# I. THE BLOOD VOLUME AND THE TOTAL AMOUNT OF HAEMOGLOBIN IN ANAEMIC RATS.

By JAMES MATTHEWS DUNCAN SCOTT

(*John Lucas Walker Student*)

AND JOSEPH BARCROFT.

*From the Physiological Laboratory, Cambridge.*

(*Received October 5th, 1923.*)

It has been shown [Scott, 1923, 1] that in blood regeneration after a series of haemorrhages rats fed upon white bread and whole milk show a marked contrast to rats on a similar diet with an *ad lib.* amount of green food added, the former developing a sub-chronic anaemia and the latter not. An easier method of producing this anaemia in rats has also been demonstrated [Scott, 1923, 2], that of breeding from mothers which were fed for some time before mating and during gestation and suckling on a diet of bread and milk, and rearing the young on the same diet. By such means it is an easy matter to obtain numbers of young rats which show up to about the twenty-fourth week of life an anaemia in which each cubic millimetre of blood shows a diminution of haemoglobin and a normal or increased corpuscular content, *i.e.* a fall in colour index.

It seemed probable that the deficient haemoglobin content in each $mm^3$. of blood was due to a deficiency in the material from which the pigment could be formed and it became a matter of interest to investigate whether the slow formation of haemoglobin was reflected in other characters of the blood such as its total volume, the total number of cells or the age of the corpuscle. In respect to the last point it will be clear that even if the haemoglobin was more slowly formed and the colour index was depressed the body might accumulate a normal quantity of haemoglobin were there a lower death-rate and a greater longevity of the corpuscle; in such a condition the whole number of corpuscles in the body would increase as would the volume of the blood. Apart from these considerations it was a matter of great interest in view of the work of Lorrain Smith [1900] on human chlorosis and that of Haldane on ankylostomiasis to determine in these rats what the total amount of haemoglobin in the body was, whether there was, as in the case of Lorrain Smith's subjects, an increase in blood volume sufficient to compensate for the diminution in the haemoglobin percentage and to bring the total amount of haemoglobin in the body up to the normal proportion per 100 g. body weight, or whether there was no such increase in blood volume so that the total haemoglobin in the body fell *pari passu* with the haemoglobin percentage of the blood. This question was therefore the first to be taken up by us.

## METHODS.

The method of determining the blood volume was the CO method and in principle was the same as that used by Haldane, Lorrain Smith, Boycott, Douglas and others. Instead, however, of determining the CO percentage of the blood colorimetrically, as they did, we used Hartridge's reversion spectroscope for its determination.

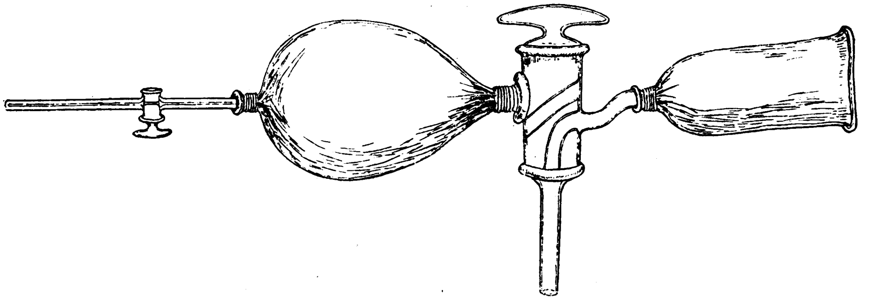

Fig. 1. Apparatus used.

*Apparatus* (Fig. 1). A mask was made of a size 5 finger-stall which fitted over the head and ears of the rat. In the case of thin finger-stalls a ring cut from a piece of rubber tubing was used as a muzzle; no such muzzle was found necessary in the case of stout finger-stalls. An opening in the tip of the finger-stall connected with a three-way stopper tap on the other limb of which was a balloon of 100 cc. capacity. The bore of the three-way tap and the tubing attached to it were of ample diameter and the tap was disposed so that the rat could breathe either from the open air or the balloon. In an opening at the other end of the balloon was a straight piece of capillary glass tubing with a tap for the introduction of the gas. This apparatus is rather more simple in construction than that used by Boycott and Douglas [1909] in their work on the rabbit. When the apparatus was to be used about 10 g. of soda lime were placed in the balloon and it was wired on to the tap; 15 cc. of oxygen were introduced into it and a carefully measured quantity of CO, usually about 0·5 cc. at N.T.P. One operator holding the rat then thrust its head into the mask, which was held stretched by the other operator and was adjusted by him to cover the head and ears of the rat. The rat was then plunged into a bell-jar of warm water at about 38° up to and covering the free edge of the mask on its neck, so that any leakage of gas might be detected; but care was taken to keep the rat's nose above the level of the water lest water might enter and it should drown. The tap, through which the mask had hitherto communicated with the external air, was turned so that the mask communicated with the

