THE EFFECT OF HAEMORRHAGE UPON RED CELL SIZE AND RED CELL DISTRIBUTION*

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In 1940 Brennan reported that "as a result of haemorrhage individual red corpuscles increase very appreciably in size, often by 30 to 50%." From his observations, which consisted in making red cell counts and in haematocrit determinations, he concluded that "following haemorrhage many patients' red cells become side-tracked—probably in the muscle capillaries; often more than 20%." Brennan also claimed that transfusion of 500 to 1,000 c.cm. of plasma mobilized these cells once more, so that the red cell count actually rose after transfusion. Such fundamental changes in the distribution of the circulating cells have an important bearing on the reliability of observations such as red cell count, haemoglobin, and haematocrit in the recognition of "shock," the response to treatment, and blood-volume determinations.

Each of us, independently, has made careful investigation into Brennan's claims, and has concluded that, though under certain conditions there may be minor degrees of swelling of red cells following upon haemorrhage, the order of the change is so much smaller than that stated by Brennan that it cannot have the physiological significance deduced from his results. There is no evidence to support the claim for the immobilization of cells following upon haemorrhage, nor for their mobilization after plasma transfusion. All published results concerning plasma transfusion (Kekwick *et al.*, 1941; Grant and Reeve, 1941) are opposed to the claim.

Sources of Error

Of the four haematological procedures available for this work, direct measurement of cells and the haemoglobin estimation can be considered the most accurate. As to the haemoglobin, it has been shown that in a normal person there is no significant difference between venous and capillary blood (Price-Jones *et al.*, 1935), but under conditions in which a large quantity of blood is withdrawn from the circulation this normal finding might not hold. This point has been checked by simultaneous estimations on blood taken from the ear and the median basilic vein, after bleeding, and no significant difference found. The haemoglobin estimation provides a useful check on the mean corpuscular volume figure and any deductions made therefrom, because the mean corpuscular haemoglobin concentration can be calculated and, assuming there is no change in red cell population, should change inversely to the mean corpuscular volume. The red cell count is admitted to have no more accuracy than $\pm 5\%$, and because an alteration of as little as 2% in the red cell count can

influence the mean corpuscular volume figure by as much as 3%, it will be obvious that this figure does not provide reliable evidence of change in red cell size unless it is grossly altered. Alterations in red cell count of less than 200,000 per c.mm. are of no significance unless they are sustained. As to the haematocrit reading, the variability with ten estimations of the same sample of blood has been found to be $\pm 0.7\%$ (Price-Jones *et al.*, 1935). But there is much more fluctuation in successive samples from the same person. In Table I is shown an example of this in which the pre-bleed fluctuation in mean corpuscular volume amounts to 2%. From the whole series it has been calculated that the influence of the bleed on the mean corpuscular volume cannot be regarded as statistically significant unless the increase in size exceeds 6.4%—that is, three times the standard error of the pre-bleed figures. Absolute accuracy with the haematocrit is not possible unless the centrifuge is efficient so that packing is complete, unless the anticoagulant is isotonic, and unless the procedure is carried out without delay. We have considered especially the question of the anticoagulant itself, and particularly the influence of delay before spinning the tube. In our hands potassium oxalate, Wintrobe's ammonium oxalate, potassium oxalate mixture (Wintrobe and Landsberg, 1935), the binoxalate used by Brennan, and heparin have all given consistent results. But with binoxalate delay is fatal. Changes occur rapidly within the first two hours and progress slowly to completion in twenty-four to forty-eight hours. For instance, a sample spun immediately had a mean corpuscular volume of 82 c. μ , after two hours 88 c. μ , and after twenty-four hours 92 c. μ .

Scope of Investigation

The state of the circulation with regard to the cell number, haemoglobin, red cell volume, red cell diameter, and reticulocytes has been investigated after an acute single haemorrhage (animals and man), sustained haemorrhage due to surgical operation (man), and repeated small haemorrhages (animals). The results enable conclusions to be made concerning changes in red cell size, changes in red cell population, and the recording of some facts concerning the speed and efficiency of normal haemodilution.

