.
19. Frothingham, T. E., Virology, 1963, v19, 583.
20. Kumagai, T., Shimizu, T., Ikeda, S., Matumoto, M., J. Immunol., 1961, v87, 245.
21. Koprowski, H., Wiktor, T., Kaplan, M. M., Virology, 1966, v28, 754.
22. Peterson, R. D. A., Hendrickson, R., Good, R. A., Proc. Soc. Exp. Biol. and Med., 1963, v114, 517.
23. Deut, P. B., Peterson, R. D. A., Good, R. A., ibid., 1965, v119, 869.
24. Salaman, M. H., Wedderburn, N., Immunology, 1966, v10, 445.
25. Old, L. J., Clark, D. A., Fed. Proc., 1959, v18, 589.
26. Siegel, B. V., Morton, J. I., Immunology, 1966, v10, 559.
27. Gross, L., Acta Haemat., 1966, v35, 200.
28. Glasgow, L. A., J. Exp. Med., 1965, v121, 1001.
29. Medearis, D. N., Jr., Bull. Johns Hopkins Hosp., 1964, v114, 181.
30. Ho, M., Kono, Y., Proc. Nat. Acad. Sci. U. S., 1965, v53, 220.

Received October 31, 1966. P.S.E.B.M., 1967, v124.

Liver Manganese in Hemochromatosis. (31741)

LESLIE B. ALTSTATT, SIMEON POLLACK, MILTON H. FELDMAN, RICHARD C. REBA, AND WILLIAM H. CROSBY
Division of Medicine and Division of Nuclear Medicine, Walter Reed Army Institute of Research, Washington, D.C.

Hemochromatosis is a disease in which occur excessive absorption of iron and cirrhosis of the liver(1). The increased liver iron has been suggested as the cause of the cirrhosis(1,2,4). But the failure to produce cirrhosis with iron loading in animal experiments (1,13,14) and observations of transfusional iron loading of the liver without cirrhosis (1,5,6) suggest that this may not be the case.

It has recently been shown that manganese absorption in rats increases when iron absorption is increased by bleeding or dietary deprivation of iron(12). The possibility is apparent that in diseases in which iron absorption is increased manganese absorption may also be increased. Since liver cirrhosis may be produced in experimental animals by injecting manganese into the peritoneum(7) the hypothesis was considered that absorption and deposition of manganese and iron in the hemochromatotic liver is the cause of the cirrhosis.

If manganese absorption is increased in hemochromatosis then the concentration of manganese in the liver might be increased, that organ being the principal storage site (8,9) for manganese.

The concentration of manganese in liver samples from patients dying with hemochromatosis was compared with the manganese concentration in liver samples of patients dying of myocardial infarction.

Comparative concentrations were determined by neutron activation analysis. Formalin-preserved samples were studied. Two groups of hepatic samples were collected. Twenty cases of "hemochromatosis" were studied. All met these criteria:

(1) Males over 35 years of age
(2) Liver cirrhosis
(3) Liver hemosiderosis
(4) Iron deposition in other tissues of the body
(5) No history of multiple transfusions
(6) No history of excess iron ingestion

Fig. 1 shows a photomicrograph of typical

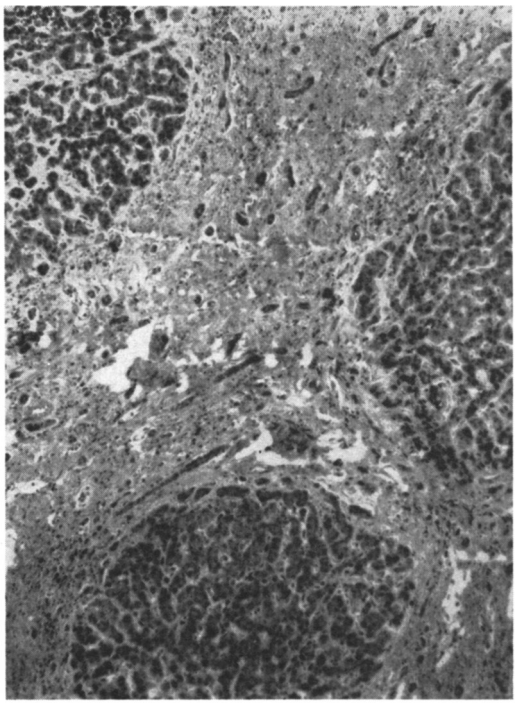

FIG. 1. Representative liver biopsy sample of patient with hemochromatosis. Cirrhosis and hemosiderosis are shown.

liver tissue selected.

Twenty control samples were selected from cases meeting these criteria:

(1) Males over 35 years of age

(2) Autopsy diagnosis of myocardial infarction

(3) No history or autopsy findings of liver disease (acute passive congestion was not disqualifying)

(4) No liver hemosiderosis

(5) No iron deposition in other tissues

(6) No history of hematologic disorders or multiple transfusions

(7) No history of excess iron ingestion

Samples selected for study were collected from plastic storage bags by chemically aseptic techniques suitable to this method of analysis. Neutron activation analysis was employed for estimation of the manganese concentration of the dried samples, and the results expressed μg/g of dry tissue. In this technique, as described by Feldman *et al* (10), the specimen for analysis is introduced into a thermal nuclear reactor with a separate tube of an aqueous sample of manganese of known concentration. In the reactor, manganese-55 in both specimen and standard is proportionally transmuted to manganese-56 by neutron capture. Manganese-56 is an unstable isotope with a half-life of 2.59 hours which decays with a gamma emission at an energy of .84 MeV. The gamma emission of specimen and standard were counted simultaneously on an appropriately calibrated 400 channel pulse-height analyzer. Manganese concentration of the sample is computed by comparing gamma emissions of standard and specimen. The chemically determined iron content of the specimens was not sufficient to contribute significantly by $Fe^{56}(np)Mn^{56}$ in the reactor flux used.