CO mixture in the balloon. The bag was given a little pressure with the hand rhythmically so as to aid the respiration of the rat in mixing the air in the bag with that in the mask, and the soda lime was continually shaken up to absorb $CO_2$. The duration of administration of CO lasted for times varying between 4 and 10 minutes but was usually between 5 and 8 minutes and was registered by a stop-watch. During this time the legs of the rat were held by the one operator, the body of the rat, the mask and apparatus by the other. The rat usually breathed normally for 2–3 minutes and then commenced to struggle. Breathing usually grew gradually more and more spasmodic and ceased after a variable interval (for what reason we never ascertained), though the heart continued beating. It was an advantage that the circulation should be maintained for some time after the respiration ceased, as the CO taken into the body before the cessation of respiration could be distributed more evenly. In order to prolong the life of some of the control rats we gave them urethane so as to obviate struggling, and this was successful; in a number of such cases we were able to maintain the breathing over a longer period. When the inhalation of CO was concluded, sometimes after respiration had ceased, but always while the heart was still beating, the rat was lifted out on to an enamelled tray; its head was snipped off instantly with a large pair of scissors, and samples of blood were taken for the reversion spectroscope and the haemoglobinometer, care being specially taken in the latter case lest water from the drenched fur should dilute the specimen. Blood was taken in this way so that a satisfactory sample from the central circulation might be obtained and to avoid the lapse of any time in which the body might lose CO. In the rat it is difficult to get from the tail a satisfactory sample of blood without such care as is precluded by the manipulations to which the rat is being subjected. Lorrain Smith's samples were of course taken from the peripheral circulation.

The percentage saturation of the blood with CO was then determined by the reversion spectroscope and the haemoglobin percentage by Haldane's haemoglobinometer. The amount of CO left in the bag was determined with a Haldane's gas analysis apparatus and the amount so left was subtracted from the amount administered. The amount of CO left in the bag was usually trifling and of the order to be expected if the blood and the gas in the bag were in equilibrium. Five quantities required measurement, viz. amount of CO administered, weight of rat, haemoglobin percentage, amount of CO left in the bag, and percentage saturation of the blood with CO. Of these one observer usually measured the first three, the other the last two; in spite of great variations in one or other of these factors the results would fall into line with remarkable consistency. In evolving the routine a number of experiments were performed on about thirty rats.

That the time given is long enough for the absorption of the CO and its equable distribution throughout the circulation is shown by the fact that no difference in blood volume per 100 g. could be detected between rats in which the administration lasted 5 and those in which it lasted 10 minutes. The "circulation" is here held to exclude the spleen pulp into which the CO scarcely entered. On the other hand, the blood in the liver seemed to be saturated to the same extent as that of the general circulation. The same was true of the maternal placental blood in pregnant females and approximately, though not entirely so, of the blood in the foetal portions of the placenta.

*Comparison with results obtained by Welcker's method.*

With the co-operation of Messrs Savage and Ellis an attempt was made to compare the results of the CO method of estimating blood volume in rats with those of the Welcker method. The latter method gave invariably smaller results and it was found that this was due to the fact that in the rat haemoglobin shows a great tendency to crystallise. When the blood was washed out of the blood vessels with warm Ringer and diluted by the addition of distilled water to the point at which the solution contains between 1 and 2 % haemoglobin, it still required further laking by alternate freezing and thawing before the solution became clear. Under such circumstances and at such a dilution there was a deposit of haemoglobin crystals in the sediment at the bottom of the receptacle. Thus the method is unsatisfactory for determining blood volume in the rat. The results we did get by the Welcker method were of the same order as those of Kronecker and Marti [1897] who find that the blood volume of a well-nourished rat determined by this method averages 4·7 % of the body weight.