Acute Single Haemorrhage

Observations have been made on 13 human subjects after the loss of 440 to 880 c.cm. of blood, on one rabbit bled 20% of its blood volume, and on two cats bled 50% and 70% of their blood volume and afterwards transfused with plasma. Details of two typical human cases are shown in Fig. 1 and Table I, and those of a plasma-transfused cat in Fig. 2.

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TABLE I.—(Human, Bled 540 c.cm.)

Time	Red Cells (Millions)	Haemoglobin % (Haldane)	Haematocrit	M.C.V. (c.μ)
10 a.m.	4.63	84	42.0	90.7
11.10 a.m. . . .	4.69	84	42.5	90.6
12.10 p.m. . . .	4.62	82	42.5	91.9
1.10 p.m.	4.70	84	43.0	91.4
Immediately after bleeding	4.66	78	42.0	90.0
½ hour after . . .	4.70	80	42.0	89.9
1 " " " " " " . .	4.20	76	42.0	100.0
1½ hours after . .	4.18	76	40.0	95.5
2 " " " " " " . .	4.24	76	38.5	90.7
4 " " " " " " . .	4.14	74	38.5	93.4
5 " " " " " " . .	4.30	74	40.0	93.0

An analysis of the 13 human cases shows that the haemodilution following upon haemorrhage is associated with small increases in red cell size in 10. The amount of increase is such that in a single experiment it would be regarded as of no significance. The maximum amount of swelling observed in any of this series was 13% in volume and 6% in diameter, the average being 8% and 4.5% respectively. Small though these changes are—small enough to be only just outside the statistically significant range and probably of no physiological importance—they are present so consistently that they cannot be entirely dismissed. The maximum increase occurs soon after bleeding—in some cases almost immediately, in others within half an hour to two hours.

The results are far below Brennan's claim of 30% to 50% increase. Careful analysis of six other red cell distribution curves, similar to Fig. 1, as well as of the reticulocyte counts,

FIG. 1.—Human. Haemodilution appreciable in 2 hours and complete in 5 (Hb 128 to 116, reds 5.7 to 4.9 mils., haematocrits 48.5 to 45.0). No evidence of change in red cell population, and red cell distribution curves are identical. There is a suggestion of slight swelling at the 2nd and 5th hours; for, though the mean diameter is only slightly increased, the observed haematocrits are higher than the calculated levels (48 instead of 47, and 45 instead of 43.9).

shows no evidence that the red cell population is altered by the mobilization of new cells during the twenty-four hours after bleeding. The red cell distribution curves are unaltered and, falling into line with the apparent slight swelling of the cells as shown by measurement in all planes, there is a parallel decrease in mean corpuscular haemoglobin concentration. This last fact is consistent with the view that the circulating cells are the same cells slightly increased in size.

The animal experiments involving an acute loss of blood—estimated from body weight at 20%, 50%, and 70%—showed changes in cell size of the same order: Fig. 2 is an example. Neither did plasma transfusion in this animal appear to mobilize cells, in that both the haemoglobin and the red cell count after transfusion approximate closely to the figures anticipated from a calculation based upon dilution; neither does the form of the

curve nor the reticulocyte count suggest the addition of new cells to the circulation. The 13 human subjects have provided information as to the speed with which haemodilution occurs in

FIG. 2.—Cat. Severely bled (70%) and immediately transfused 90 c.cm. plasma, equal to three-quarters of blood removed. No change in red cell distribution, and all the figures remain remarkably constant under these severe conditions, notably the mean corpuscular haemoglobin concentration.

what may be assumed to be normally hydrated subjects. The phenomenon is easily detectable within half an hour, is well established and sometimes complete in two to five hours, but in most cases is not complete for twenty-four hours. Intake of fluid during this time does not appear greatly to influence the speed of haemodilution in the normally hydrated subject. Observations on those who purposely drank as much as 6 litres in the subsequent twenty-four hours gave readings of the same order as those taking 2 litres.

