Diagnostic value of serum haptoglobin

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SYNOPSIS Serum haptoglobin has been estimated quantitatively in 25 patients with haemolytic disease, and its diagnostic value assessed by comparing the levels with those obtained in 110 normal subjects, in 149 patients with other forms of anaemia, and in 37 patients with non-haematological disorders. The normal range was found to be 33 to 213 mg./100 ml.; subnormal levels were found in 80% of patients with haemolytic disease or megaloblastic anaemia, patients with haemorrhage into the tissues, and occasionally in association with other diseases. When taken in conjunction with other clinical and laboratory features this simple biochemical estimation can be of diagnostic value.

Haptoglobins are a group of serum proteins, originally identified by Polonovski and Jayle (1939), which have the characteristic property of combining with haemoglobin to form a stable complex. Quantitative estimation of haptoglobins depends upon measuring the amount of this haemoglobin-haptoglobin complex formed when the test serum has been allowed to combine with free haemoglobin either as oxyhaemoglobin or methaemoglobin and is therefore a measure of their haemoglobin-binding capacity.

Neale, Aber, and Northam (1958) showed that in patients with excessive haemolysis clearance of haemoglobin from serum leads to absence or reduction of haptoglobins. Serum haptoglobin estimation may therefore be of value in the diagnosis of haemolytic disease (Nosslin and Nyman, 1958) but clinical interpretation is difficult because of low levels occurring in some normal subjects (Table I). Furthermore, Allison, Blumberg, and ap Rees (1958) found a complete absence of serum haptoglobins in 2% of normal healthy British subjects and in 30% of healthy Nigerian subjects. It has also been recognized that decreased levels are found in association with hepatocellular disease (Nyman, 1959; Owen, Mackay, and Got, 1959; Williams, Speyer, and Billing, 1961). Raised levels have been found in serum from patients with infection, malignancy, connective tissue disorder, biliary obstruction, glomerulonephritis, pyelonephritis, ulcerative colitis, arterial disease, acute rheumatic fever, myocardial infarction (Owen, Smith, Padanyi, and Martin, 1964), and haemoblastoses (Iványi, Šochman, and Iványi, 1961). Both high and low levels occur in patients receiving steroid therapy (Brus and Lewis,

1959; Borglin and Nyman, 1961). Doubt has therefore been thrown on the diagnostic value of this estimation, and so we have assessed its use in the investigation of patients with haemolytic disease and compared their levels with those from normal subjects, patients with other forms of anaemia, and patients with non-haematological disorders.

TABLE I SERUM HAPTOGLOBIN LEVELS IN NORMAL SUBJECTS			
Author	Number of Subjects	Mean (mg./100 ml.)	Range (mg./100 ml.)
Jayle and Boussier (1955)	1,000	74	12-136
Nyman (1959)	277	110	28-136
Iványi <i>et al.</i> (1961)	30	110	50-150
Owen <i>et al.</i> (1964)	152	93	10-220
Present series	110	110	33-213

SUBJECTS

NORMAL SUBJECTS One hundred and ten healthy subjects volunteered, 14 being laboratory staff, 81 blood donors, and 14 old age pensioners.

PATIENTS Two hundred and fifteen cases are grouped into the following categories:—

Haemolytic disease Twenty-five cases with a normocytic normochromic anaemia associated with reticulocytosis, erythroblastemia, and such features as splenomegaly, microspherocytosis, elliptocytosis, auto-immune red cell antibodies, increased red cell osmotic fragility, abnormal haemoglobin, decreased red cell survival: none of the cases had overt anaemia at the time the estimation was made.

Megaloblastic anaemia Thirty-three cases with megaloblastic erythropoiesis: this group included patients with pernicious anaemia, post-gastrectomy vitamin B₁₂ deficiency, intestinal malabsorption, and anaemia following treatment with anticonvulsant drugs.

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Iron-deficiency anaemia This category comprises 65 cases with hypochromic anaemia and mean cell haemoglobin concentration below 32%. In those cases where the serum iron and iron-binding capacity was determined it was found to be outside the normal ranges for the respective age and sex of the particular patient. Anaemia was due either to chronic haemorrhage, nutritional iron deficiency, or intestinal malabsorption.

Recent haemorrhage Twenty-eight cases of normocytic normochromic anaemia following recent blood loss from the gastro-intestinal tract, nose or uterus, or from bleeding into the tissues as a result of trauma, coagulation defect, or vascular disorder.