In rabbits Boycott [1912, 1] found that the blood volume given by the CO method was higher than that given by the wash-out method, but in these animals crystallisation of haemoglobin was not observed, and this observer found the explanation in the hypothesis that the CO method, unlike the other, measured extra- as well as intra-vascular haemoglobin.

## Results.

The normal and anaemic rats compared by us were the same age, viz. 2–3 months. The normals were bred from a normal stock fed with a plentiful supply of green food. The anaemic rats were bred as had been described by Scott [1923, 2]. Both sets were taken at random from large stocks of normal and anaemic rats. The figure we obtain for the average blood volume in normal rats (6·13 cc.) per 100 g. of rat, is very close to that found by Boycott [1912, 2], viz. 6·28 cc. From columns in Tables I (normal) and II (anaemic) rats, the results of which are shown graphically in Fig. 2, it will be seen that there is little difference in blood volume, as determined by the methods described, between the normal and the anaemic rats. The blood volume of the anaemic rats averages 6·65 cc. per 100 g. rat as against 6·13 cc. in the case of the normals. The blood volume in the case of these anaemic rats does not therefore increase appreciably, as would appear from Lorrain Smith's figures (confirmed by Plesch and Oerum) to be the case in human chlorosis. In other columns of Tables I and II are given the figures for the haemoglobin content per 100 g.

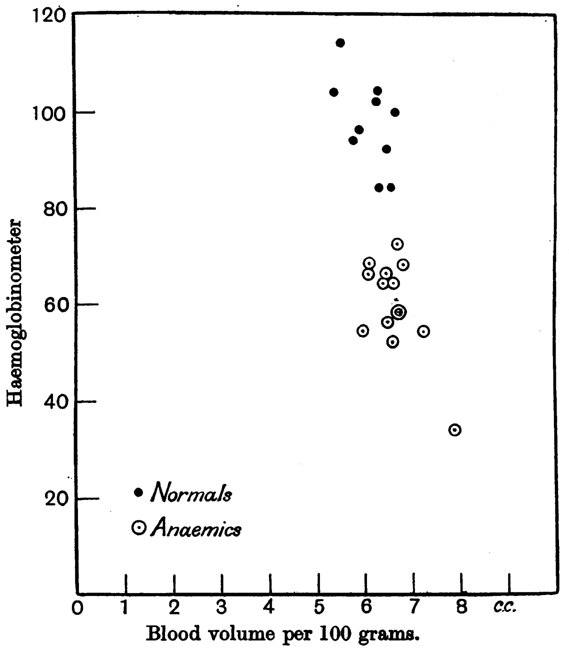
Haemoglobinometer
120
100
80
60
40
20
• Normals
⊙ Anaemics
0 1 2 3 4 5 6 7 8 cc.
Blood volume per 100 grams.

Fig. 2.

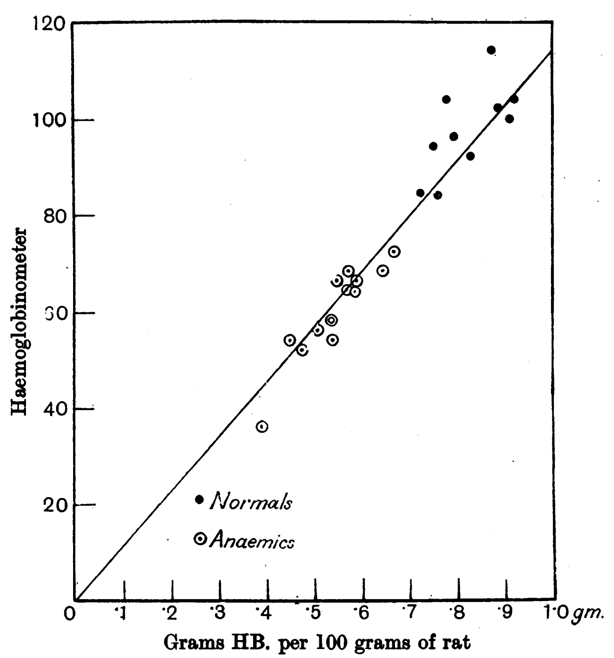
Haemoglobinometer
120
100
80
60
40
20
• Normals
⊙ Anaemics
0 ·1 ·2 ·3 ·4 ·5 ·6 ·7 ·8 ·9 1·0 gm.
Grams HB. per 100 grams of rat

Fig. 3.