Sustained Haemorrhage from Surgical Operation

Nine cases of operative blood loss have been investigated. Blood loss was estimated by taking all towels, swabs, etc., at the end of the operation, soaking them in a large volume of water, and squeezing out all possible fluid. From this the dilution of the haemoglobin in a known volume of fluid allows the volume of blood therein to be estimated. This technique must necessarily give an underestimate of blood loss, and to this must be added the continued loss of blood from the circulation which invariably occurs for some hours after an operation demanding widespread tissue-cutting. The estimated blood loss ranged from 256 to 1,026 c.cm., and the duration of operation from one-half to three hours, but observations were continued for three or four days. Table II is an example of the maximum changes found, which are an increase of 13% in mean corpuscular volume one hour after beginning an operation causing a blood loss estimated at 745 c.cm.

TABLE II.—Radical Mastectomy. Blood Loss estimated at 745 c.cm. after an Operation lasting Two Hours

Time	Red Cells (Millions)	Haemoglobin % (Haldane)	Haematocrit	M.C.V. (c.μ)
Before operation . . .	4.94	100	40	81
1 hour	5.01	102	43	86
1½ " " " " " " . .	4.91	99	45	92
2 " " " " " " . .	—	98	41	—
2½ " " " " " " (end of operation) . . .	4.82	98	40	83
5 hours	4.67	96	40	85
7 " " " " " " . .	4.85	98	42	87
25½ " " " " " " . .	4.33	90	40	92
50 " " " " " " . .	3.78	78	34	90
74 " " " " " " . .	3.56	74	31	87
97½ " " " " " " . .	3.55	72	32	90

In the whole series of 9 there have been 7 who have shown an increase in mean corpuscular volume, the average increase being 6%; the other two have shown an actual decrease in cell size. Under operative conditions the time required for complete haemodilution appears to be from three to four days, a figure

very similar to that observed in injury cases by Cannon *et al.* (1919) but longer than that found in the series of cases of acute haemorrhage reported herein. An important factor, and one difficult to estimate, is the state of hydration subject; it is generally believed to be low by reason of sweating, perhaps vomiting, and reduced fluid intake immediately after operation.

In this series blood volume was estimated with Evans's blue in three cases, and appeared to be definitely reduced immediately after operation. The total observed loss of red cells from the body as estimated from the blood volume was equivalent to the volume of red cells in the blood lost, within the limits of experimental error. There was no evidence that red cells had been mobilized. Furthermore, if it be assumed that the total blood volume at the time of the final reading three to four days later was the same as the pre-operative blood volume, then the estimated total cell volume at the end of the investigation was approximately the same as that at the end of operation.

Repeated Small Haemorrhages

Experiments were carried out on cats anaesthetized with a 5.5% solution of nembutal 0.7 c.cm./kg., administered intraperitoneally. Some animals received 500 units of heparin/kg. intravenously (sodium salt of heparin, Connaught Laboratories, of strength 1,000 units/c.cm., approximately 10 mg. heparin/c.cm.); the blood was then collected in tubes containing 0.05 c.cm. of the same heparin solution. In other animals no heparin was given, but the blood samples were collected into 0.5 c.cm. of Wintrobe's ammonium oxalate-potassium oxalate mixture (Wintrobe and Landsberg, 1935). In all experiments 5 c.cm. of blood was withdrawn from the carotid artery at intervals of ten minutes until the animal died. From each sample a Wintrobe tube was filled for the haematocrit determination. When four tubes had been filled they were immediately spun at 8,000 r.p.m. for fifteen minutes. Haemoglobin was determined in terms of CO capacity in the manometric van Slyke apparatus. Hayem's fluid was found unsuitable for counting cat's cells as it caused agglutination; most counts were therefore carried out with 0.9% saline, making a 1 in 200 dilution of the blood. In eight of nine experiments there was complete parallelism between the changes in the cell:plasma ratios and the haemoglobin content of the

the remaining red cells. In all experiments successive bleedings have produced notable changes in the cell:plasma ratio: in three experiments it declined steadily from the beginning of the bleeding (Fig. 3); in three it remained almost unchanged until

