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THE IRON RESERVE OF A NORMAL MAN

BY

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No one seems to have attempted to measure the reserve of iron available for haemoglobin synthesis in man. Hahn (1937) estimated that 57 per cent of the total iron in a dog was contained in the blood haemoglobin, 20 per cent was stored in a form potentially available for haemoglobin synthesis, and 23 per cent was bound in myohaemoglobin, cell enzymes, etc., and was not available for haemoglobin formation. The available iron reserve of the dog was thus sufficient to replace about one-third of its circulating haemoglobin, and clinical experience of unassisted recovery from acute haemorrhage suggests a similar figure in man.

The rate of haemoglobin regeneration after haemorrhage depends upon the stimulus to and response of the marrow as long as iron is freely available. A normal man can draw upon his iron reserve as well as upon dietary iron for blood regeneration after acute haemorrhage, but if his iron reserve is depleted by repeated haemorrhage the rate of haemoglobin regeneration is limited by the dietary iron available. Thus if a man is kept anaemic by repeated bleeding, his rate of haemoglobin regeneration will fall progressively until, when his iron reserve is exhausted, a constant level is attained. This principle has been used in this experiment to calculate the size of the available iron reserve of a normal man.

Material and Methods

The subject was a man 37 years old weighing 63 kg. He had lived on the normal English civilian diet since his discharge from the Army sixteen months before the experiment began.

He was bled every weekday an hour and a half after breakfast, having sat down for 30 minutes before the venepuncture. Anaemia was established by two blood donations of 800 and 900 ml. respectively. The amount of blood removed daily for routine tests was at different times, 5.5, 6.5, and 20 ml., as indicated in Figs. 1 and 2. In all, 2,750 ml. of blood were removed during the 136 days of the experiment.

The vein for venepuncture was not compressed in any way. Wintrobe's dry oxalate mixture was used as anticoagulant, and pipettes were filled immediately, before sedimentation had begun. All estimations were made in duplicate. Haemoglobin was estimated photo-electrically as oxyhaemoglobin. The optical density of a 1/201 dilution of blood in 0.4 per cent ammonia was measured in a Spekker absorptiometer calibrated against iron determinations on washed red cells, with a suitable correction for the plasma content of whole blood. Red cell counts, Wintrobe haematocrit determinations, and reticulocyte counts were made in the usual way. Serum and plasma specific gravities were estimated by the copper sulphate specific gravity technique of Phillips and van Slyke (1945).

The daily rate of haemoglobin regeneration was calculated from the best-fitting polynomials to the observed haemoglobin levels in each period of the experiment.

Results

The course of the blood haemoglobin level is shown in Figs. 1 and 2. (No blood was taken during the interval of nine days between the two charts.) It will be observed that there was a relatively wide day-to-day fluctuation in the haemoglobin level. This was closely paralleled by fluctuations in the haematocrit value and serum specific gravity, showing that even under the standard conditions of the experiment there was a day-to-day variation of about ± 5 per cent in the plasma volume. These random fluctuations were excluded from the calculation of haemoglobin regeneration by the statistical fitting of curves to the data.

The falls in haemoglobin level after the two large venesections corresponded to a blood volume of 5.3 and 5.5 litres respectively, in good agreement with a value of 5.45 litres previously obtained by the Evans blue technique. It will be observed that the haemoglobin level did not reach its nadir for a few days after the venesection ; evidently the compensatory increase in plasma volume occupied this interval.

If the blood volume and amount of blood withdrawn daily are known, it is easy to calculate the

