LIVER CATALASE ACTIVITY IN PATIENTS WITH IRON DEFICIENCY ANEMIA*

Şinasi Özsoylu, M.D.**

Catalase is one of the iron containing enzymes. Although its activity is much higher in erythrocytes and liver and kidney cells, almost all of the mammalian cells contain this enzyme. The prosthetic group, hematin, is similar to the prosthetic group of methemoglobin, methmyoglobin and ferricytochrome.[1] There are four protoporphyrin iron complexes (hematin) in one molecule of catalase and its iron content is 0.1 per cent, or less than a third that of hemoglobin.[2]

The decreased activity of erythrocyte catalase in patients with iron deficiency anemia was shown for the first time by us,[3] and recently confirmed by Balcerzak and Jensen.[4] Human liver catalase activity in patients with liver diseases has been, to the best of our knowledge, determined only by Dale.[5] All changes of catalase activity in various pathological conditions of the liver of animals have been determined.[6–13]

In this report, the assay results of catalase activity from liver biopsy specimens obtained from patients with iron deficiency anemia will be presented.

Material and Method

Liver catalase activity was determined in 36 subjects whose ages ranged from six months to 65 years. The serum iron (SI) and serum iron binding capacity (SIBC) determinations of 24 patients who had anemia were compatible with iron deficiency anemia in 17 of these patients, aged 21 months to 34 years. Two of the 24 anemic patients had normal SI and SIBC values. Although SI and SIBC were not determined in five patients of the anemic group, their peripheral smears were compatible with iron deficiency anemia. The hemoglobin (Hb), hematocrit (Hct), SI, SIBC, liver function tests, liver biopsy results and clinical diagnosis of this group of 24 are given in Table 1.

From Ankara University Hacettepe Faculty of Medicine and Hacettepe Children's Hospital.

* This study was supported by Türkiye Bilimsel ve Teknik Araştırma Kurumu.

