IRON-TOLERANCE TESTS IN DIFFERENTIATING BETWEEN HAEMOCHROMATOSIS AND LIVER CIRRHOSIS

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The study of the results of the oral and intravenous iron-tolerance tests can help in establishing whether alterations in the metabolism of iron occur in different diseases. Little comparative research has been done on the metabolism of iron in patients with haemochromatosis and liver cirrhosis (Gitlow and Beyers, 1952; Gitlow, Beyers, and Colmore, 1952).

During the course of personal research on haemochromatosis (Napolitano and Scuro, 1954a, 1954b, 1956), it seemed to us worth while studying this problem by simultaneously determining the serum iron, the oral and intravenous iron tolerance, and the serum transferrin in patients suffering from the above-mentioned diseases.

We have also analysed the serum-iron data of Gitlow and Beyers (Napolitano and Scuro, 1956), some of which have proved very useful in differentiating haemochromatosis from liver cirrhosis.

Materials and Methods

Our studies were carried out on 5 patients with haemochromatosis, 12 with liver cirrhosis, and 5 normal subjects as controls. Of the 12 with liver cirrhosis, 7 suffered from Morgagni-Laennec atrophic cirrhosis and 5 had hypertrophic splenomegalic cirrhosis with hyperhaemolysis. In the cirrhotic patients, except Cases 11, 13, 20, and 22, slight anaemia and macrocytosis were present; Case 14 was intensely jaundiced, and subjaundice was present in Cases 11, 12, 13, and 15.

In all subjects the behaviour of serum iron, oral iron, intravenous iron, and serum transferrin has been studied.

The serum iron was calculated according to the method of Bothwell and Mallett (1955), which is based on the lines of the Hellmeyer and Plötner method and calls for the use of the Beckman spectrophotometer on 520 m μ wavelength.

For the study of the oral iron tolerance 120 mg. of Fe⁺⁺ was administered in the form of ferrous sulphate and vitamin C (three pills of "ferro redoxon"); the serum iron was determined before and 2, 4, 6, and 10 hours after administration of the salt.

In carrying out the intravenous iron tolerance tests 10 mg. of Fe⁺⁺ was injected in the form of ferrous gluconate with vitamin C; the serum iron was determined before and 5 minutes, 30 minutes, 2 hours, and 3 hours after the intravenous injection of the salt.

The unsaturated serum transferrin (U.I.B.C.) was determined *in vitro* according to the method of Ventura (1952), which is an adaptation of the original technique of Cartwright and Wintrobe, and calls for the use of the Beckman spectrophotometer on 520 m μ wavelength.

In the study of the graphic curve of the intravenous iron-tolerance tests the following data of Gitlow and Beyers were taken into consideration: (1) T.I.B.C.=total iron-binding capacity (specimen drawn five minutes after the injection of iron); (2) INC.=increase from the initial serum iron level

(SeFe) to the T.I.B.C. (INC.=T.I.B.C.-SeFe); (3) DEC.=decrease in the serum iron level from the five-minute to the 120-minute specimen (DEC.=T.I.B.C.-120-minute specimen); (4) DEC./INC. ratio; (5) SeFe/T.I.B.C. ratio; (6) S.D.I.=addition of the DEC./INC. and SeFe/T.I.B.C. values $\left(S.D.I. = \frac{DEC.}{INC.} + \frac{SeFe}{T.I.B.C.} \right)$.

Results

Our research (see Table and Figs. 1 and 2) gave the following results:

Serum Iron.—The serum iron was constantly high in the patients with haemochromatosis and in those with hypertrophic splenomegalic liver cirrhosis; on the other hand, it was normal or lower than normal in the patients with Morgagni-Laennec atrophic cirrhosis.

Oral Iron-tolerance Tests.—In the cases of haemochromatosis the oral administration of iron did not provoke any significant increase of serum iron, and the graphic curve of the iron-tolerance test showed a clearly level pattern (Fig. 1).

FIG. 1.—Oral iron-tolerance tests with 120 mg. Fe⁺⁺ in patients with haemochromatosis and in patients with (hypertrophic or atrophic) liver cirrhosis.

