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THE DIURNAL VARIATION OF THE SERUM IRON LEVEL IN ERYTHROPOIETIC DISORDERS

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Heilmeyer and Plötner (1937), Skouge (1939), and Moore, Minnich, and Welch (1939) recognized that there were fluctuations in the level of serum iron during the day. Their examples were few, and, while it was apparent that the fluctuations were independent of the food intake, these did not possess the characteristics of a constant daily trend. Vahlquist (1941) first clearly demonstrated that there was a fall in the serum iron level of normal subjects during the day: 15 normal male subjects had a mean serum iron level of $135 \pm 10.6 \mu\text{g./100 ml.}$ at 8 a.m.; by 6 p.m. a mean fall of $36.3 \pm 9.2 \mu\text{g./100 ml.}$ had occurred. Vahlquist considered his results to show "*eine Tendenz zu einem Tagesrhythmus*," a diurnal rhythm which has subsequently been confirmed by other authors (Hemmeler, 1944; Høyer, 1944a, 1944b; Waldenström, 1946; Schafer and Boenecke, 1949; Hamilton, Gubler, Cartwright, and Wintrobe, 1950; and Paterson, Marrack, and Wiggins, 1952). Hamilton *et al.* (1950) have reviewed the considerable body of information concerning this *intra diem* variation, but there remains little or no agreement as to its cause and significance.

Vahlquist (1941) and later Waldenström (1946) have suggested that the erythropoietic activity of the bone marrow influenced the serum iron level in such a way as to cause the diurnal fluctuation. In this paper we present data obtained as opportunity arose from patients suffering from various disorders of erythropoiesis in order to determine whether in these circumstances any typical alterations occur which might support Vahlquist's hypothesis. A secondary study of the effect upon the serum iron levels of A.C.T.H. and cortisone administration was made.

Procedure and Methods

The subjects of this investigation comprised two patients with idiopathic aplastic anaemia, four

with pernicious anaemia, six with polycythaemia vera, two with congenital haemolytic anaemia, two with nocturnal haemoglobinuria, and two with iron-deficiency anaemia. Diagnoses were established during their stay in hospital. The two patients suffering from aplastic anaemia had had previous blood transfusions; Case 1 was the more severe, aspiration biopsy showing the bone marrow to be virtually acellular, and reticulocytes on repeated daily counts were frequently absent from the peripheral blood; Case 2 was less severe, and, since the peripheral blood showed 0.6 to 1.0% reticulocytes, some erythropoiesis was going on. Case 3 was an example of severe pernicious anaemia, Case 4 of moderate severity, and Cases 5 and 6 were very mild. In these last two cases marrow biopsy showed only slight megaloblastic change, while the complaint of the last patient (Case 6) which brought her to hospital was that of sore tongue rather than of symptoms attributable to anaemia *per se*. Case 12 is included amongst the cases of polycythaemia vera, although no longer polycythaemic. Three months previously 7 millicuries of radioactive phosphorus (P^{32}) had been given intravenously, when the red cell count was 7.9 m./c.mm., haemoglobin 22.9 g./100 ml., and packed cell volume 80%.

The procedure and chemical methods employed have been previously described (Paterson *et al.*, 1952). Haemoglobin was estimated by a modification of the standard oxyhaemoglobin methods employing a photoelectric colorimeter. The method is checked monthly by comparison with a blood sample supplied by the Medical Research Council Haemoglobin Standards Scheme.

Results

Diurnal Serum Iron Levels.—Haematological data and serum iron determinations are shown in the Table.

Aplastic Anaemia.—The serum iron levels were high, as expected. In Case 1 there was no real

