DETERMINATION OF BLOOD VOLUME BY A SIMPLE ACCURATE TECHNIQUE AND ITS APPLICATION IN ASSESSING PATIENTS FOR MAJOR SURGERY

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The routine investigations for the assessment of the patient for surgery include haemoglobin and haematocrit determinations. These express only a percentage concentration and do not give any quantitative evaluation. Owing to this lack of information the blood volume is not taken into account and, provided the haemoglobin is not less than 12 g. (or 80%), no correction of the blood volume is usually made. The replacement of fluid, however, and of blood in particular, is one of the essential factors in reducing the mortality of major surgical procedures.

A further reduction in morbidity and post-operative mortality can be achieved where the blood volume, based on an accurate quantitative determination, is corrected pre-operatively.

In recent surgical literature many reports have emphasized the need for blood-volume estimation; it would seem that the one factor which has limited its general

application has been the lack of a simple accurate technique. Many methods have been described, particularly using the dye technique, but they have been cumbersome and difficult. Usually the syringe has to be weighed before and after the injection, and the dye has had to be made up afresh for each investigation.

In this communication we shall describe a simplified technique which dispenses with these difficulties and which can be used in any institution; a plea will be made for the routine use of blood-volume studies to assess the pre-operative and post-operative needs of surgical patients.

The first recorded attempt to determine the blood volume was made experimentally in 1854 by Welcker, who bled animals to death. Since this method was only applicable experimentally it was replaced by several indirect procedures during the past century. These methods measure (1) the circulating intravascular fluid (plasma), using Congo Red (Griesbach), Neutral Red (Rowntree *et al.*); Brilliant Vital Red Evans dye (Keith *et al.*); Evans Blue (Dawson *et al.*); radio-active ^{131}I (Koster); Dextran (Birch *et al.*); or (2) the total haemoglobin, using carbon monoxide (Sjöstrand); or (3) the red-cell mass by means of radio-active ^{59}Fe (Gibson *et al.*); radio-active ^{32}P (Reid *et al.*); radio-active ^{51}Cr (Gray *et al.*). Of these methods, the estimation of circulating intravascular fluid has been widely adopted and the use of Evans Blue (T-1824) has come to be regarded as most satisfactory. It has several advantages:

1. The dye is non-toxic and is eliminated slowly from the blood stream. The dye has been reported to enter the reticulo-endothelial system and leaves the circulation slowly in comparison with other dyes. It has been estimated that 5-9% of injected dye disappears from the circulation within the 1st hour of injection and that 50-53% has disappeared by the end of 24 hours (Birch *et al.*). This behaviour of the dye is due to the fact that it is firmly bound to the albumin, and is therefore found only in the plasma (Gregerson *et al.*, 1935).
2. It is non-haemolytic.
3. It is uniformly distributed in the blood after 5-6 minutes (Remmer) and it retains its colour on entering the blood stream (Koster).
4. Slight haemolysis does not affect this method (Remmer), especially when using a Beckman DU Spectrophotometer, which measures the optical density selectively at 620 mμ.

It has been stated that the disadvantages of this test are (1) It cannot be repeated at short intervals because of the retention of the dye by the reticulo-endothelial system (Rawson). Remmer, however, says that the results are made even more accurate if the dye injection is repeated after $\frac{1}{2}$ hour to enable blockage of the reticulo-endothelial system to occur, and estimation performed after the double injection.

(2) The patient may become unpleasantly discoloured. Results agreeing closely with those obtained with T-1824 were given by human serum-albumin linked with ^{131}I (Crispell *et al.*). Recently the use of dextraven for measuring blood volume has come into prominence (Birch *et al.*); the results however are at variance with those obtained with the Evans Blue and ^{131}I techniques, possibly owing to the inevitable plasma volume expansion. On the whole, however, the Evans Blue dye dilution method was considered as the best available technique for our purposes.

MATERIAL AND METHOD

1. *The Dye Solution.* Evans Blue dye T-1824, obtained from British Drug House, is dissolved in 0·9% saline in a concentration of 300 mg. %. Ampoules containing 10 ml. of the dye were standardized and sterilized.

