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Research Article

THE VARIABILITY OF NON-HEMOGLOBIN IRON [1]

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In this paper data on the range of variation of non-hemoglobin iron in human blood will be presented. The significance of these variations in relation to (a) the calculation of hemoglobin, or oxygen capacity from the determinations of total blood iron, and reciprocally, (b) the deduction of values for total blood iron from determinations of hemoglobin or oxygen capacity, will be considered.

Because of the difficulties inherent in the direct determination of hemoglobin there has been a widespread interest in the indirect methods of approach. Of these the oxygen capacity is the most accurate, but there has been an idea in the minds of many that it is a formidable procedure and, as an alternative, there has been a growing tendency to arrive at values for hemoglobin by measurements of total blood iron. Wong (1), Fowweather (2), Sackett (3), Dupray (4), and others have calculated hemoglobin values from determinations of total blood iron. Kennedy (5) believes that total blood iron can be correlated with oxygen capacity and hemoglobin content as determined by colorimetric methods. Murphy, Lynch and Howard (6) have proposed a new index, the "iron index," obtained by dividing the milligrams of iron in 100 cc. of blood by the number of red blood cells in the same volume, as an expression of the concentration of iron in the red blood cells. The validity of these values rests on the assumption that all the iron of the blood is contained within the red blood cells, or combined with hemoglobin, and behaves as a single variable.

In 1898 Abderhalden (7) first showed that there was more iron in the blood of various animals than could be attributed to the hemoglobin alone. He found that the non-hemoglobin iron amounted to 10 per cent of the total blood iron in the ox, 3.6 per cent in the horse, and 6 per cent in the rabbit. Since then Ehrlich and Lazarus (8), Rosin and Jellinek (9), Erben (10), Seiller (11), Lintzel (12), Freund (13), Barkan (14), Brugsch (15), Warburg and Krebs (16), Langer (17), Locke, Main and Rosbash (18), Dominici (19), McIntosh (20) and many others have verified the presence of non-hemoglobin iron in human blood. The values have varied from minute amounts to 10 per cent of the total blood iron. Barkan (14) showed that there was no relation between the level of hemoglobin and non-hemoglobin iron among twenty-one different individuals nor in the same person under varying conditions. Riecker and Winters (21) found no direct relationship between serum iron and the level of hemoglobin in dogs. Schultze and Elvehjem (22) could obtain no agreement between total blood iron and hemoglobin in fowl.

Because of the fundamental importance of this question to the problems of iron metabolism, iron transport, and the relations between iron and hemoglobin, it seemed desirable to study the magnitude and the variations of non-hemoglobin iron in human beings. With this in mind seventy-two determinations of total blood iron and oxygen capacity were made on the blood of fifty-seven individuals. Patients with a wide variety of disease conditions, as well as normals, were included, in order that the results might give, as far as possible, a picture of the range of both physiologic and pathologic variations. From the work of Barkan (14, 23) who showed that all but a small fraction of the non-hemoglobin iron was contained within the red blood cells, it is obvious that valid figures for non-hemoglobin iron cannot be derived from analyses of blood serum or plasma. Since one gram-molecule of hemoglobin combines with one gram-molecule of iron and one of oxygen, values for hemoglobin-iron can be calculated from measurements of blood oxygen capacity, with the high degree of accuracy inherent in this procedure. To obtain the value for hemoglobin iron the figure for oxygen capacity is multiplied by the factor 2.495. By subtracting this value from the total blood iron determined by analysis, a figure for