Table I. *Blood volume and haemoglobin content per* 100 *g. body weight of* normal *rats about* 2 *months old.*

| No. | Weight in g. | Sex | Duration of administration in mins. | CO in cc. Given | CO in cc. Left | CO in cc. Absorbed | % saturation of blood with CO | Hb % | Per 100 g. body weight: Blood volume in cc. | Per 100 g. body weight: Haemoglobin in g. |
|---|---|---|---|---|---|---|---|---|---|---|
| 49 | 145 | F. | 5 | ·571 | ·040 | ·531 | 34·5 | 96 | 5·98 | ·793 |
| 50 | 140 | F. | 5½ | ,, | ·037 | ·534 | 32·2 | 102 | 6·26 | ·884 |
| 51 | 95 | F. | 8 | ,, | ·044 | ·527 | 47·5 | 114 | 5·55 | ·874 |
| 52 | 96 | F. | 5 | ,, | ·083 | ·488 | 41·5 | 100 | 6·64 | ·916 |
| 53 | 154 | M. | 6 | ,, | ·027 | ·544 | 34·7 | 84 | 6·57 | ·760 |
| 54 | 108 | F. | 6 | ·556 | ·008 | ·548 | 41·5 | 104 | 6·36 | ·913 |
| 55 | 134 | M. | 8 | ,, | ·033 | ·523 | 38·7 | 94 | 5·80 | ·754 |
| 56 | 136 | M. | 6 | ,, | ·030 | ·526 | 39·5 | 84 | 6·32 | ·721 |
| 57 | 128 | F. | 8 | ,, | ·010 | ·546 | 41·0 | 104 | 5·40 | ·780 |
| 58 | 106 | M. | 8 | ,, | ·060 | ·496 | 42·5 | 92 | 6·47 | ·822 |
| | | | | | | | Average | 97·4 | 6·13 | ·822 |

Table II. *Blood volume and haemoglobin content per* 100 *g. body weight of* anaemic *rats about* 2 *months old.*

| No. | Weight in g. | Sex | Duration of administration in mins. | CO in cc. Given | CO in cc. Left | CO in cc. Absorbed | % saturation of blood with CO | Hb % | Per 100 g. body weight: Blood volume in cc. | Per 100 g. body weight: Haemoglobin in g. |
|---|---|---|---|---|---|---|---|---|---|---|
| 32 | 118 | M. | 6 | ·581 | ·055 | ·526 | 60·0 | 66 | 6·09 | ·555 |
| 33 | 96 | M. | 7 | ,, | ·054 | ·527 | 76·2 | 58 | 6·72 | ·538 |
| 34 | 106 | M. | 6 | ,, | ·029 | ·552 | 68·0 | 64 | 6·46 | ·571 |
| 35 | 127 | M. | 5½ | ,, | ·045 | ·536 | 70·5 | 54 | 6·00 | ·447 |
| 36 | 107 | M. | 5½ | ,, | ·002 | ·579 | 62·7 | 68 | 6·85 | ·643 |
| 37 | 111 | F. | 6½ | ,, | ·014 | ·567 | 64·5 | 66 | 6·50 | ·590 |
| 38 | 98 | F. | 5 | ,, | ·027 | ·554 | 72·5 | 64 | 6·57 | ·582 |
| 39 | 128 | M. | 5 | ·577 | ·064 | ·513 | 76·0 | 36 | 7·92 | ·392 |
| 40 | 113 | M. | 7 | ,, | ·024 | ·553 | 72·0 | 56 | 6·56 | ·507 |
| 41 | 100 | F. | 8 | ,, | ·013 | ·564 | 63·2 | 72 | 6·70 | ·666 |
| 42 | 96 | F. | 5 | ,, | ·066 | ·511 | 73·5 | 54 | 7·24 | ·540 |
| 43 | 123 | M. | 8 | ,, | ·007 | ·570 | 60·0 | 68 | 6·14 | ·576 |
| 45 | 88 | M. | 8 | ·493 | ·024 | ·469 | 83·9 | 52 | 6·60 | ·474 |
| 46 | 83 | M. | 10 | ·492 | ·055 | ·437 | 73·5 | 58 | 6·70 | ·535 |
| | | | | | | | Average | 59 | 6·65 | ·544 |