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TABLE
HAEMATOLOGICAL DATA AND DIURNAL SERUM IRON LEVELS

Case No.	Diagnosis	Age and Sex	Therapeutic Data	R.B.C. (m./c.mm.)	Reticu- locytes (%)	Hb. (g./100 ml.)	P.C.V. (%)	Serum Iron Level (μ g./100 ml.)					Maximum Decrease (μ g./100 ml.)
								9 a.m.	1 p.m.	5 p.m.	9 p.m.	9 a.m.	
1	Aplastic anaemia	44 F		1.69	0.1	7.5	17.5	233	234	230	225	231	8
2		57 M		1.4	1.0	4.1	13.0	278	253	251	248	277	30
3	Pernicious anaemia	37 F	Untreated 7 days after 80 μ g. vitamin B ₁₂ i.m. 6 months later	1.14 2.1 4.8	3.2 39.0	6.0 15.4	14.0 45.0	197	196	174	197	192	23
4		75 F	Untreated 4 months later	2.5 4.4	2.0	9.3 12.8	30.0 41.0	122	125	129	108	136	14
5		68 F	Untreated 8 days after 100 μ g. vitamin B ₁₂ i.m. 6 months later	3.2 3.6 4.2	3.3 9.8	12.4 13.5	36.0 42.5	148	144	107	114	172	31
6		59 F	Untreated 8 days after 160 μ g. vitamin B ₁₂ i.m. 5 months later	3.9 3.8 4.2	2.4 4.2	13.5 12.9 12.7	38.5 38.0 38.5	120	118	120	115	118	5
7	Po'ycythaemia	52 M	Remission	7.8	2.8	17.9	65.0	120	107	125	113	125	13
8		74 M		10.1	1.2	20.0	80.0	85	81	73	57	58	28
9		70 F		7.5	2.8	17.3	62.0	59	47	55	47	48	12
10		66 F		6.8	18.8	18.8	68.0	117	104	104	92	124	25
11		55 M		7.9	2.2	14.0	51.0	71	81	69	70	76	2
12		52 F		3.2	1.6	10.2	32.0	115	102	77	115	131	38
13	Congenital haemolytic anaemia	15 F		4.2	12.0	12.6	34.0	88	87	80	88	86	8
14		16 M		4.3	17.0	13.7	37.5	105	102	105	102	91	14
15	Nocturnal haemoglobinuria	35 F		2.3	15.2	9.9	31.0	88	80	75	82	96	13
16		49 M		3.7	24.8	11.1	39.0	98	98	94	95	97	4
17	Iron-deficiency anaemia	25 F	Untreated 48 hours after 100 mg. sacch. iron oxide i.v. 6 months later	4.4 4.6		9.8 12.8	34.0 40.0	46	40	36	42	36	10
18		26 F	Untreated 48 hours after 800 mg. sacch. iron oxide i.v. 6 months later	2.8 4.6	12.0	6.8 9.5 13.9	24.0 41.0	42	40	36	36	32	8
								99	97	105	106	93	2
								110	72	70	64	90	46

"Maximum fall" refers to the maximum fall of the iron level from the 9 a.m. level of the same day.

variation of the iron level throughout the day; a fall of 30 $\mu\text{g./100 ml.}$ occurred in Case 2, i.e., a relatively small change in relation to the height of the initial level.

Pernicious Anaemia.—In one case only (Case 3), the most severe example of the disorder, were the serum iron levels raised. The diurnal serum iron curves were significantly flatter in all four cases before treatment than after a full remission had occurred. Thus, with a remission, there was a return to the normal pattern of diurnal rhythm. It is known that there is a fall of the serum iron level in pernicious anaemia after the administration of liver extract (Moore, Doan, and Arrowsmith, 1937). The diurnal levels at the height of the reticulocyte response in Cases 3, 5, and 6 were likewise low, and the curves were, in addition, flat. The data from Case 3 are shown graphically in Fig. 1.

FIG. 1.—Pernicious anaemia (Case 3) showing diurnal serum iron levels (A) before treatment, (B) at the height of the reticulocyte response, and (C) in remission.

Polycythaemia Vera.—The diurnal serum iron levels were generally low and the curves flat in the five cases where the disorder was active (Cases 7–11), but in Case 12 (no longer polycythaemic) there was a more marked diurnal variation.

Haemolytic Anaemia.—In Cases 13 and 14 (congenital haemolytic anaemia) and 15 and 16 (nocturnal haemoglobinuria) erythropoiesis was active, as shown by the reticulocyte counts of 12 and 17% and 15.2 and 24.8% respectively. In all four cases the serum iron levels were low and showed little variation throughout the day.

Iron-deficiency Anaemia.—The very low levels with little or no variation throughout the day return to normal with pronounced variation as the result of full treatment with iron. Serum iron levels were

determined in each case after partial iron therapy, i.e., when it might be assumed that the iron was being rapidly utilized. Thus in Case 17 (48 hours after a single intravenous injection of 100 mg. saccharated iron oxide) and in Case 18 (48 hours after the eighth consecutive daily intravenous injection of 100 mg. saccharated iron oxide) the serum iron levels were intermediate between the “untreated” and “fully treated” levels and the curves were flat throughout the day. Fig. 2 shows the serum iron curves in Case 17.

FIG. 2.—Iron-deficiency anaemia (Case 17) showing diurnal serum iron levels (A) before treatment, (B) 48 hours after a single intravenous injection of 100 mg. saccharated iron oxide, and (C) after full treatment.