** Hematologist and Associate Professor of Pediatrics.

TABLE 1

LIVER FUNCTION TESTS, SI, SIBC, Hb, Hct VALUES IN PATIENTS WITH IRON DEFICIENCY ANEMIA

Age	and sex	Name	Hb/Hct	SI/SIBC	Catalase U/mg.	Bilirubin % mg. D/T*	Prothrom-bin Time (seconds)	Alkaline Phosphatase Bodansky Units	CCF	SGOT	Cholesterol E/T mg % •	Thymol Turbidity	Albumin %	Globulins % α_1	α_2	β	γ	A/G gr %	Liver Biopsy	Clinical Diagnosis	Others
9	M	I. B.	5.45/20	12/516	67		18		3+	26	/106	1	39	5.8	12.4	15.6	23	4.5/2.2	Normal	Iron deficiency, Ascariasis, Hepatosplenomegaly	FA : 3■
12	F	F. A.	6.95/22	27/308	41	0,3/0.7	17	1.9	1+	22	/116	0.8						5/2.7	Focal fatty infiltration	Pica, iron deficiency anemia	FA : 55
7	M	M. B.	8.45/34	30/417	39.5	0.3/0.5	13	1	4+	20	78/184		42.7	3.5	16.7	12.7	24.1	4.2/2.8	Insufficient	Pica, anemia, Lung tbs.	
34	M	H. B. S.	8.5/	33/417	34														Normal	Portal hypertension	
2	F	A. Ü.	6.5/	30/417	35.5														Veno-occusive disease	Hepatomegaly	
8	F	S. Ş.	8.15/	16/208	20		17												Cloudy swelling & hydropic degeneration	Meningococcal meningitis Fe deficiency anemia	
10	M	A. T. S.	10/28	46/408	38		13			50	94/154	1.3						6.2/1.9	Normal	Portal hypertension, anemia	
5	M	H. Ç.	6.2/	64/387	37		13		4+	60		7.5	32.3	2.8	12.4	24.7	27.78	3.1/2.6	Normal	Anemia, Hepatosplenomegaly, Trombocytopenia	Cu++: 75 FA: 10
3	F	G. A.	6.2/20	16/362	39.6	0.3/1.3	19	10.3	3+	134	60/340	2	41	7	16	13	23	3.5/3.5	Insufficient	Anemia	Cu++: 60 FA : 1
4	M	H. E.	6.34/		26.7	0.3/0.7	17			26		0.8						3.1/3.7	Mild fatty infiltration	Urinary tract infection, Pancytopenia	
6	M	Ç. Ö.	6.65/	32/279	32.7	0.4/0.6	18		4+	20	/54	8.8						3.1/6.6	Cloudy swelling	Kala-Azar, bronchitis, hypersplenism	
13	F	N. S.	6.95/26	18/487	48.4		14		3+	20	/152	1.3						5/2.3		Anemia	FA : 18
5	F	A. O.	6.95/24	37/437	37	0.3/0.5	13	1.6	1+	12	/164	2.5	39.8	4.5	14	19.6	22.1	3.7/2.7	Normal	Anemia, thrombocytopenia	Cu++: 100
3	F	N. T.	4.65/16	40/350	21.4	0.4/0.7	19		1+	28	38/68	1.4						2.5/2.2	Normal	Anemia	Cu++: 82
1 9/12	F	E. K.	5.15/24		31.4	0.2/0.5	16	8.3	1+	16	68/116							4.8/2.2	Mild cloudy swelling	Anemia, Rickets	
11	M	Y. Ş.	7.4/	33/445	37.3		13	4.6		42	118/166	2.3						5.7/2.6	Hydropic degeneration	Anemia, Hepatosplenomegaly	FA : 20
11	M	H. G.	5.45/	44/450	12.2				3+	8	/128	2.5						4.8/2.2	Mild hydropic degeneration	Anemia, Pica, Hepatosplenomegaly	
10	F	D. Y.	9.65/31	50/417	34.5		16	1.9		16	38/86	2.3						5.2/	Normal	Anemia, Hepatosplenomegaly, pica	
14	F	E. O.	8.15/	26/417	43			1.7	3+	8	/122		43	3	8	13	32	4.8/2.7	Normal	Anemia	
9	M	A. Ç.	7.5/27		44.6														Precirrhotic changes	Nephrotic syndrome	
3	F	Ü. K.	6.5/		56.5														Cloudy swelling	Anemia	
11	M	M. K.	6/		40.9														Cloudy swelling	Anemia	Cu++: 66 ■■
8	F	S. O.	9.6/	107/308	41.3		16		1+	6	/265	1.1	52.7	36	18.3	13.6	11.5	5.3/1.9	Mild periportal fib.	Myocarditis, convulsion microcephaly	
3	F	N. D.	9.20/	92/310	14.3		17		1+	24	50/78	1.9						4.2/2.8	Congestion	Heart failure	

* Bilirubin D/T = Direct/Total

• Cholesterol E/T = Ester/Total

■ F. A. = Folic acid m_μ/ml

■■ Cu++ = Copper = γ %

TABLE 2

CATALASE ACTIVITY, LIVER FUNCTION TESTS, SI, SIBC, Hb, HCT AND BIOPSY RESULTS IN CONTROL PATIENTS WITHOUT ANEMIA

Age and sex		Name	Hb/Hct	SI/SIBC	Catalase	Bilirubin mg % D/T	Prothrom-bin time	Alkaline phosphatase Bodansky Units	CCF	SGOT	SGPT	Cholesterol E/T mg %	A/G	Liver Biopsy	Clinical diagnosis	Others
9	M	A. Ş.	13/	208/488	18.7	2.6/3.5	52	6	3+	180		/112	3.3/3.8	Cirrhosis	Wilson Cirrhosis	Cu : 40 %
6/12	F	N. K.	10.45/	88/303	9.7	0.8/1.1	13			120		/360	4.6/2.6	Hemangioendothelioma + Acute non specific hepatitis	Hepatomegaly	
16	F	E. A.	12.4	85/269	44.6		13							Normal	Heart Failure	
11	F	E. I.	12/	208/529	34		16			1800 (later 36)		58/88	2.3/2.2	Normal	Convulsion, Myocarditis	
35	M	H. K.	13.8/40	100/320	45	0.2/0.5	13			32			4.4/2.6	Normal, Hydatic cyst	Cyst Hydatic	
65	F	Z. O.	12.2/	78/268	39.4									Cholangiolitis, Necrobiose	Cholecystitis	
4	M	M. B.	12.30/	75/350	33		24					382/504	3.4/2.6	Diffuse fatty infiltration	Nephrotic syndrome, bronchopneumonia, heart failure	
18	M	Ç. T.	14.05/42		30		17	13.9	1+	28	17		5.1/2.1	Dilation of central veins		
18	M	A. A.	13.8/43		34		15	3.2	1+	20	15		5.1/2.6	Mild vacuolisation		
18	M	S. A.	13.8/41		38		16	3.3	1+	18	11		5.9/2.7	Normal		
13	F	A. T.	13.8/41		35.8		16	5	1+	22	19		5 /2.5	Hydropic Degeneration		
19	F	Z. K.	13.35/42		28.4		17	3.7	2+	156	260		5 /4.8	Postnecrotic cirrhosis		