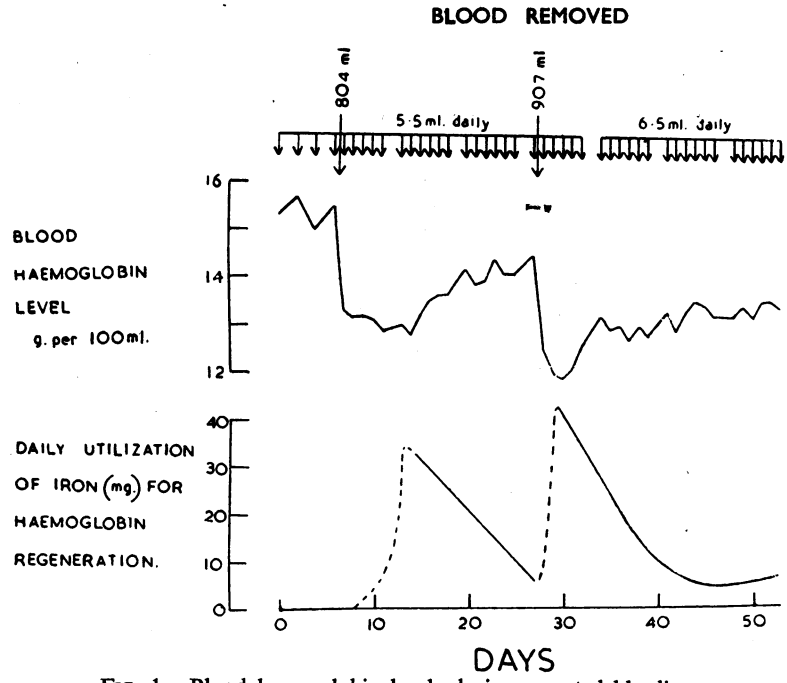

FIG. 1.—Blood haemoglobin levels during repeated bleeding.

daily utilization of iron for haemoglobin regeneration in excess of the normal replacement of effete red cells. The lower curves on Figs. 1 and 2 show this value, which fell to a minimum on day 45 and thereafter remained constant.

We may deduce that the subject's iron reserve was finally exhausted at this time.

The size of the available iron reserve was calculated as follows: The initial blood volume at day 6 was 5.3 litres, the haemoglobin level 15.2 g. per 100 ml., and the total circulating haemoglobin iron $53 \times 15.2 \times 3.34 = 2{,}690$ mg. The final blood volume at day 125 was 5.7 litres (see below), the haemoglobin level 13.2 g. per 100 ml., and the total circulating haemoglobin iron $57 \times 13.2 \times 3.34 = 2{,}510$ mg.

A total of 1,190 mg. of iron was removed by venepuncture during the interval. We therefore have the equation:

$$R + 2{,}690 - 1{,}190 + x = 2{,}510$$
$$\text{or } R = 1{,}010 - x \quad \ldots\ldots\ldots (1)$$

where R is the initial iron reserve in mg., and x the total iron absorbed and retained from the food.

We may obtain an estimate of x by considering the periods from days 62 to 102 and 104 to 123 inclusive, when the iron reserves were completely exhausted and iron absorption from the food was

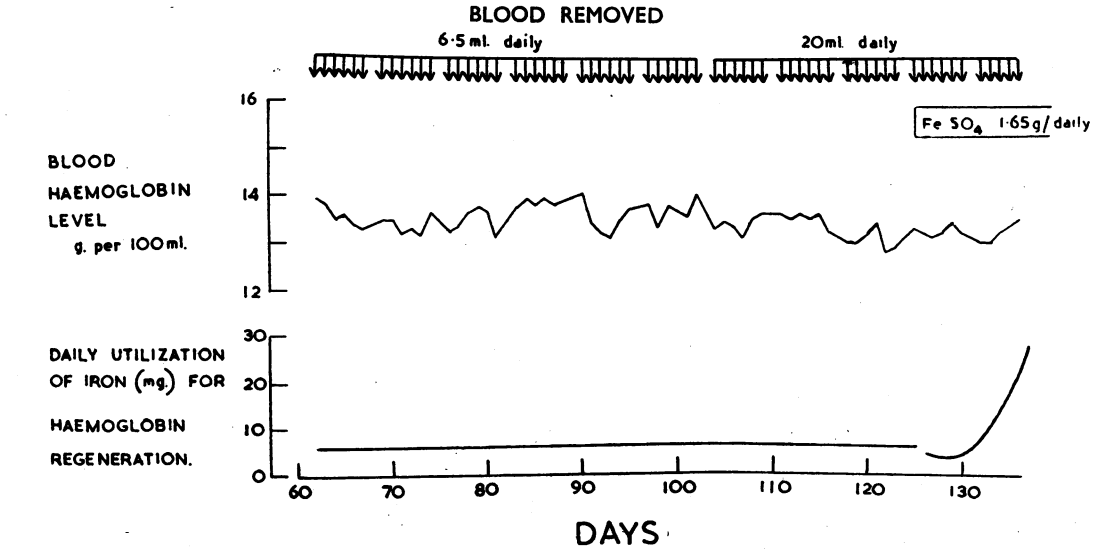

FIG. 2.—Response of blood haemoglobin to repeated bleeding after exhaustion of the iron reserve.