2. *Syringes.* Luer-Lok syringes and needles are used. In order to facilitate the injection of the dye into any vein, it was decided to use No. 15 needles only. For collecting blood any needle of suitable size is satisfactory.

All syringes used for injecting the dye were accurately calibrated. About 0·5 cm. from the tip of the piston of a 10-ml. syringe a diamond pencil mark about $\frac{1}{2}$ inch long was made parallel to the circular edge. The syringe was filled with normal saline and pushed forward until the mark on the piston coincided with the 8 ml. mark on the barrel.

By means of accurate weighing, exactly 5 ml. of saline was ejected through an empty No. 15 needle. The starting and end points were carefully marked on the barrel by means of a diamond pencil. For injecting the dye the syringe was (carefully) filled with dye. After all the air had been ejected the piston was pushed in until the mark on the piston coincided with the first mark on the barrel. Exactly 5 ml. of dye are injected by pushing the piston in until the second mark on the barrel coincides with the mark on the piston.

3. *Construction of the Standard Curve.* In order to construct a standard curve, 5 ml. of the dye solution were injected into known volumes of saline, ranging from 1 litre to 5 litres. After thorough mixing the optical densities were read on a Beckman DU Spectrophotometer at 620 mμ. The data thus obtained were used for the construction of a standard curve (Fig. 1). The curve was found to be a hyperbola which could be represented by the equation:

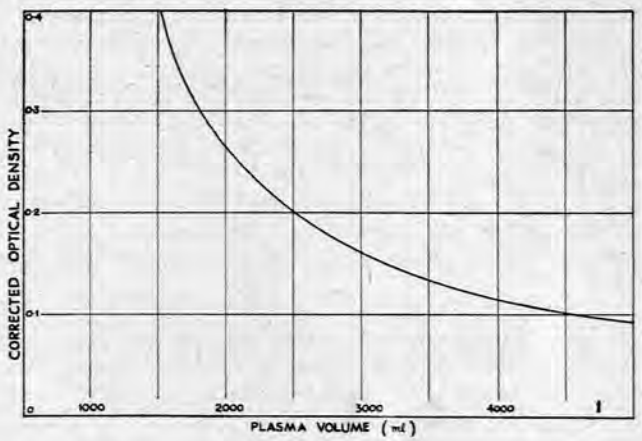

Fig. 1

$$(OD)\,(V)=K. \quad \ldots\ldots (1)$$

where OD=optical density at 620 mμ, V=volume in ml., and K=a constant.

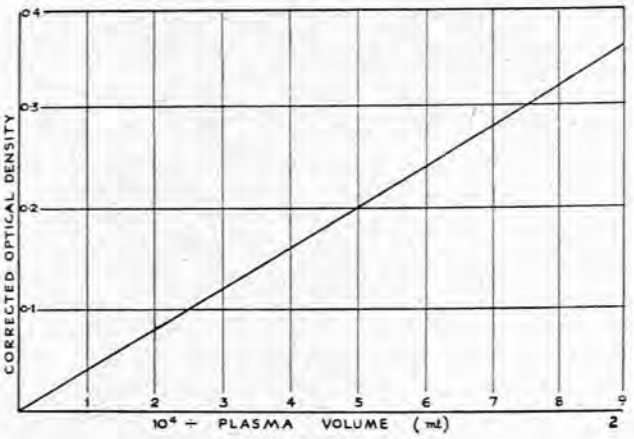

Fig. 2

From (1) $OD = K \div V$ it therefore follows that if the optical density is plotted against the reciprocal of the volume a straight line of slope K will be obtained (Fig. 2). This graph was used in all determinations in preference to that shown in Fig. 1.