Reticulosis Twenty cases in which the diagnosis was either acute leukaemia, myeloid leukaemia, myelofibrosis, lymphatic leukaemia, lymphosarcoma, reticulum-cell sarcoma, or Hodgkin's disease.

Miscellaneous anaemias Three cases of 'hyper-splenism', one of which was secondary to hepatic cirrhosis, two cases of aplastic anaemia, and two of sideroblastic anaemia.

Other diseases Thirty-seven cases with a non-haematological disorder: this group included patients with conditions such as bronchitis, pneumonia, Hammon-Rich syndrome, subacute bacterial endocarditis, pyelonephritis, pyogenic abscess, cholecystitis, hepatitis, hepatic cirrhosis, pancreatitis, hypothyroidism, rheumatoid arthritis, benign or malignant tumours.

is measured by the colour developed when added to hydrogen peroxide and guaiacol. This technique measures the total serum haptoglobin and makes no attempt to differentiate between the types of haptoglobin demonstrated by starch gel electrophoresis (Smithies, 1955). Quantitative estimation was made up to 250 mg./100 ml., but where the level was in excess of this concentration it was recorded as greater than 250 mg./100 ml.

In 19 selected cases red cell survival was determined by the radioactive chromium technique (Veall and Vetter, 1958), the patients' own cells being labelled by ^{51}Cr , washed in saline, and later reinjected. Blood samples were taken after 15 minutes, 24 hours, 48 hours, and then at three-day intervals until the ^{51}Cr half-life had been passed. The results were expressed in terms of half-time for the disappearance of ^{51}Cr from the circulation ($T_{1/2}^{51}\text{Cr}$), without correction for chromium elution. Due to uncertainties with regard to the activities of some of the 15-minute specimens, the 100% value was taken as the activity found after 24 hours. The mean $T_{1/2}^{51}\text{Cr}$ by this method is 25.5 days. In later studies the labelled red cells were washed in a phosphate-citrate buffer, as recommended by Mollison (1961). This procedure decreases the elution of chromium and prolongs the $T_{1/2}^{51}\text{Cr}$ to 32.5 days (range 28-33 days).

Haemoglobin, serum bilirubin, and other haematological data were obtained by methods described by Dacie and Lewis (1963).

METHODS

Serum haptoglobin was measured by the method of Owen, Better, and Hoban (1960). Here the peroxidase activity of the haemoglobin-haptoglobin complex obtained when haptoglobin is bound by methaemoglobin

RESULTS

The serum haptoglobin levels have been charted according to their respective clinical groups (Fig. 1).

FIG. 1. Levels of serum haptoglobin in normal subjects and different clinical groups.

FIG. 2. Distribution of serum haptoglobin levels in 110 normal subjects.

NORMAL SUBJECTS In 54 males the serum haptoglobin range was 40-213 mg./100 ml. (mean 108 mg./100 ml.) and in 56 females 33-190 mg./100 ml. (mean 111 mg./100 ml.). The mean for both sexes was 110 mg./100 ml. The distribution showed a positive skew curve (Fig. 2). In only one subject was the level below 40 mg./100 ml. so this has been taken as an arbitrary lower limit of normal in this investigation. None of the 14 subjects under 20 years of age had a level above 120 mg./100 ml. and none of the 14 subjects over 65 years of age had a level below 80 mg./100 ml. Apart from this suggestion of

an increase in level with age it was not possible to demonstrate a change with age for the whole series.

HAEMOLYTIC DISEASE The range of serum haptoglobin varied from 0 to 85 mg./100 ml. (Table II). Of the 25 patients investigated, 14 had levels of 20 mg./100 ml. or below, while four were above 40 mg./100 ml., cases 5, 14, and 19 having marked anaemia with reticulocytosis and raised levels of unconjugated bilirubin, while case 12 had a decreased red cell survival time. In the group as a whole, there was little correlation between serum haptoglobin and bilirubin levels, the latter being within normal limits in eight of the 25 cases, all of which had low levels of haptoglobin, five with marked anaemia. There was little general correlation between the levels of serum haptoglobin and the haemoglobin concentration.

MEGALOBLASTIC ANAEMIA The range in the 33 cases was 0-110 mg./100 ml., 13 having levels below 20 mg./100 ml. and eight above 40 mg./100 ml. The patient with a level of 110 mg./100 ml. had haemoglobin concentration of only 6.6 g./100 ml. but also suffered from pyelonephritis. The one with a level of 75 mg./100 ml. had classical pernicious anaemia of mild degree (haemoglobin 10.5 g./100 ml.).