The results of this study are tabulated in Table I. Mean concentration of liver manganese was significantly higher in the hemochromatosis samples (4.25 μg/g dry tissue) than in liver samples free of cirrhosis and siderosis (2.13 μg/g dry tissue). To further define the relationship between iron and manganese content of the liver in hemochromatosis, duplicate specimens of liver tissue from the hemochromatotic group were studied for iron content. Tissues were digested in nitric acid and the digestant analyzed for iron according to the method described by Conrad and Williams(3). The values for iron con-

TABLE I

Sample	Patient's age	Liver Mn (μg/g)	Sample	Patient's age	Liver Mn (μg/g)
Hemochromatosis			Normal		
1	58	2.6	1	68	1.1
2	57	5.4	2	65	3.5
3	42	2.7	3	74	2.4
4	66	2.7	4	45	5.5
5	56	3.2	5	77	1.9
6	59	7.6	6	66	1.6
7	65	5.4	7	65	1.9
8	63	8.2	8	66	1.3
9	63	1.5	9	64	1.8
10	51	3.3	10	60	1.5
11	40	5.2	11	40	2.8
12	61	5.5	12	56	2.6
13	57	4.9	13	68	2.6
14	61	3.6	14	37	1.3
15	57	1.9	15	55	1.2
16	58	6.1	16	47	1.2
17	55	1.9	17	98	1.7
18	66	6.3	18	43	2.7
19	53	4.2	19	70	3.2
20	68	1.9	20	40	1.7

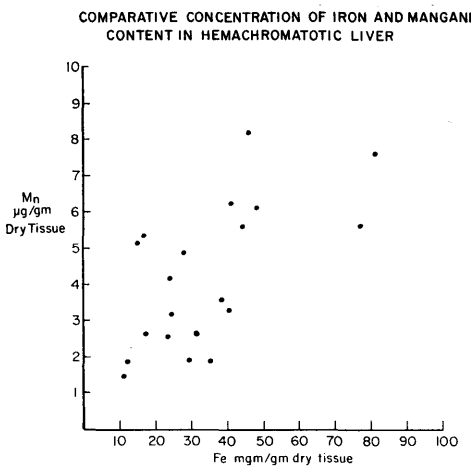

FIG. 2. Correlation found from a plot of the manganese and iron content of each hemochromatotic sample studied.

tent of the tissue were then plotted against the values for manganese content of the respective tissues (Fig. 2). This plot shows a significant correlation between manganese and iron content in hemochromatosis (correlation coefficient = .632). In the age groups studied, there was no dependent relationship between age of the patient at death and concentration of manganese in the liver. In the tissues studied, there was no dependent relationship between the time the tissues were in storage and their concentration of manganese.

The observation that manganese content of the liver in hemochromatosis is higher than normal liver contrasts with that of Butt *et al* (11). It is to be noted that the study reported here employed a different technique of analysis using a different experimental design. This study was designed as a comparative experiment in which the normal, abnormal and standard specimens were activated simultaneously and the unknown activated specimens and the standard were then counted simultaneously. The differences reported are therefore thought to be real.

This study indicates the need for investigation regarding the role of trace metals in other types of cirrhosis. It supports the proposal(12) that iron and manganese share a common absorptive pathway in the gut. If it is presumed that manganese shares a common absorptive mechanism with iron and that increased manganese absorption may produce cirrhosis, an explanation of the seemingly ubiquitous appearance of cirrhosis in some iron storage diseases is possible.

It is concluded that hemochromatosis is associated with increased liver manganese. A causal relationship between the cirrhosis and the manganese has not been established.

Summary. A comparative study was performed in which the manganese concentration in liver samples from cases of hemochromatosis was compared with the manganese concentration of normal liver. A higher mean concentration of liver manganese was found in the former group. The possibility that manganese is involved in the cirrhosis of hemochromatosis is discussed.

1. Bothwell, T. H., Finch, C. A., Iron Metabolism, Little, Brown & Co., Boston, 1962.
2. Bothwell, T. H., Cohen, I., Abrahams, O. L., Perold, S. M., Am. J. Med., 1959, v27, 730.
3. Conrad, M. E., Williams, H., J. Lab. Clin. Med., 1966, v67, 171.
4. Finch, S. C., Finch, C. A., Medicine, 1955, v34, 381.
5. Higginson, J., Gerritsen, T., Walker, A. R. P., Am. J. Path., 1953, v29, 779.
6. MacDonald, R. A., Mallory, G. K., AMA Arch. Int. Med., 1960, v105, 686.
7. Findlay, G. M., Brit. J. Exp. Path., 1924, v5, 92.
8. Gallup, W. D., Walters, L. E., McOsker, D. E., Proc. Oklahoma Acad. Sci., 1951, v32, 71.
9. Von Oettingen, W. F., Physiol. Rev., 1953, v15, 175.
10. Feldman, M. H., Reba, R. C., Battistone, G. G., Reported at International Congress on Activation Analysis (NATO), Glasgow, Scotland, Aug. 1964.
11. Butt, E. M., Nusbaum, R. E., Gilman, T. C., Didio, S. L., Am. J. Clin. Path., 1956, v26, 225.
12. Pollack, S., George, J., Reba, R., Kaufman, R. M., Crosby, W. H., J. Clin. Invest., 1965, v44, 1470.
13. Brown, E. B., Smith, D. E., Duback, R., Moore, C. V., J. Lab. Clin. Med., 1953, v53, 591.
14. Brown, E. B., Jr., Dubach, R., Smith, D. E., ReynFaye, C., ibid., 1957, v50, 862.

Received May 23, 1966. P.S.E.B.M., 1967, v124.