maximal. In the first of these periods, when 6.5 ml. of blood were removed daily, the haemoglobin level was increasing at the rate of 0.0844 g./litre/day, and the iron utilized daily for this increase amounted to $0.0844 \times 3.34 \times V$ mg., where V is the blood volume in litres. The blood removed during these 41 days contained 104 mg. of iron. If, therefore, F mg. is the mean daily amount of iron utilized for haemoglobin regeneration in excess of the normal replacement of effete red cells, we have:

$$F - \frac{104}{41} = 0.0844 \times 3.34V \quad \ldots\ldots\ldots (2)$$

From days 104–123, when 20 ml. of blood were removed daily, the haemoglobin level fell at the rate of 0.2075 g./litre/day; 161 mg. of iron was withdrawn in blood in the 20 days, and consequently:

$$F - \frac{161}{20} = -0.2075 \times 3.34V \quad \ldots\ldots\ldots (3)$$

Combining equations (2) and (3) we have:

$V = 5.7$ litres $\qquad F = 4.1$ mg.

We may assume that the iron absorption was maximal from day 46, when the iron reserves were exhausted, so that the minimum value to be assigned to x in equation (1) is $80 \times 4.1 = 328$ mg. The value of F in the first part of the experiment remains unknown, since it rose from zero on day 6, when the blood count was normal, to a maximum value of 4.1 on or before day 46. If we take F as zero for these 40 days, x in equation (1) will be underestimated as 328 mg., whereas if we take F as 4.1 mg. throughout the experiment, x will be overestimated as 492 mg. The iron reserves therefore lie between:

$R = 1{,}010 - 328 = 682$ mg. and $R = 1{,}010 - 492 = 518$ mg.

The intermediate value of 600 mg. may be taken as a reasonable approximation.

Discussion

This experiment would have required much more stringent control of the dietary iron intake but for the fortunate fact that our present rationing system does not permit any substantial variation of the diet. It is thus reasonable to assume that the iron intake was the same over any period of two or three weeks during the course of the experiment.

The conclusions drawn from this experiment are based upon the assumption that the supply of iron was the limiting factor in blood formation. The marrow must normally replace effete red cells at a rate corresponding to the production of 45 ml. of blood daily, and the loss of a further 6 or 7 ml. daily would not impose any great strain if the supply of raw materials were adequate. Moreover, it is common experience that the daily increase in the red cell count in the cure of anaemia corresponds to the production of 15 g. of haemoglobin a day, and only 0.9 g. was withdrawn daily during most of this experiment. Even the final withdrawal rate of 2.5 g. of haemoglobin

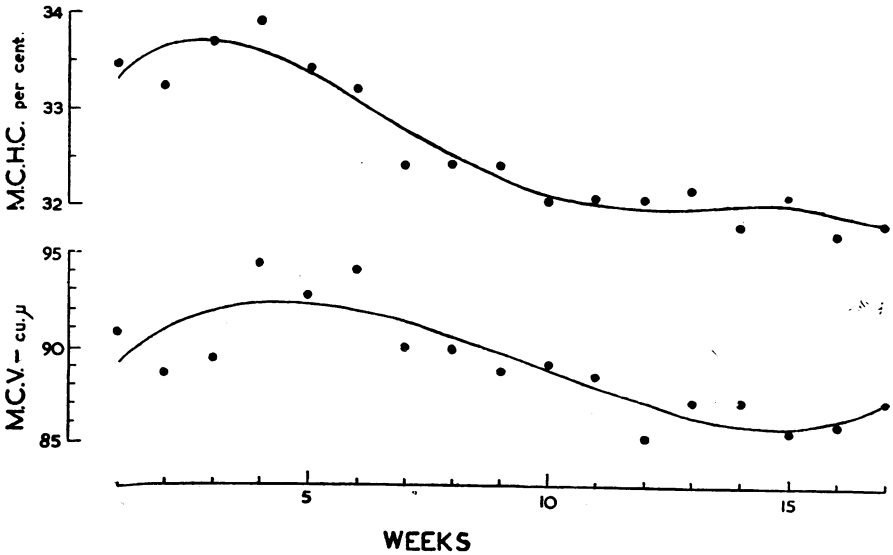

FIG. 3.—The decline in mean corpuscular volume and haemoglobin concentration with repeated bleeding

a day would scarcely strain the capacity of a marrow already made hypertrophic by 96 days of anaemia. The supply of accessory factors would presumably be adequate, for nutritional macrocytic anaemia is rare in this country.