Also in 10 out of 12 cases of liver cirrhosis these tests gave similar results, with no or very little increase of serum iron. Only in two cirrhotic patients was a moderate increase of serum iron observed (respectively +66% and +80%); it was, however, lower than in the control subjects (Fig. 1).

Intravenous Iron-tolerance Tests.—In the patients with haemochromatosis the increase of serum iron, which is considerable in normal subjects, was very little; it was moderate in only one case. The graphic curve of these tests showed a level pattern (Fig. 2). In the patients with liver (hypertrophic or atrophic) cirrhosis, however, the curve showed a quick increase, but it was lower than in the controls; besides, the serum iron values tended to return to the initial levels more quickly than in the controls (Fig. 2).

Data Drawn from Intravenous Iron-tolerance Test.—From the Table we can observe the following data: (a) T.I.B.C. was normal or slightly reduced both in cases of haemochromatosis and in those of hypertrophic or atrophic liver cirrhosis. (b) INC. was very little in the patients with haemochromatosis and in three out of five patients with hypertrophic hyperhaemolytic liver cirrhosis; it was remarkable in all the patients with atrophic liver cirrhosis. (c) DEC. was variable in all the subjects tested. (d) DEC./INC. (index of response of the body to the intravenous iron charge) was always increased in the patients suffering from haemochromatosis or hypertrophic splenomegalic liver cirrhosis with hyperhaemolysis; it was lower in those suffering from atrophic liver cirrhosis. (e) SeFe/T.I.B.C. (degree of saturation of the serum iron-binding capacity) was always considerably increased in the cases of haemochromatosis or hypertrophic splenomegalic liver cirrhosis with hyperhaemolysis; it was lower in

the cases of atrophic liver cirrhosis. (f) S.D.I. was always higher than 1.35 in those with haemochromatosis and lower than 1.35 in cases of Morgagni-Laennec atrophic cirrhosis. In three out of five patients suffering from hypertrophic hyperhaemolytic liver cirrhosis the behaviour of S.D.I. was the same as in those with haemochromatosis.

Transferrin (Unsaturated Fraction, or U.I.B.C.).—The level of the unsaturated serum iron-binding capacity, or transferrin, was very low in the patients with haemochromatosis. In 7 out of 12 with liver cirrhosis (2 with hypertrophic hyperhaemolytic cirrhosis and 5 with atrophic cirrhosis) it was normal; in the other 5 it was lower than in normal controls.

Discussion

The results are in close agreement with those of Gitlow and Beyers. They show that the study of iron metabolism can supply significant data for the differential diagnosis between haemochromatosis and liver cirrhosis. Three indices derived from breaking down the curve of intravenous iron-tolerance tests are particularly useful from this point of view. They are: (1) DEC./INC., which shows the capacity of response of the body to the intravenous iron charge; (2) SeFe/T.I.B.C., which shows the degree of saturation of the serum iron-binding capacity; (3) S.D.I.—that is, the addition of the two preceding indices (DEC./INC. + SeFe/T.I.B.C.).

In haemochromatosis the values of the first two indices are constantly higher than in normal subjects. In patients with Morgagni-Laennec atrophic cirrhosis they are significantly lower and their levels are not substantially different from the limits registered in normal subjects.

Data from Intravenous Iron-tolerance Test and Transferrin Values in Normal Subjects and in Patients with Haemochromatosis and Liver Cirrhosis