4. *Resting Sample and Injection of Dye.* The patient must have fasted for at least 12 hours before the test in order that clear serum may be obtained. Throughout the test the patient has to lie flat in bed; out-patients were rested for 1 hour before the test. A No. 15 needle attached to a syringe was inserted into an antecubital vein, and 12 ml. of blood were withdrawn with the tourniquet released. The syringe was detached, leaving the needle *in situ*. At this stage it will be seen that blood will drip slowly out of the needle, indicating that the needle is well placed in the lumen of the vein.

About 3 ml. of blood are placed into a sedimentation-rate tube for the first haematocrit reading, and thoroughly mixed with the anti-coagulant contained in the tube; the remainder is put into a sterile tube, allowed to clot, and marked 'Resting' sample. A calibrated syringe filled to the upper mark with sterile dye from an ampoule is now securely screwed into the needle. A stop-watch is started as soon as the dye injection is begun. Exactly 5 ml. of the dye solution are injected over a period of 30-40 seconds by pushing the plunger into the barrel until the second mark on the barrel coincides with the mark on the piston.

5. *Samples after Dye Injection.* After 10 minutes 12 ml. of blood are withdrawn into a dry syringe from the other arm with the tourniquet released. About 3 ml. are used for a second haematocrit reading and the remainder is placed into a sterile tube and marked No. 1. The correct time of collection is noted to the nearest quarter-minute.

A last blood specimen is collected 20 minutes after injection, and the exact time is noted to the nearest quarter-minute.

6. *Colorimetry.* The blood samples are spun and the sera transferred to clean dry tubes and centrifuged again. Of each serum 2 ml. are diluted to 5 ml. with 0·9% saline and again centrifuged. The optical densities are read at 620 mμ on a Beckman DU Spectrophotometer.

7. *Calculation.* The optical densities of the coloured solutions are corrected by subtracting the optical density of the resting specimen.

A correction is made for dye disappearance during the tests by plotting the logarithm of the corrected optical densities against time (linear) and determining the extrapolated density value (Ellis). This value (at time = 0 minute) is used in conjunction with Fig. 2 in determining the serum volume. The total blood volume and red-cell volume are calculated from the serum volume and the corrected average haematocrit readings. Haemoglobin determinations are carried out on the two samples collected for haematocrit determinations and an albumin-globulin determination is done on the serum from the resting blood specimen.

It must be remembered that blood is not a homogeneous fluid, but a suspension of corpuscles of varying size in a fluid which is undergoing variation in volume, so that the concentration of corpuscles is not constant throughout the circulation. Methods dependant upon the injection and dilution of a known amount of substance into the blood stream measure only the volume through which the blood carrying the injected substance circulates and it is not possible to differentiate between changes in circulating volume due to fluid loss or to vasomotor or circulatory changes (Tice, editor). It is obvious that, despite all improvements, the dye-dilution method cannot be considered a precise method for measuring or following plasma volume (Courtice and Gunton). It is, nevertheless, useful for comparing values obtained in the same individual at various intervals and is more accurate in this respect than the concentration of plasma protein or the relative volume of packed red-cells.

ILLUSTRATIVE CASES

During the past 2 years blood-volume estimations have been increasingly used in our surgical wards and now it has become largely routine in major surgical cases. Over 80 cases have been fully investigated and a follow-up blood volume done in most of them.

It will be seen that in practically all cases cited the haemoglobin determination, even where corrected by the P.C.V., is misleading. A haemoglobin reading of 13 or 14 g.% does not exclude a red-cell volume of less than 25 ml./kg as seen in cases 1, 2 and 3.

The correction of the blood volume was estimated on the basis of a 3·6 ml./kg red-cell deficiency. (i.e. 10% of red-cell volume) requiring one pint (575 ml.) of whole blood. Where, however, a total of more than 3 pints was required this was given by two separate transfusions. It is better to err on the side of under-estimations, for any further deficiencies can be made up during the operation as required.

Case 1. E.J., female aged 57. Admitted for gastrectomy for gastric ulcer on lesser curvature.