Twelve subjects without anemia served as controls. SI and SIBC were recorded for seven of these 12 subjects. In the remaining five, no hypochromia was detected on examination of peripheral smears. These five patients had been previously admitted to this hospital with porphyria Turcica and had been free of the disease for at least one year (Table 2).

SI and SIBC were determined according to the methods of Simmonds [14] and Peters et al,[15] respectively. Hemoglobin and hematocrit were measured by cyanmethemoglobin (Klett-Summerson photometer method) and microhematocrit. The methods of Herbert et al,[16] Fisher [17] and Quick [18] were used in determinations of serum folic acid, copper, and one-stage prothrombin time, respectively.

Liver specimens were obtained from 31 subjects by using the Wim-Silverman needle, and from five patients by open surgical biopsy. Enzyme assays were repeated in four patients; in two at the time of operation, and in two because previous specimens were insufficient for microscopic examination. Catalase activity was assayed as soon as the biopsy specimens were obtained. The surgical biopsy material was cut into pieces approximating in size those obtained by needle biopsy. The specimens were blotted on filter paper to absorb as much blood as possible, weighed on tissue balance, and then homogenized at room temperature in a sinter- walled all-glass homogenizer with distilled water. The number of cubic centimeters of water added was equal to one half the weight of the specimen, in milligrams.

One ml of the homogenate was introduced with a blowout pipete into a 50 ml beaker containing a 5 ml solution of 0.01 M hydrogen peroxide and 0.01 M phosphate buffer with a pH of 8.6 which had been kept refrigerated and prewarmed to 37°C in a constant temperature water bath. After 15 to 20 seconds of incubation (the incubation time was recorded precisely), the reaction was stopped by adding 2 ml of 2 N sulfuric acid. A second beaker, to which the same amount of sulfuric acid was added before the homogenate, was used to determine the initial hydrogen peroxide concentration. (In five instances, instead of using 1 ml homogenate, 0.2 ml or 0.5 ml homogenates were used, and the substrate and amount of sulfuric acid were decreased proportionately.) The hydrogen peroxide concentration in each beaker was determined by titration with 0.025 N potassium permanganate and all of these determinations were performed in duplicate. When the results of

these duplicates were dissimilar, the whole experiment was repeated. Under these assay conditions, the liver catalase activity is calculated from this equation: $K_{cat} = \frac{10^3}{t} \log \frac{Xo}{X}$. Xo is the initial hydrogen peroxide concentration expressed in milliliters of permanganate and X is the concentration after t seconds (between 15 and 20 precisely recorded seconds) incubation. Since catalase is a protein, after measuring the catalase activity of the homogenate, its activity was expressed as a unit for each milligram of protein. To accomplish this, the protein concentration of the homogenate was determined by the method of Lowry et al.[19] (Liver catalase activity was represented by K_{cat} per milligram of liver protein.)

Results

Forty determinations were carried out in 36 cases. In the four cases requiring repeat biopsies, catalase activity was no different in the repeat assay from the initial one.

The mean liver catalase activity was 33.538 ∓ 2.679 U/mg in 11 patients without iron deficiency, and was 36.359 ∓ 2.890 U/mg in 17 patients with iron deficiency anemia on whom SI and SIBC were determined. (One member of the control group, patient 2 in Table 2, who had liver hemangioendothelioma was not included in the statistical study as we were not sure how much tumoral tissue was included in the biopsy specimen.)