Effect of A.C.T.H. and Cortisone Administration.—Following the determination of the initial (drug-free) diurnal serum iron curve, the curve after hormone administration was obtained either consecutively or after an interval of 24 hours, except in the cases of aplastic anaemia (Cases 1 and 2), where the curves were determined at various times over a period of two months. In these A.C.T.H. and cortisone were employed to diminish the purpuric manifestations. In each case A.C.T.H. was administered intramuscularly or cortisone orally immediately after withdrawing the 9 a.m. blood sample. In no case was there any detectable change in the diurnal serum iron curve following single doses of A.C.T.H. and cortisone. Representative curves are shown in Figs. 3–5.

Discussion

Three features of interest are discernible in the above results. First, there is a tendency for the diurnal variation of the serum iron to disappear in erythropoietic disorders, and in those disorders which can be treated full treatment restores the

FIG. 3.—Diurnal serum iron levels in aplastic anaemia (Case 1); control levels and levels following the administration of 100 mg. A.C.T.H. intramuscularly and of 100 mg. cortisone orally.

normal diurnal pattern. Secondly, there is much similarity between the low serum iron levels with little diurnal variation found in the cases of pernicious anaemia at the height of the reticulocyte response, the untreated cases of polycythaemia vera, the haemolytic anaemias, and the cases of iron-deficiency anaemia given only a limited amount of iron. These low and flat curves thus appear characteristic of increased red bone marrow activity with enhanced iron utilization for new haemoglobin synthesis. Since these low levels correspond well with the lower parts of the normal diurnal curve, it may be inferred that in the presence of increased red cell formation (presumably continuous through-

FIG. 4.—Diurnal serum iron levels in pernicious anaemia (Case 3); control levels and levels following the administration of 50 mg. A.C.T.H. intramuscularly.

FIG. 5.—Diurnal serum iron levels in iron-deficiency anaemia (Case 17); control levels and levels following the administration of 25 mg. A.C.T.H. intramuscularly.

out the 24-hour period) there is no nocturnal rise of the serum iron level. Thirdly, it is evident that in erythropoietic disorders A.C.T.H. and cortisone in single doses have no significant effect upon the diurnal serum iron levels. This is in harmony with our previous findings in normal subjects (Paterson *et al.*, 1952).

Hahn, Bale, Ross, Balfour, and Whipple (1943) have drawn attention to the difficulties encountered in the interpretation of serum iron levels. Since the serum iron represents that part of the iron in transport, the level of the serum iron may be altered both by removal of iron for the synthesis of haemoglobin, myoglobin, cytochromes, peroxidases, or ferritin, and by its recovery from these sources. Thus, the diurnal variation of the serum iron level might depend upon varying iron utilization, or varying pigment and enzyme disintegration, or upon both. Cruz, Hahn, and Bale (1942) have presented evidence that iron liberated in the ordinary processes of daily wear and tear is utilized for the formation of new haemoglobin in preference to iron from storage depots. The total quantity of serum iron at any given moment, however, is generally less than one-sixth of the daily requirement for haemoglobin synthesis alone, and hence the rate of turnover of the serum iron is of great significance. It is not known whether a diurnal variation occurs in the rate of serum iron turnover. Huff, Hennessy, Austin, Garcia, Roberts, and Lawrence (1950) studied the rate of disappearance of injected radioactive iron (^{59}Fe) from the plasma and were able to demonstrate an increased rate of plasma iron turnover in polycythaemia vera, untreated pernicious anaemia, and the haemolytic anaemias. These increased rates were restored to normal by appropriate treatment of the erythropoietic disorder. In refractory anaemias the iron

turnover rate was increased in some and normal or reduced in others. It is of interest that the conditions in which there is an increased rate of plasma iron turnover should also be those in which there is diminished diurnal variation of the serum iron level.

Summary

Diurnal variation of the serum iron level was found to be diminished in aplastic anaemia, untreated pernicious anaemia, and untreated iron-deficiency anaemia. Full treatment of the last two conditions restores a normal diurnal variation.

Diurnal variation was also diminished and the serum iron levels were low in various conditions associated with increased erythropoiesis, viz., polycythaemia vera, congenital haemolytic anaemia, nocturnal haemoglobinuria, pernicious anaemia at the height of the reticulocyte response, and iron-deficiency anaemia treated with a limited quantity of iron.

It is suggested that the decreased diurnal variation of the serum iron levels in erythropoietic disorders may be related to the increased plasma iron turnover observed by Huff *et al.* (1950) in similar disorders.

The administration of A.C.T.H. and cortisone had no significant influence upon the diurnal serum iron levels in those disorders.

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