Pre-operative Investigations (9 June 1954):

Weight = 51·4 kg.	Corrected P.C.V. = 41%
Serum total proteins = 7·2 g.%	Total blood volume = 68·1 ml./kg.
Serum albumin = 3·4 g.%	Plasma volume = 40·2 ml./kg.
Serum globulin = 3·8 g.%	Red-cell volume = 27·9 ml./kg.
Haemoglobin = 14·1 g.%	

Correction. Two pints of whole blood were given. A 90% gastrectomy for two high gastric ulcers was performed on 10 June.

Subsequent Investigations (26 July):
Weight=42·6 kg. Corrected P.C.V.=39%
Total serum protein=5·9 g.% Total blood volume=66·5 ml./kg.
Serum albumin=3·5 g.% Plasma volume=40·5 ml./kg.
Serum globulin=2·4 g.% Red-cell volume=26·0 ml./kg.
Haemoglobin=15·3 g.%

8 September:
Weight=46·4 kg. Corrected P.C.V.=39%
Total serum protein=6·5 g.% Total blood volume=57·9 ml./kg.
Serum albumin=4·9 g.% Plasma volume=35·2 ml./kg.
Serum globulin=1·6 g.% Red-cell volume=22·7 ml./kg.
Haemoglobin=14·4 g.%

No further correction was done before the patient was discharged.

Case 2. M.B., female aged 58. Admitted for gastrectomy for duodenal ulcer.

Pre-operative Investigations (23 June 1954):
Weight=46·4 kg. Corrected P.C.V.=40%
Total serum protein=5·7 g.% Total blood volume=58·2 ml./kg.
Serum albumin=3·1 g.% Plasma volume=34·3 ml./kg.
Serum globulin=2·6 g.% Red-cell volume=23·9 ml./kg.
Haemoglobin=13·8 g.%

Correction. Three pints of whole blood were given.

No lesion was found at laparotomy and no further procedure was carried out. This case illustrates an over-enthusiastic correction with some congestion of neck veins which subsided within twelve hours.

The pre-operative correction of anaemia has long been accepted as necessary in skin-graft surgery. Case 3 shows how misleading a picture a haemoglobin determination gives and further illustrates the rapidity of healing after restoring the blood volume to normal limits.

Case 3. M.M., female aged 50. Admitted for a skin graft for an intractable calf ulcer resulting from a burn from a hot-water bottle following a panhysterectomy for carcinoma of the cervix 4 months earlier.

Pre-graft Investigations:
Clinically she appeared well. Haemoglobin=13·3 g.%.
P.C.V.=38·5%.

A routine skin-grafting operation was done. Five days after operation the grafted area revealed a loose slough of skin indicating complete failure of the graft.

Investigations:
Weight=44 kg. Plasma volume=34·0 ml./kg.
Corrected P.C.V.=34·5% Red-cell volume=17·9 ml./kg.
Total blood volume=51·9 ml./kg.

Correction. 4½ pints of whole blood were transfused in 24 hours without any signs of overloading. Three weeks after the transfusion, the large chronic ulcer was nearly completely epithelialized without further recourse to skin grafting.

There is little doubt in our experience that the pre-operative correction of blood volume is the only accurate means of preparing cases when actual loss of blood has occured. Case 4, a case of long-standing melaena, demonstrates this well. Case 5 (Hodgkin's hypersplenism) also illustrates that one transfusion can rapidly restore haemoglobin levels to normal while red-cell volume is still dangerously low.

Case 4. H.G.H., male aged 67. Admitted with a history of weakness, loss of weight and melaena stools for the past 3 months. There was no history of alcohol.

Pre-operative Investigations (28 May 1954):
Weight=62·5 kg. Total blood volume=73·6 ml./kg.
Haemoglobin=13·8 g.% Plasma volume=47·1 ml./kg.
Corrected P.C.V.=36% Red-cell volume=26·5 ml./kg.

Barium meal revealed a chronic penetrating duodenal ulcer.

Correction. Two pints of whole blood were transfused.

Subsequent Investigations (3 June):
Weight=63 kg. Plasma volume=39·2 ml./kg.
Corrected P.C.V.=42·5% Red-cell volume=29·0 ml./kg.
Total blood volume=68·2 ml./kg.