Case No.	Sex and Age	Diagnosis	SeFe (γ/100 ml.)	T.I.B.C. (γ/100 ml.)	INC. (γ/100 ml.)	DEC. (γ/100 ml.)	DEC./INC.	SeFe/T.I.B.C.	S.D.I. (DEC + SeFe / INC + TIBC)	Transferrin (U.I.B.C.) in vitro Determination (γ/100 ml.)
1	F 45	Normal	132	363	231	63	0.27	0.36	0.63	240
2	F 50	"	116	370	254	74	0.29	0.31	0.60	270
3	M 45	"	148	375	227	59	0.25	0.39	0.64	220
4	M 49	"	144	320	176	68	0.38	0.45	0.83	170
5	M 47	"	123	298	175	60	0.34	0.41	0.75	180
Mean			132.6 ± 6.0	345.2 ± 15.2	212 ± 15.8	64.8 ± 2.7	0.30 ± 0.02	0.38 ± 0.02	0.69 ± 0.04	216 ± 18.6
6	F 54	Haemochromatosis	220	270	50	34	0.68	0.81	1.49	65
7	M 32	"	178	251	73	51	0.69	0.70	1.39	80
8	M 45	"	259	294	35	30	0.85	0.88	1.73	0
9	F 61	"	171	285	114	105	0.92	0.60	1.52	120
10	F 58	"	232	400	168	160	0.95	0.58	1.53	0
Mean			212.0 ± 16.5	300 ± 26.0	88 ± 24	76 ± 24.8	0.81 ± 0.05	0.71 ± 0.05	1.53 ± 0.05	53 ± 23.4
t			4.54 P < 0.01	1.49 P > 0.1	4.32 P < 0.01	0.44 P > 0.6	8.39 P < 0.001	5.32 P < 0.001	12.0 P < 0.001	5.45 P < 0.001
11	M 53	Hypertrophic liver cirrhosis	188	350	162	63	0.38	0.53	0.91	180
12	M 49	"	160	244	84	60	0.71	0.65	1.36	80
13	M 64	"	231	304	73	44	0.60	0.76	1.36	65
14	M 54	"	228	280	52	40	0.76	0.81	1.57	0
15	M 61	"	160	308	148	88	0.59	0.51	1.10	160
Mean			193.4 ± 15.5	297.2 ± 17.4	103.8 ± 21.6	59 ± 8.4	0.60 ± 0.06	0.65 ± 0.05	1.26 ± 0.11	97 ± 32.8
t			3.65 P < 0.01	2.06 P > 0.05	4.06 P < 0.001	0.65 P > 0.5	4.37 P < 0.01	4.18 P < 0.01	4.67 P < 0.01	3.07 P < 0.02
16	M 57	Atrophic liver cirrhosis	154	336	182	75	0.41	0.45	0.86	190
17	M 56	"	132	356	224	104	0.46	0.39	0.85	190
18	M 58	"	92	270	178	66	0.37	0.34	0.71	180
19	M 42	"	148	292	144	40	0.27	0.50	0.77	30
20	M 56	"	76	260	184	108	0.58	0.29	0.87	180
21	F 53	"	88	280	192	60	0.31	0.31	0.62	200
22	M 58	"	128	192	64	32	0.50	0.66	1.16	60
Mean			116.9 ± 11.7	283.7 ± 20.2	166.6 ± 19	69.2 ± 11	0.41 ± 0.05	0.42 ± 0.07	0.83 ± 0.09	147 ± 38
t			1.07 P > 0.3	2.27 P < 0.05	1.72 P > 0.1	0.33 P > 0.7	2.07 P > 0.05	0.58 P > 0.5	1.69 P > 0.1	1.93 P > 0.05

Statistical significance of the differences of the mean values between haemochromatosis and liver cirrhosis:

Haemochromatosis-hypertrophic liver cirrhosis. t	0.83 P > 0.4	0.09 P > 0.9	0.46 P > 0.6	0.64 P > 0.5	2.44 P < 0.05	0.72 P > 0.4	2.12 P > 0.05	1.09 P > 0.3
Haemochromatosis-atrophic liver cirrhosis. t	4.87 P < 0.001	0.52 P > 0.6	2.55 P < 0.05	0.28 P > 0.7	5.97 P < 0.001	3.81 P < 0.01	7.86 P < 0.001	2.51 P < 0.05

The standard error of the mean was calculated according to the formula $s = \sqrt{\frac{s(\bar{y} - \bar{y})^2}{n(n-1)}}$.

The t was calculated according to the procedure given in J. H. Burn's *Biological Standardization* (Oxford Univ. Press, London, 1950).

The statistically significant differences of the mean values are printed in italics.

FIG. 2.—Intravenous iron-tolerance tests with 10 mg. Fe⁺⁺ in patients with haemochromatosis and in patients with (hypertrophic or atrophic) liver cirrhosis.

The third index is generally higher than 1.35 in haemochromatosis and lower than 1.35 in atrophic liver cirrhosis.