The statistical difference between these two groups was only about five per cent ($0.01 < p < 0.05$). The mean catalase values were 37.104 ∓ 3.319 in 22 patients with iron deficiency and the difference between the anemic and non-anemic groups was insignificant ($p < 0.05$).

When liver catalase activity of patients with normal liver histology was compared with that of patients with pathological changes, no statistically significant difference was found. The mean value of the former group (12 subjects) was 39.458 ∓ 3.078 U/mg; that of the latter group (20 subjects) was 32 ∓ 2.395 U/mg; $p < 0.05$ (Tables 3 and 4).

Although liver enzyme activity is higher in male animals than in females, we could find no statistically significant differences in catalase activity between human males and females in our study (Table 5).

TABLE 3
LIVER CATALASE ACTIVITY IN SUBJECTS WITH NO LIVER PATHOLOGY

Name	Age and Sex	Liver Catalase U/mg)
E. A.	16 y. Female	44.6
E. I.	11 y. Female	34
I. B.	9 y. Male	67
H. K.	35 y. Male	45
H. B. S.	34 y. Male	34
A. T. S.	10 y. Male	38
H. Ç.	5 y. Male	37
A. Ö.	5 y. Female	37
N. T.	3 y. Female	21.4
D. Y.	10 y. Female	34.5
E. Ö.	14 y. Female	43
S. A.	18 y. Male	38
	Mean value	39.458 ∓ 10.680 U/mg

Comments

Catalase has been crystallized from bacteria,[20] beef liver,[21] human liver and human erythrocytes.[2] The species specificity of this enzyme is determined by the composition of the apoenzyme. Although the rate of synthesis and the half-life of the enzyme in different organs are not the same,[22] the enzyme itself is identical.[6]

The half-life of liver catalase is only a few days [22] and four to five days is enough for the incorporation of injected radioactive iron into the molecule.[6] Corticosteroids, and testicular and thyroid hormones have been shown to affect liver catalase activity.[9, 12] Again, in animals, it has been shown that starvation, sedormid and INH decrease the liver catalase content without affecting erythrocyte activity.[8, 13, 22] The inhibitory effect of INH can be reversed with vitamin B_6.[13]

Schultze and Kuiken [7] had reported decreased liver catalase activity in iron deficient rats. The deficiency of the eznyme in the liver was even more marked in rats with copper deficiency. However, a study by Adams did not show decreased liver catalase activity in mice with iron deficiency,[10] and his results were confirmed by Lahey et al.[11] The effect of copper deficiency on liver catalase activity is also not certain,[10, 11] but enzyme activity has been reported to be markedly decreased in animals in a state of inanition.[5]

Tissue catalase activity in all cells was found to be markedly depressed in cases of acatalasia.[25-27] The activity of this enzyme is also reported to be depressed in patients with infectious and nutritional hepatitis;[5] however, we could find no marked difference between subjects with or without liver pathology.

As catalase is the most active enzyme known, optimal hydrogen peroxide concentration and short incubation periods are advised. For this reason, we used 15 to 20 seconds of incubation in our assay system.

TABLE 4

LIVER CATALASE ACTIVITY IN CASES IN WHICH SOME PATHOLOGY WAS DETECTED UPON LIVER BIOPSY

Name	Age and sex	Liver Catalase U/mg.	Diagnosis
A. S.	9 y. Male	18.7	Cirrhosis (Wilson)
S. Ö.	8 y. Female	41.3	Mild periportal fibrosis
N. D.	3 y. Female	14.3	Congestion
Z. O.	65 y. Female	39.4	Cholangiolitis necrobiose
M. B.	4 y. Female	33	Diffuse fatty infiltration
F. A.	12 y. Female	41	Focal fatty infiltration
A. Ü.	2 y. Female	35.5	Veno-occlusive disease
S. Ş.	8 y. Female	20	Cloudy swelling and hydropic degeneration
H. E.	4 y. Male	26.7	Mild fatty infiltration
Ç. O.	6 y. Male	32.7	Cloudy swelling
E. K.	21 Mos. Female	31.4	Mild cloudy swelling
Y. Ş.	11 y. Male	37.8	Hydropic degeneration
H. G.	11 y. Male	12.2	Mild hydropic degeneration
A. Ç.	9 y. Male	44.6	Pre-cirrhotic changes
Ü. K.	3 y. Female	56.5	Cloudy swelling
M. K.	11 y. Male	40.9	Cloudy swelling
Z. K.	19 y. Female	28.4	Post necrotic cirrhosis
A. T.	13 y. Female	35.8	Hydropic degeneration
A. A.	18 y. Male	34	Mild vacuolisation
Ç. T.	18 y. Male	30	Vacuolisation and dilatation of central veins
N. K.	6 mos. Female	9.7 (not included)	Liver hemangioendothelioma
	Mean value	32.710 ∓ 10.730 U/mg	