Correction. Two pints more of whole blood were transfused and after this his general condition improved markedly.

A subtotal extensive gastrectomy was performed. The patient made an uninterrupted recovery except for the development of a mild pulmonary atelectasis.

Case 5. E.C., female aged 54. Referred by the physicians for splenectomy because hypersplenism had occured in the course of generalized Hodgkin's disease.

Pre-operative Investigations (7 June 1954):
Weight=48·6 kg. Corrected P.C.V.=27%
Total serum protein=5·6 g.% Total blood volume=68·7 ml./kg.
Serum albumin=3·5 g.% Plasma volume=50·2 ml./kg.
Serum globulin=2·1 g.% Red-cell volume=18·5 ml./kg.
Haemoglobin=9·6 g.%

Correction. As the plasma volume was already high 2 pints of packed cells were given.

Subsequent Investigations (16 June):
Weight=47·7 kg. Corrected P.C.V.=37%
Total serum protein=6·0 g.% Total blood volume=66·7 ml./kg.
Serum albumin=3·4 g.% Plasma volume=42·0 ml./kg.
Serum globulin=2·6 g.% Red-cell volume=24·7 ml./kg.
Haemoglobin=14·5 g.%

Correction. As the red-cell volume was still low a further pint of packed cells was given and a transthoracic splenectomy was then performed.

Investigation 1 month after operation (20 July):
Weight=46·6 kg. Corrected P.C.V.=48%
Total serum protein=4·4 g.% Total blood volume=79·5 ml./kg.
Serum albumin=3·0 g.% Plasma volume=41·3 ml./kg.
Serum globulin=1·4 g.% Red-cell volume=38·2 ml./kg.
Haemoglobin=16·8 g.%

The convalescence was remarkably smooth and on leaving hospital the blood studies were quite normal.

POST-OPERATIVE CORRECTION

These cases illustrate the rapid and marked improvement that takes place after a stormy convalescence when the blood volume is restored to normal.

Case 1. S.S., female aged 62. Eleven years' history of peptic ulcer with repeated haematemeses over the past 6 years. An emergency gastrectomy for uncontrollable bleeding was performed. At the time of operation the patient was in a poor condition. Pathology showed a chronic gastric ulcer. At operation 2½ pints of whole blood were transfused.

Post-operative Investigations:
9 April 1954. Haemoglobin=13·4 g.%. The patient began to have melaena stools.
20 April. Haemoglobin=15·2 g.%.
1 May. A small localized abdominal abscess developed and was drained.
12 May. Five weeks after the operation the patient was still making a very slow recovery.
Weight=45·7 kg. Plasma volume=25·2 ml./kg.
Haemoglobin=15·8 g.% Red-cell volume=14·2 ml./kg.
Corrected P.C.V.=36% Red-cell count=5,250,000 per
Total blood volume=39·4 ml./kg. c.mm.
15 May. Two pints of whole blood were transfused.
24 May. Patient had lost a further 3 lb. in weight and therefore another 2 pints of whole blood were given. The patient then began to gain steadily and was soon fit enough for discharge.

Case 2. C.B., male aged 58. A diabetic admitted with a large carbuncle of the neck. He showed very slow improvement on a variety of antibiotics.

Investigations 5 weeks after admission:

Weight=58·2 kg.	Corrected P.C.V.=40·5%
Total serum protein=8·0 g.%	Total blood volume=53·3 ml./kg.
Serum albumin=2·7 g.%	Plasma volume=31·8 ml./kg.
Serum globulin=5·3 g.%	Red-cell volume=21·5 ml./kg.
Haemoglobin=14·4 g.%	

The very slow healing here is partly explained by the low red-cell volume.

Case 3. K.S., male aged 50. An abdomino-perineal excision of the rectum was performed. Post-operatively he had a stormy passage. He had a burst abdomen and a partial retraction of the colostomy, with widespread infection of the abdominal wall. After 4 weeks a large ulcerated area round the colostomy showed little signs of healing.