The above-mentioned data do not seem to be of value in differentiating haemochromatosis from hypertrophic splenomegalic liver cirrhosis with hyperhaemolysis. In these diseases the values of the indices referred to can be equal. From the Table it will be seen that, except the DEC./INC. ratio, none of the mean values of the indices taken into consideration is statistically different in the two groups of patients. However, other clinical features and laboratory tests are available in this differential diagnosis.

The determination of serum iron alone, of oral iron-tolerance, and of serum transferrin has little diagnostic value in differentiating haemochromatosis from liver cirrhosis.

Serum iron is usually normal or lower than normal in liver cirrhosis, but can often reach very high values in various conditions (acute hepato-cellular damage, hyperhaemolysis, etc.). Also, the oral iron-tolerance test has no significantly differential value; the level pattern of the graphic curve of this test is probably related to an increased

tendency to store iron in the body. This increased iron storage occurs both in haemochromatosis and in liver cirrhosis.

The unsaturated serum transferrin is constantly very low in haemochromatosis. This feature can be observed also in liver cirrhosis, not only in hypertrophic splenomegalic cirrhosis with hyperhaemolysis, but also in atrophic cirrhosis, probably in relation to the usual decrease of plasma β_1 -globulins, to which the transferrin belongs.

Summary

A comparative study has been made of serum iron, of oral and intravenous iron-tolerance, and of unsaturated transferrin (U.I.B.C.) in 5 cases of haemochromatosis, in 12 cases of liver cirrhosis (7 cases of Morgagni-Laennec atrophic cirrhosis, 5 cases of hypertrophic splenomegalic liver cirrhosis with hyperhaemolysis), and in 5 normal controls.

The results obtained prove that the study of iron metabolism can give some significantly differential data between haemochromatosis and liver cirrhosis. Three indices derived from breaking down the graphic curve of intravenous iron-tolerance tests are particularly useful. The first of these (DEC./INC.) expresses the capacity of response to the intravenous iron charge; the second (SeFe/T.I.B.C.) expresses the degree of saturation of serum iron-binding capacity; both are greatly increased in haemochromatosis. Generally such marked increase is absent in atrophic liver cirrhosis, but sometimes values are found to be slightly higher than in normal subjects. The third index (S.D.I.) is obtained by the addition of the two preceding data. S.D.I. is always higher than 1.35 in haemochromatosis and lower than 1.35 in atrophic liver cirrhosis.

The above-mentioned data are of no use in the differential diagnosis between haemochromatosis and hypertrophic splenomegalic liver cirrhosis with hyperhaemolysis.

The determinations alone of serum iron and unsaturated transferrin, and the oral iron-tolerance test, have little value in differentiating haemochromatosis from liver cirrhosis.

REFERENCES

- Bothwell, T. H., and Mallett, B. (1955). *Biochem. J.*, **59**, 599.
- Gitlow, S. E., and Beyers, M. R. (1952). *J. Lab. clin. Med.*, **39**, 337.
- and Colmore, J. P. (1952). *Ibid.*, **40**, 541.
- Napolitano, L., and Scuro, L. A. (1954a). *Policlinico, Sez. prat.*, **61**, 1265.
- (1954b). *Progr. med. (Napoli)*, **10**, 676.
- (1956). *Policlinico, Sez. prat.*, **63**, 1823.
- Ventura, S. (1952). *J. clin. Path.*, **5**, 271.

The annual conference of the National Smoke Abatement Society was held in Hastings this week (October 2-4). The first three sessions of the conference dealt with air pollution from road vehicles, the administration of the Clean Air Act, 1956, and industry and the Clean Air Act. In his presidential address Dr. R. LESSING, Ph.D., stressed the importance of extensive cleaning of raw coal. He contended that a large proportion of the dust discharged from both industrial and domestic chimneys was due to "natural," removable dust in coal. Dr. Lessing also emphasized the importance of washing coal to remove sulphurous impurities. Papers on air pollution from road vehicles dealt with methods of reducing the amount of sulphur released from diesel fuel and of carbon monoxide from petrol. Mr. G. GRAHAM DON (London School of Hygiene and Tropical Medicine) said that the smoke control area was the backbone of the new attack on air pollution. He considered it more profitable to control a suburban area with mainly domestic chimneys than the centre of an industrial city. He suggested that a "clean air ring," on the "green belt" principle, should be instituted in industrial cities, spreading gradually from the edge towards the centre.