TABLE 5

LIVER CATALASE ACTIVITIES COMPARED ACCORDING TO SEX*

	Total	With iron deficiency	Without iron deficiency	With normal liver biopsy	With liver pathology
Male	35.8 ∓ 2.828 (17)	37.264 ∓ 3.964 (11)	33.116 ∓ 3.575 (6)	43.166 ∓ 4.987 (6)	30.844 ∓ 3.466 (9)
Female	35.894 ∓ 2.421 (18)	37.118 ∓ 3.221 (11)	36.44 ∓ 4.634 (5)	35.750 ∓ 3.33 (6)	34.236 ∓ 3.388 (11)
t	0.0261	0.029	0.717	1.237	0.694
p	0.05	0.05	0.05	0.05	0.05

* Numbers in parentheses indicate the number of cases in each group.

The lowest catalase activity in our series was found in a six month old child (N. K.) who had liver hemangioendothelioma and acute non-specific hepatitis (Table 2). Results of this patient's tests were not included in the statistical analysis, since we were not sure how much tumoral tissue was contained in the biopsy specimen. The low level of activity could be the result of the tumor's inhibitory effect on liver catalase. It has been reported that malignant tumors of the liver and other tissues inhibits liver catalase activity in animals.[28] Nakahara and Fukuoka have indicated that material isolated from human cancer and injected into rats inhibits the liver enzyme activity of these animals.[29]

According to our results, enzyme activity is not related to age in human beings beyond the age of 21 months. We have also determined enzyme activity post mortem on three occasions: one, immediately after death; another, one hour after death; and a third, one day after death. One of these subjects was a premature baby whose liver catalase activity, assayed from a postmortem liver specimen which had been refrigerated and protected from evaporation, did not show any changes in four days. The postmortem enzyme activity was not different from the controls in any of the studies. It might then be concluded that this enzyme activity in human beings, contrary to observations in animals,[10] does not appear to be related to age.

In this study, contrary to Dale's [5] report, we could find no statistical difference in the activity of this enzyme in patients with liver pathology. We were not able to confirm decreased activity of this iron-containing enzyme in patients with iron deficiency; nor did serum copper levels, determined in six cases, appear to be related to liver catalase activity. Liver catalase activity of five subjects who had previously had porphyria Turcica was found to be similar to the normal controls.

Summary

In this study, human liver catalase activity of 36 subjects was assayed 40 times. The study showed that liver catalase activity does not decrease in patients with iron deficiency anemia and that it is neither related to the sex, age, liver pathology nor to possible serum copper deficiency in human beings.