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TABLE I

Outline of experimental data

Subject number	Age	Diagnosis	Hemoglobin iron calculated from O_2 capacity	Total iron by analysis	Non-hemoglobin iron	Non-hemoglobin iron / Total iron
	years		*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*
1.	55	Pernicious anemia, diabetes mellitus	44.8	46.2	1.4	3.0
2.	85	Diabetes mellitus, arteriosclerotic heart disease	51.5	51.7	0.2	0.4
3.	44	Pregnancy, acute nephritis	46.5	48.8	2.3	4.7
4.	69	Cardiovascular disease, dehydration	43.8	45.7	1.9	4.2
5.	63	Diabetes mellitus, arteriosclerosis, generalized	49.0	52.7	3.7	7.0
6.	40	Pernicious anemia	27.4	29.4	2.0	6.8
7.	70	Diabetes mellitus	48.5	51.4	2.9	5.6
8.	65	Paget's disease of bones	27.7	33.7	6.0	17.8
9*a*.	45	Apoplexy, arteriosclerosis, generalized	44.8	47.8	3.0	6.3
9*b*.	45	Apoplexy, arteriosclerosis, generalized	45.6	51.0	5.4	10.6
10.	45	Diabetes mellitus, perianal abscess	43.4	45.5	2.1	4.6
11.	48	Cardiac decompensation, severe	40.9	48.1	7.2	15.0
12.	38	Nephritis, subacute; anemia, hypochromic	23.6	25.4	1.8	7.1
13*a*.	20	Chlorosis	10.8	11.7	0.9	7.7
13*b*.	20	Chlorosis, iron therapy	29.7	30.4	0.7	2.3
14.	60	Diabetes mellitus, abscess of hand	33.6	36.4	2.8	7.7
15.	16	Acute nephritis	41.9	45.1	3.2	7.1
16.	43	Acute nephritis	35.6	41.0	5.4	13.2
17.	30	Anxiety neurosis, obesity	47.8	53.6	5.8	10.8
18.	73	Diabetes mellitus, arteriosclerosis, generalized anemia, hypochromic	18.8	19.4	0.6	3.1
19.	39	Chronic nephritis with edema, anemia, hypochromic	31.6	34.3	2.7	7.9
20.	57	Leukemia	14.3	14.4	0.1	0.7
21.	60	Arteriosclerosis with heart disease	61.5	62.4	0.9	1.4
22.	17	Hyperthyroidism	52.5	53.1	0.6	1.1
23.	46	Diabetic acidosis	51.0	53.3	2.3	4.3
24*a*.	56	Polycythemia vera	62.4	64.3	1.9	3.0
24*b*.	56	Polycythemia vera	62.9	63.2	0.3	0.5
25*a*.	67	Banti's disease	26.0	26.4	0.4	1.5
25*b*.	67	Banti's disease	25.4	26.5	1.1	4.2
26.	55	Pernicious anemia, nephritis, acute	34.2	33.0	−1.2	
27.	76	Arteriosclerosis, generalized; anemia, hyperchromic	30.3	29.5	−0.8	
28*a*.	45	Anemia, hypochromic, due to chronic blood loss	12.6	13.7	1.1	8.0
28*b*.	45	Anemia, hypochromic, due to chronic blood loss; iron therapy	36.3	36.7	0.4	1.1
29.	43	Cardiovascular renal disease; hypochromic anemia, secondary	34.3	35.4	1.1	3.1
30.	39	Idiopathic hypochromic anemia	28.0	29.2	1.2	4.1
31.	63	Arteriosclerosis, generalized; apoplexy	54.1	55.4	1.3	2.3
32.	63	Polycythemia vera	51.8	53.0	1.2	2.3
33.	39	Idiopathic hypochromic anemia; iron therapy	24.4	25.9	1.5	5.8
34.	44	Idiopathic hypochromic anemia	25.1	28.7	3.6	12.5
35*a*.	41	Bleeding peptic ulcer	21.5	23.1	1.6	6.9
35*b*.	41	Bleeding peptic ulcer	38.3	39.1	0.8	2.0
36*a*.	44	Pernicious anemia	17.2	18.7	1.5	8.0
36*b*.	44	Pernicious anemia, liver therapy	26.0	30.5	4.5	14.8
37*a*.	58	Emphysema, polycythemia	59.0	60.7	1.7	2.8
37*b*.	58	Polycythemia, phenylhydrazine therapy	50.4	51.2	0.8	1.6
37*c*.	58	Polycythemia, phenylhydrazine continued	43.5	43.8	0.3	6.9
38*a*.	60	Polycythemia vera	70.7	71.5	0.8	1.1
38*b*.	60	Polycythemia vera, phenylhydrazine therapy	56.3	63.6	7.3	11.5
39.	30	Normal	51.0	52.9	1.9	3.6
40*a*.	28	Normal	49.6	52.0	2.4	4.6
40*b*.	28	Normal	52.2	56.0	3.8	6.7
41.	30	Normal	46.6	47.3	0.7	1.5
42*a*.	30	Normal	44.2	49.7	5.5	11.1
42*b*.	30	Normal	49.2	54.3	5.1	9.4

TABLE I (*continued*)

Subject number	Age	Diagnosis	Hemoglobin iron calculated from O_2 capacity	Total iron by analysis	Non-hemoglobin iron	Non-hemoglobin iron / Total iron
	years		*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*per cent*
42*c*.	30	Normal	47.8	50.4	2.6	5.2
43*a*.	31	Normal	50.2	52.7	2.5	4.7
43*b*.	31	Normal	47.3	49.2	1.9	3.9
44.	35	Normal	48.3	50.7	2.4	4.7
45*a*.	25	Normal	45.4	48.5	3.1	6.4
45*b*.	25	Normal	49.1	49.1	0.0	0.0
46.	21	Normal	44.2	46.8	2.6	5.6
47.	25	Normal	54.7	58.6	3.9	6.7
48.	25	Normal	51.1	54.7	3.6	6.6
49.	26	Normal	48.8	51.4	2.6	5.1
50.	26	Normal	47.2	48.3	1.1	2.3
51.	21	Normal	52.8	57.0	4.2	7.4
52.	38	Normal	57.4	60.2	2.8	4.7
53.	26	Normal	45.7	46.3	0.6	1.3
54.	21	Normal	42.2	43.3	1.1	2.5
55.	22	Normal	45.1	47.1	2.0	4.2
56.	28	Normal	45.2	45.9	0.7	1.5
*57.	25	Normal	54.2	69.5	15.3	22.0

Mean non-hemoglobin iron	2.2 mgm. per cent	Standard deviation ± 1.74 mgm. per cent
Non-hemoglobin iron / Total iron	5.3 per cent	Standard deviation ± 3.9 per cent
Mean error of duplicate determinations of total iron	0.38 mgm. per cent	Standard deviation ± 0.46 mgm. per cent
Mean error of duplicate determinations of oxygen capacity in terms of hemoglobin iron	0.38 mgm. per cent	Standard deviation ± 0.31 mgm. per cent

*Omitted from statistical consideration.

true non-hemoglobin iron can be obtained. Barkan (24) attempted to arrive at values for non-hemoglobin iron by ultrafiltration of hemolyzed blood, and Winegarden and Borsook (25) by dialysis; but in view of the unstable nature of hemoglobin when subjected to manipulation, these methods cannot be considered reliable.