FIG. 4.—Human. Showing effect of rapid haemodilution following taking of alcohol, with corresponding reduction in plasma protein concentration but no effect on M.D. or M.C.V. Note quick excretion of alcohol, after which haemodilution proceeds naturally.

blood equivalent to 0.02% of body weight had been lost, and afterwards declined. In these two types of response death occurred when the cell:plasma ratio was reduced to some 60% of its original value. In two experiments the haemorrhage was followed by a concentration of the blood, which reached 20% in excess of its original value, and thereafter declined until the death of the animal.

Discussion

In none of these experiments, of varying type, do the results confirm those obtained by Brennan. Small increases in red cell size occur under certain conditions of bleeding, more especially soon after a large acute haemorrhage. But these increases are scarcely significant statistically and are probably immaterial from the physiological aspect. The results, however, show that a haematocrit estimation by itself is in no way accurate for following changes in blood volume: the reading needs to be checked and correlated with an accurate red cell count and haemoglobin estimation.

There remains to be discussed the mechanism whereby these minor increases in red cell size are produced. Two simple explanations can be put forward, bearing in mind that the red cell is extremely labile and may undergo alterations in shape and size in response to numerous artificially induced external stimuli (Price-Jones, 1921; Dacie and Vaughan, 1938). The first is that the process of haemodilution causes a reduction in the plasma osmotic pressure, so that the equilibrium between it and intracorporeal fluid is disturbed. The second is that haemorrhage may, under certain conditions, disturb the acid-base equilibrium, to which the red cell is known to be sensitive. With acidosis there is a definite increase in the size of the red cells (Paxton, 1935).

It has indeed long been known in clinical medicine that after alimentary haemorrhage there may be a transient macrocytosis, but so definite as to lead to an erroneous diagnosis of pernicious anaemia. It is also well known that alimentary haemorrhage is apt to be accompanied by azotaemia and disturbances of acid-base equilibrium, whilst an acidosis similarly occurs in the later stages of circulatory failure through haemorrhage.

FIG. 3.—The effect of repeated small haemorrhages. Cat, 2.7 kg., decerebrate.

blood; the changes in red cell count have been less regular, but the divergencies have been within the limits of experimental error (Fig. 3). There is therefore no evidence that repeated small bleedings cause any significant change in the size of

The following preliminary observations were designed to test these views. Fig. 4 shows the results on a subject in whom rapid haemodilution was induced by taking two pints of lager beer in the half-hour following bleeding. There was marked haemodilution—measured by a fall in red cells and haemoglobin—in the first hour, with a corresponding fall in plasma protein but no increase in cell size. Between the first and second hours there was a big diuresis, during which time all the quickly absorbed fluid appears to have been excreted, so that the haemoglobin and plasma protein rose to their original figure. Thereafter haemodilution proceeded normally. Evidence of the ability of the diluted plasma to cause swelling of the cells was sought for by exchanging cells and plasma in a haematocrit tube from samples obtained before and after bleeding and observing the effect on the haematocrit reading. Table III shows the negative effect of making these interchanges in this case, which is perhaps not surprising as no swelling of the cells was observed. But the same result was obtained in four animals more severely bled.

TABLE III

Corpuscles	Plasma	Haematocrit
Before bleeding	Before bleeding	46.5
" "	1 hour after	46.0
" "	5 hours after	46.0
1 hour after	1 hour after	44.0
1 " "	Before bleeding	43.8
5 hours after	5 hours after	44.0
5 " "	Before bleeding	43.8

It is clear from this case that considerable haemodilution with plasma protein reduction can occur without swelling of the red cells. It is clear also that in this case there was neither acidosis (Fig. 4) nor swelling. Furthermore, the observations made on animals subjected to repeated small haemorrhages, already described, show that considerable haemodilution can occur without alteration in cell size. The balance of this preliminary evidence is therefore against a simple osmotic phenomenon due to plasma protein dilution. More positive evidence from similar observations needs to be obtained from cases which exhibit definite cell swelling before the acid-base explanation can be accepted.