rat and in Fig. 3 is shown the result of plotting this figure against the haemoglobin percentage of the blood. The average haemoglobin percentage in this series of anaemic rats is 59·7 % as against a normal of 97·4 %, and the average total haemoglobin content per 100 g. of rat is 0·544 g. as against a normal of 0·822, a fall to 66 % of the normal. There is therefore no doubt that the animals show a very definite deficiency of haemoglobin. In Fig. 4 are plotted the points representing the averages for normal and anaemic rats respectively. From these charts it will be seen that the total amount of haemoglobin per 100 g. of rat falls in direct proportion to the haemoglobin percentage of the blood, and that this rule holds alike for normal and anaemic rats, so that the points as plotted lie along a straight line the equation of which is

$$y = 115 \begin{matrix} (+15) \\ (-21) \end{matrix} x$$

or

$$x = \cdot 0087 \begin{matrix} (+\cdot 0019) \\ (-\cdot 0010) \end{matrix} y,$$

in which $x$ = the weight of haemoglobin in grams per 100 g. of rat and $y$ = the haemoglobinometer reading.

Therefore in the case of the rats rendered anaemic by the method described by Scott [1923, 2] it is clearly demonstrated that the total haemoglobin content of the body is not maintained but falls in direct proportion to the haemoglobin percentage of the blood.

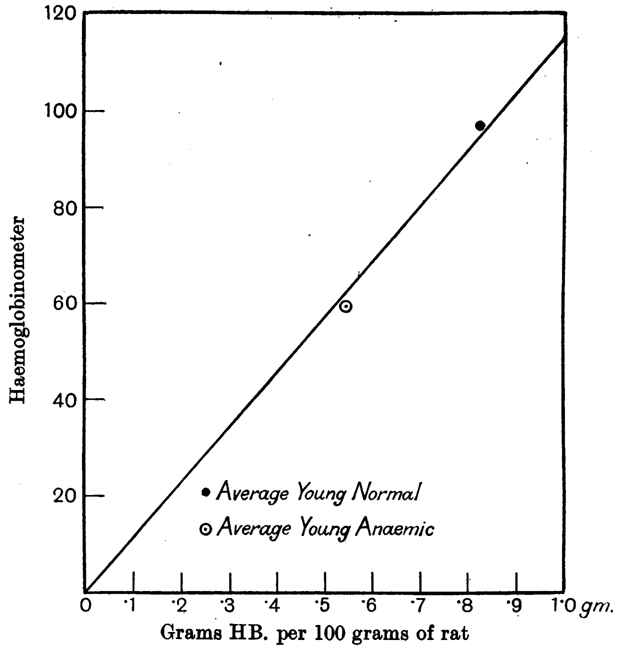


Fig. 4.

## Conclusion.

In the anaemia described in rats by Scott [1923, 2] there is a fall in the haemoglobin percentage of the blood, the blood volume per 100 g. remains practically normal, and there is a deficiency in the weight of haemoglobin per 100 g. body weight corresponding and directly proportional to the fall in haemoglobin value.

This relationship is expressed in the equation given in the text.

This rule holds even in the case of such slight diminutions of haemoglobin percentage as occur in normal rats.

### *Note.*

In the course of this work we by chance had the opportunity of noting that pregnant rats resembled anaemic rats in that they showed a slight fall in the haemoglobin percentage of the blood without increase in blood volume, so that per 100 g. weight of rat (including foetuses) they showed a proportional deficiency in the total haemoglobin content of the body. This is being made the subject of further investigation.

The expenses of this research were in part defrayed by a grant from the Medical Research Council to one of us (J. B.).

## REFERENCES.

Boycott (1912, 1). *J. Path.* **16**, 485.
—— (1912, 2). *J. Path.* **16**, 263.
Boycott and Douglas (1909). *J. Path.* **13**, 256.
Kronecker and Marti (1897). *Verh. d. XV, Kongress f. Innere Med. zu Berlin.*
Lorrain Smith (1900). *Trans. Path. Soc. Lond.* **51**, 311.
Scott (1923, 1). *Biochem. J.* **17**, 157.
—— (1923, 2). *Biochem. J.* **17**, 166.