Oxygen capacity was determined in duplicate by the carbon monoxide method of Van Slyke and Hiller as described by Peters and Van Slyke (26). Total blood iron was measured in duplicate or triplicate by volumetric titration with titanium according to the technique recently described (27). Data concerning the sensitivity of this method as well as recovery experiments are given in that paper.

RESULTS

The experimental findings are presented in Table I.

The value for non-hemoglobin iron of Case 57 was omitted in the mathematical treatment of results because it lies so far beyond the range of the other findings that it is highly probable that some gross technical error was undetected.

In this series an average of 5.3 per cent of the total iron was found uncombined with active hemoglobin. This is in close agreement with the work of Barkan (24) who reports that the free iron of the blood amounts to from 5 to 6 per cent of the hemoglobin iron. In Figure 1 the values for non-hemoglobin iron are plotted against oxygen capacities. It can be readily seen that there is no correlation between non-hemoglobin iron and oxygen capacity. These results indicate that attempts to arrive at values for hemoglobin by determination of total blood iron or vice versa, are subject to an average error of 5.3 per cent with a standard deviation of 3.9 per cent, and a possible error as great as 17.8 per cent. The scatter of the actual values of non-hemoglobin iron is given in Figure 2. The wide range of variations of non-hemoglobin iron is apparent.

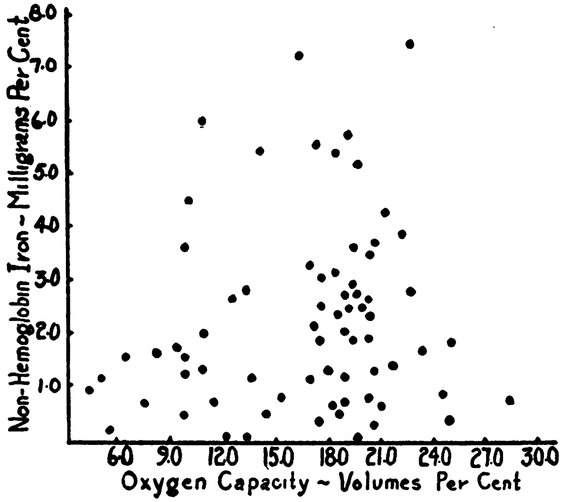

FIG. 1. CORRELATION CHART.

The relation between non-hemoglobin iron and oxygen capacity.

In agreement with Barkan (23), it was evident from simultaneous determinations of total blood iron, oxygen capacity, and serum iron, that the serum contained only a fraction of the non-hemoglobin iron. This will be considered in detail in another report.

SUMMARY

From determinations of oxygen capacity and total blood iron in fifty-seven subjects under various conditions, values for non-hemoglobin iron were obtained. It was found that non-hemoglobin iron varies widely and is a significant fraction of total iron. For these reasons, attempts to correlate values for hemoglobin and oxygen capacity with determinations of total blood iron are fruitless.

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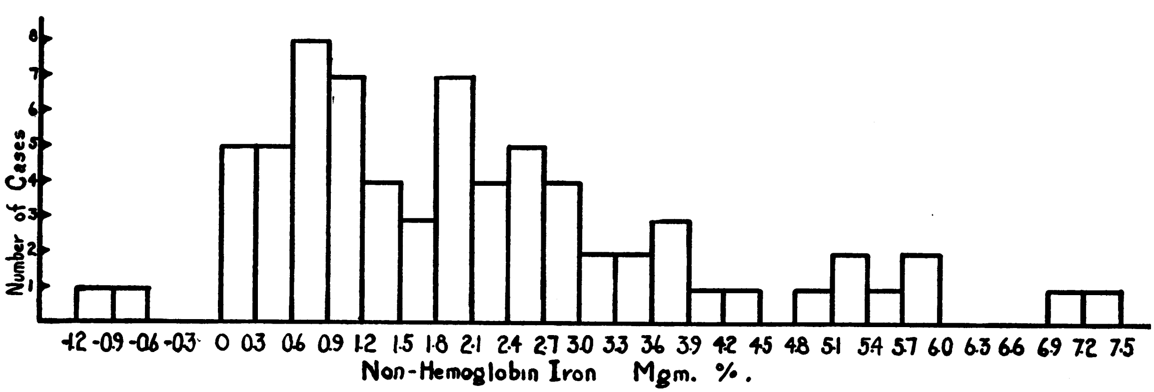

FIG. 2. THE DISTRIBUTION OF VALUES FOR NON-HEMOGLOBIN IRON.

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