These assumptions are confirmed by the fact that both the mean corpuscular haemoglobin concentration and mean corpuscular volume after a preliminary rise fell steadily during the experiment (Fig. 3). Both the rise and the subsequent fall were statistically significant, for the data could only be satisfactorily fitted by cubic and quartic curves respectively. The final proof, however, was when, after day 123, the daily withdrawal of 20 ml. of blood was maintained, but full doses of iron were given by mouth. After a short interval the rate of haemoglobin regeneration rose steeply.

It is sometimes believed that iron-deficiency anaemia due to haemorrhage is accompanied by a reticulocytosis which serves to distinguish it from other forms of hypochromic anaemia. This view was not, however, borne out by this experiment. The reticulocyte count did rise from 0.2 per cent at the beginning of the experiment to 0.4 per cent when the rate of haemoglobin regeneration was maximal, but it fell again thereafter, and was only 0.1 per cent when 20 ml. of blood was being withdrawn daily and the haemoglobin level was falling. When iron was administered the reticulocyte count rose to 0.7 per cent.

The estimated value of 600 mg. for the iron reserve available for haemoglobin synthesis was sufficient to replace about one-fifth of the subject's red cells. The relative smallness of this reserve emphasizes the importance of iron therapy after any substantial haemorrhage.

The experiment further showed that the present British diet can afford some 4 mg. of iron a day for haemoglobin synthesis, in addition to the 1 mg. or so needed daily to replace iron excreted in the bile and urine (Hynes, 1948). The subject's estimated total iron intake was of the order of 15 mg. a day, so that about one-third of this total was physiologically available for absorption and haemoglobin synthesis.

This potential dietary surplus of 4 mg. of iron a day is a very small margin—it would be balanced by a loss of only 8 ml. of blood a day. The body must depend almost entirely on its available iron reserve for rapid recovery from haemorrhage, for the dietary iron suffices for a haemoglobin increase of only 0.15 g. per 100 ml. per week.

There is still some doubt as to the safe interval between blood donations for transfusion. If, as is usual, 450 ml. of blood is taken from a normal man, it will contain some 240 mg. of iron. This amount will be rapidly taken from the available reserve to reconstitute the haemoglobin level, whilst the reserve will be more gradually replenished from the dietary iron. If iron absorption were immediately maximal the process would take 60 days. A normal woman requires about 1 mg. of iron a day to replace menstrual losses, so that the replacement of 220 mg. of iron from a blood donation of 450 ml. would require 73 days if iron absorption were maximal from the beginning. Thus a cumulative iron deficiency would not follow even in normal women, unless blood were donated more than five times a year. Even moderate menorrhagia, however, might of itself cause iron losses equivalent to more than 4 mg. daily.

Summary

1. The iron reserve of a normal man was estimated by bleeding him until his rate of haemoglobin regeneration fell to a constant level. The daily absorption of iron from the food, in excess of the normal requirement to replace effete red cells, was calculated from the different rates of haemoglobin regeneration when 6.5 and 20 ml. of blood respectively were withdrawn daily after exhaustion of the iron reserve.

2. The iron reserve was thus calculated to be about 600 mg.

3. It was calculated that the present English diet can yield 4 mg. of iron daily for haemoglobin synthesis in addition to the normal needs for replacing effete red cells.

4. This extra 4 mg. of iron would replace a loss of only 8 ml. of blood a day by an ordinary man.

5. The iron reserve would not be depleted by blood donations of 450 ml. as often as five times yearly by a man or by a woman with normal menstrual loss. Moderate menorrhagia, however, could of itself demand more than the available 4 mg. of extra iron.

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