REFERENCES

1. White, A., Handler, P. and Smith, E. L.: Principles of Biochemistry, ed. 2, New York, McGraw-Hill, 1959, pp. 343 - 345.
2. Herbert, D. and Pinsent, J.: Crystalline human erythrocyte catalase, Biochem. J. 43 : 203, 1948.
3. Özsoylu, Ş.: Decreased activity of the iron-containing enzyme catalase in human iron deficiency anemia, Turkish J. Pediat. 6 : 200, 1964.
4. Balcerzak, S. P. and Jenson, W. N.: Erythrocyte catalase activity in Thalassemia, Scand. J. Haemat. 3 : 245, 1966.
5. Dale, B. G.: Catalase and esterase content of human liver obtained by needle biopsy, Am. J. M. Sc. 226 : 42, 1953.
6. Theorell, H., Beznak, M., Bonnichsen, R. K., Paul, K. G. and Akeson, A.: On the distribution of injected radioactive iron in guinea pig and its rate of appearance in some hemoproteins and ferritins, Acta Chemica Scandinavica 5 : 445, 1951.
7. Schultze, M. O. and Kuiken, K. A.: Effect of deficiencies in copper and iron on catalase activity of rat tissues, J. Biol. Chem. 137 : 727, 1941.
8. Miller, L. L.: Changes in rat liver enzyme activity inanition, Federation Proceedings 6 : 279, 1947.
9. Adams, D. H.: Hormonal factors influencing liver catalase activity in mice. Testicular and adrenal factors, Biochem. J. 50 : 486, 1952.
10. Adams, D. H.: Effect on mouse liver catalase activity and blood hemoglobin level of milk diet deficient in iron, copper and manganese, Biochem. J. 54 : 328, 1953.
11. Lahey, M. E., Gubler, C. J., Chase, M. S., Cartwright, G. E. and Wintrobe, M. M.: Studies of copper metabolism; hematologic manifestations of copper deficiency in swine, Blood 7 : 1053, 1953.
12. Girkin, G. and Kampschmidt, R. F.: Studies on the effect of thyroxine administration on catalase activity (abstract), Endocrinology 67 : 839, 1960.
13. Hawkins, J. E. and Steenken, W. Jr.: Inhibition of catalase activity by isoniazid and the effect of vitamin B_6, Proc. Soc. Exper. Biol. Med. 112 : 30, 1963.
14. Simmons, J. S.: Determination of serum iron, in Simmons, J. S. and Gentzkow, C. J., Medical and Public Health Laboratory Methods, ed. 6, Philadelphia, Lea and Febiger, 1955, p. 416.
15. Peters, T., Giovanniello, T. J., Apt., L. and Ross, J. F.: New method for determination of serum iron binding capacity, J. Lab. and Clin. Med. 48 : 274, 1956.
16. Herbert, V.: The assay and nature of folic acid activity in human serum, J. Clin. Invest. 40 : 81, 1961.
17. Fisher, H. J.: Copper method C 35 P. I., in Manual of Standard for Spectrographic Chemistry, New York, Standard Scientific Supply Corporation, 1950.
18. Quick, A. J. : Prothrombin in hemophilia and in obstructive jaundice, J. Biol. Chem. 109 : 73, 1935.

19. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. Protein measurements with the folin phenol reagent, J. Biol. Chem. 193 : 265, 1951.

20. Herbert, D. and Pinsent, J. : Crystalline bacterial catalase, Biochem. J. 43 : 193 1949.

21. Sumner, J. B. and Dounce, A. L.: Crystalline catalase, J. Biol. Chem. 121: 417, 1937.

22. Schmid, R., Figen, J. F. and Schwarts, S. : Experimental porphyria; studies of liver catalase and other heme enzymes in sedormid porphyria, J. Biol. Chem. 217 : 263, 1955.

23. Richardson, M., Huddleson, I. F. and Bethea, R. : Study of catalase in erythrocytes and bacteria; procedure for determination of catalase activity of erythrocytes, Arch. Biochem. 42 : 114, 1953.

24. Bonnichsen, R. K., Chance, B. and Theorell, H.: Catalase activity, Acta Chemica Scandinavica 1 : 685, 1947.

25. Takahara, S., Hamilton, H. B., Neel, J. V., Kobara, T. Y., Ogura, Y. and Nishimura, E. T.: Hypocatalasemia; a new genetic carrier state, J. Clin. Invest. 39 : 610, 1960.

26. Wyngaarden, J. B. and Howell, R. R.: Acatalasia, in Stanbury, J. B., Wyngaarden, J. B. and Frederickson, D. S.: The Metabolic Basis of Inherited Disease, New York, McGraw-Hill, 1960, pp. 1398 - 1415.

27. Aebi, H., Heiniger, J. P., Buetler, R. and Haessig, A.: Two cases of acatalasia in Switzerland, Experientia 17 : 466, 1961.

28. Greenstein, J. P.: Effect of cancer on liver enzymes, J.A.M.A. 148 : 697, 1952.

29. Nakahara, W. and Furuoka, F. : Toxohormone, a characteristic toxic substance produced by cancer tissue, Gann 40 : 45, 1949.