Summary

Minor increases in red cell size, probably of no physiological importance, occur after a single large acute haemorrhage.

Smaller increases occur after more sustained haemorrhage, but no increase at all with repeated small haemorrhages.

There is no evidence for the immobilization of swollen cells in muscle capillaries, as described by Brennan, nor of their mobilization by plasma transfusion.

The minor increases in size which do occur must be taken into account when using the haematocrit for following changes in blood volume caused by haemorrhage. The haematocrit figure should be controlled with a red cell count and haemoglobin determination.

Haemodilution is complete in about twenty-four hours in normal subjects, but may occupy three to four days after surgical operation.

Red cell swelling following haemorrhage is probably not due to mere reduction in plasma protein consequent upon haemodilution.

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TREATMENT OF RHEUMATOID ARTHRITIS IN CHILDREN

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In reading through the literature on the treatment of chronic infectious arthritis and noting the host of remedies proposed—each of them having enthusiastic advocates but few having stood the test of time—one cannot be surprised at the almost universal scepticism among physicians regarding any remedy. As Keating (1938) has aptly said, "The treatment of this disease has varied from the local application of rattlesnake oil and salicylic acid to the injection of anything, from salicylates, heavy metals, and dyes, down to the removal of almost every organ in the body that isn't padlocked."

Methods of Treatment

The following therapeutic items are, in the opinion of most physicians primarily interested in rheumatism, of value in the treatment of chronic infectious arthritis: management of infected foci; vaccines or filtrates; foreign proteins; diet, vitamins, blood transfusion; gold salts; artificial jaundice; physical therapy.

Focal Removal.—The conservative removal of obviously infected foci is generally recommended (Thompson *et al.*, 1938), as good results following this procedure have occurred too often to be coincidental. Teeth, tonsils, prostate, sinuses, and cervix should be examined for the presence of infection. However, indiscriminate surgical intervention should be condemned. In dealing with infected foci it should be remembered that, while many arthritics probably benefit from the removal of such infected material (Buckley, 1938), still more seem to receive no help at all, and the number of dramatic cures is relatively very small. The general physician and not the "focal specialist" should decide what foci are to be removed (Hench *et al.*, 1940).

Vaccines, Filtrates.—Vaccine therapy is considered about as valuable as focal eradication. An editorial comment in the sixth Rheumatism Review (1940) states that "it is difficult to understand why vaccines should be expected to cure this disease of unknown origin when no infectious disease of known aetiology is cured by vaccine." However, other rheumatologists are convinced of the value of vaccines (Thompson and Wyatt, 1938; Crowe, 1937). Overholt and Mortensen (1937) consider that vaccines have a place in the treatment of this disease, but not as a specific. They recommend stock vaccines of "arthrotropic" streptococci—i.e., vaccines which when injected into rabbits will produce joint swellings (Lederle's *Streptococcus haemolyticus* vaccine)—and consider them to be preferable to autogenous vaccines of unknown tropism.

Foreign Proteins.—The value of the injection of foreign protein probably results from a general stimulation of metabolism consequent on the fever produced. This form of therapy is difficult to control and is always associated with varying degrees of discomfort and danger (Copeman and Tegner, 1937). Weekly doses of the protein which do not cause the patient's temperature to rise above 102° F. are considered most likely to yield good effects. Drastic shock therapy does not justify the results obtained.

Diet, Vitamins, Blood Transfusion.—There is no "arthritis diet," *per se*, specifically beneficial in arthritis, but on account of the general systemic effects of the disease the diet should provide for optimal nutrition and relief of