IRON-DEFICIENCY ANAEMIA In the 65 cases of this group the range varied from 20 to over 250 mg./100 ml. The patient (case 26) with the level of 260

TABLE II

SERUM HAPTOGLOBIN, BILIRUBIN, AND HAEMOGLOBIN LEVELS IN 25 CASES OF HAEMOLYTIC ANAEMIA

Case	Diagnosis	Serum Haptoglobin (mg./100 ml.)	Serum Bilirubin (mg./100 ml.)	Haemoglobin (g./100 ml.)
1	Hereditary spherocytosis	10	1.1	10.4
2	Hereditary spherocytosis	24	3.4	12.0
3	Hereditary spherocytosis	20	2.5	12.4
4	Hereditary spherocytosis	0	3.0	9.7
5	Hereditary spherocytosis	62	1.3	8.0
6	Hereditary spherocytosis	18	0.8	14.9
7	Hereditary spherocytosis	12	2.5	8.2
8	Hereditary spherocytosis	33	1.0	10.0
9	Hereditary spherocytosis	40	0.6	14.6
10	Hereditary elliptocytosis	20	1.0	12.0
11	Hereditary elliptocytosis	33	0.4	4.8
12	Hereditary elliptocytosis	50	0.5	13.1
13	Sickle cell disease	24	0.9	11.2
14	Sickle cell disease	47	2.4	8.6
15	Auto-immune haemolytic anaemia	0	3.3	8.8
16	Auto-immune haemolytic anaemia	10	3.1	6.6
17	Auto-immune haemolytic anaemia	23	2.7	5.3
18	Auto-immune haemolytic anaemia	8	0.3	4.6
19	Auto-immune haemolytic anaemia	85	3.2	10.4
20	Auto-immune haemolytic anaemia	5	0.9	12.7
21	Auto-immune haemolytic anaemia	0	0.7	9.7
22	Septicaemia	31	1.4	7.0
23	Pyelo-nephritis, with uraemia	27	0.4	5.9
24	Haemolytic anaemia of unknown cause	8	1.2	11.1
25	Haemolytic anaemia of unknown cause	0	0.7	5.6

mg./100 ml. was found to have a normal red cell survival time (Table III). In those with a high level of haptoglobin the iron-deficiency anaemia was associated with such disorders as pyelitis, ulcerative colitis, hepatic cirrhosis, rheumatoid arthritis, or disseminated lupus erythematosus.

TABLE III

COMPARISON OF SERUM HAPTOGLOBIN LEVEL WITH RED CELL SURVIVAL MEASURED BY ^{51}Cr TECHNIQUE

Case	Diagnosis	Serum Haptoglobin (mg./100 ml.)	$T_{\frac{1}{2}}$ ^{51}Cr (days)
7	Hereditary spherocytosis	12	10 (25.5) ¹
11	Hereditary elliptocytosis	33	22 (32.5)
12	Hereditary elliptocytosis	50	27 (32.5)
18	Auto-immune haemolytic disease	8	14 (32.5)
24	Haemolytic anaemia of unknown cause	8	25 (32.5)
25	Haemolytic anaemia of unknown cause	10	16 (32.5)
26	Iron-deficiency anaemia	28	25 (25.5)
27	Idiopathic thrombocytopenia	20	30 (32.5)
28	Haemoptysis (pulmonary haemosiderosis)	7	29 (32.5)
29	Hodgkin's disease	230	18 (25.5)
30	Lymphosarcoma	40	25 (25.5)
31	Myeloid leukaemia	72	14 (25.5)
32	Myelofibrosis	100	25 (25.5)
33	Myelofibrosis	105	28 (32.5)
34	Hypersplenism (hepatic cirrhosis)	102	28 (32.5)
35	Hypersplenism (cause unknown)	138	28 (32.5)
36	Hypersplenism (cause unknown)	33	16 (32.5)
37	Sideroblastic anaemia	108	30 (32.5)
38	Sideroblastic anaemia	40	21 (32.5)
39	Infective hepatitis	0	28 (32.5)
40	Carcinomatosis	50	32 (32.5)
41	Hammon-Rich syndrome	145	33 (32.5)

¹Figures in brackets are mean normal $T_{\frac{1}{2}}$ for the method used in each case.

RECENT HAEMORRHAGE The levels in these 27 cases covered the whole range from 0 to over 250 mg./100 ml. Those with haemorrhage into the tissues—ovarian cyst, ectopic gestation, haemoptysis, thrombocytopenic purpura, cerebral haemorrhage, fractured femur—all had haptoglobin levels below 20 mg./100 ml. Cases 27 and 28, with thrombocytopenic purpura and haemoptysis due to idiopathic pulmonary haemosiderosis respectively, had normal red cell survival times. The patient with the level of over 250 mg./100 ml. had an infected haematoma.

RETICULOSIS Of these 20 cases only one had a serum haptoglobin level below 40 mg./100 ml. This patient showed no evidence of haemolytic disease or haemorrhage into the tissues. All three patients with high levels had Hodgkin's disease. Cases 29 and 31, who had levels of 230 and 72 mg./100 ml. respectively, had clinical and haematological evidence of haemolytic anaemia confirmed by reduced red cell survival times (Table III). In both cases

of myelofibrosis, haptoglobin levels were within the normal range, which was in keeping with their normal red cell survival times.

MISCELLANEOUS ANAEMIAS Of the three cases with 'hypersplenism', two (cases 34 and 35) had normal haptoglobin levels, while the third (case 36) had a level of 33 mg./100 ml. suggesting a haemolytic element in the anaemia. The red cell survival studies confirmed these observations, being normal in the two former cases and decreased in the one with the low level of haptoglobin. Both patients with aplastic anaemia had haptoglobin levels within the normal range. One patient with sideroblastic anaemia (case 37) showed a normal level of haptoglobin and normal red cell survival time, while the other (case 38) had a low level of haptoglobin and reduced red cell survival time (Table III).

OTHER DISORDERS The 44 cases in this group had levels ranging from 22 to over 250 mg./100 ml. Both patients with levels below 40 mg./100 ml. had infective hepatitis. Case 39 had an unconjugated serum bilirubin level of 1.6 mg./100 ml., reticulocytosis, no anaemia, and a red cell survival time within normal limits. The other patient had a moderate anaemia (haemoglobin 10.2 g./100 ml.) with reticulocytosis and a small amount of unconjugated bilirubin in the serum but she underwent a rapid spontaneous remission before it was possible to estimate the red cell survival time. A further patient with this condition had a serum haptoglobin level of over 250 mg./100 ml. High levels were also observed in another four patients of this group who had either a pyogenic abscess, acute bronchitis, or rheumatic fever.

DISCUSSION

The clinical value of any biochemical estimation depends upon the frequency of levels occurring outside the normal range in a particular group of disorders and upon the absence of such abnormal values in other conditions. We have found the level of serum haptoglobin to be subnormal in about 80% of cases with hereditary haemolytic disease, acquired haemolytic anaemia, or megaloblastic anaemia, but some of those whose haptoglobin level was within the normal range had evidence of increased haemolysis. Furthermore, a patient with Hodgkin's disease and another with myeloid leukaemia had normal levels of haptoglobin coincident with haemolytic anaemia. A normal haptoglobin level therefore does not exclude the possibility of haemolytic anaemia, especially when it occurs in patients

who have conditions which are likely to give rise to abnormally high levels.

Apart from patients with known haemolytic disease or megaloblastic anaemia, in which there is also an increased rate of haemolysis, subnormal levels of haptoglobin were sometimes found in other disorders. In those with either hypersplenism or sideroblastic anaemia low levels correlated with reduced red cell survival. Several of the patients in whom the recent haemorrhage was into the tissues had absent or reduced levels of haptoglobin. It has previously been suggested that the mechanism causing these low levels may be similar to that which occurs with haemolysis (Richardson, Glick, Bates, and Shinton, 1963), the haemoglobin-haptoglobin complex in this instance being formed from haemoglobin which has entered the plasma following organization of blood clot. Reduced levels in hepatobiliary jaundice is a well recognized feature, but it is possible that in some cases it may be the result of increased haemolysis, as decreased red cell survival has been demonstrated by Pitcher and Williams (1963) in patients with hepatitis. Two of our cases with this condition showed haematological evidence of increased haemolysis but the red cell survival time in one of them was found to be normal. Williams *et al.* (1961) could find no correlation between serum haptoglobin and red cell survival times in patients with hepatitis but neither is there evidence that low levels are due to decreased synthesis (Owen *et al.*, 1964). The patients who had iron-deficiency anaemia or lymphosarcoma and had low levels of serum haptoglobin showed no evidence to suggest that these were a consequence of release of haemoglobin into the plasma from any cause and so it is probable that these were extreme examples of the normal range.

Red cell survival studies using radio-active isotope techniques are probably the most accurate method of assessing whether anaemia is due to increased haemolysis or not, but they are time-consuming procedures which require special equipment and take several weeks to complete. On the other hand, serum haptoglobin can be estimated in any hospital laboratory.

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