Investigations (12 May 1954):

Weight=50·0 kg.	Plasma volume=43·5 ml./kg.
Corrected P.C.V.=30%	Red-cell volume=18·3 ml./kg.
Total blood volume=61·8 ml./kg.	

Correction. 5½ pints of whole blood were given over a period of 10 days with a marked improvement in his general condition and in about 3 weeks he began to gain weight and the ulcerated area was clean and practically healed.

DISCUSSION

Our chief concern has been to show that blood-volume studies are of fundamental importance in assessing the fluid requirements of patients for major operations. We would stress that the haemoglobin and haematocrit values are too frequently misleading to be the sole guide to the quantitative state of the patient's circulating fluid and red-cell mass.

Berlin and his co-workers, using radio-active phosphorus to measure blood volume, give their normal values for males and females as 38 ml. per kg. for plasma and 28·5 ml. per kg. for red-cell volume. Ellis's figures for the Witwatersrand (5,740 ft. above sea level), using the Evans Blue method, are 39 ml. per kg. for plasma and 36 ml. per kg. for red-cell volume. At sea level the corresponding figures are 45 ml. and 38 ml. respectively. These blood-volume figures at high altitudes agree closely with those reported by Huey and Holmes at a similar altitude.

In a man of average size a 10% blood volume is equivalent to one pint or 575 ml. of blood. Grant and Reeve have shown that the average blood loss from moderate wounds of skin and muscle implies a 30% loss of blood volume, while very large wounds indicate a 50% blood-volume loss.

Using the described dye technique to determine the plasma and red-cell volumes, and the stated average normal values, the amount of blood to be administered for correction was calculated from the difference between the present blood volume and that calculated for the ideal weight of the patient.

All patients are weighed on admission and the ideal weight is then read from a chart where the calculation is made from height and habitus. In the majority of our patients the ideal weight has been within 10 kg. of the weight on admission. In these cases the ideal weight has been taken as a basis for fluid requirements. In cases where obesity or thinness results in a weight differing widely from the ideal we have in the obese taken the ideal weight, and in the thin the usual weight of the patient. By 'usual weight' is meant the weight over the past few years before the illness. In both cases, therefore, the danger of excess fluid is avoided.

We are investigating further the relation of blood volume to marked overweight and underweight.

Overloading of the circulation and consequent cardiac failure does not appear to be a serious hazard in patients who have proven plasma or red-cell deficiencies. However, in view of the frequent objections raised by anaesthetists that the patient has been or will be 'overloaded' with fluids, it is worth noting that the inevitable loss at operation usually corrects this. It is probably wiser to under-correct by 500 ml. than to over-correct. Frequently 3 or 4 pints of blood have been given pre-operatively without any untoward complications.

During the past 5 years we have noted that the mortality rate in elderly patients undergoing surgery is still high from delayed peripheral circulatory collapse and pulmonary complications. Although care has been taken with the administration of fluids and electrolytes patients die despite all treatment.

Clark *et al.* use the term 'chronic shock' to describe the prolonged peripheral circulatory failure which occur in the post-operative course of those patients who are underweight and who have low blood volumes; they ascribe this to the low reserves of haemoglobin and plasma proteins, which cannot be rapidly mobilized. A few hours' low blood pressure can render important organs, particularly the liver, anoxic; this in turn gives rise to vaso-depressor materials (V.D.M.) which cause irreversible shock (Zweifach *et al.*). Death often occurs in 4-6 days, and at autopsy no macroscopic cause is found.

We prefer the term 'latent shock' to express this concept of chronic shock in the elderly mal-nourished or emaciated patient, because empirical clinical experience shows that poor surgical risks are given a better chance of survival when their haemoglobin and protein reserves are increased by pre-operative blood transfusions.

According to Whipple, when a deficiency of both haemoglobin and tissue protein occurs the formation of new haemoglobin has priority. Consequently the restoration of protein deficits is expedited by the initial correction of a masked anaemia.

We have frequently noted a marked drop in haemoglobin level and fall of the red-cell mass about the 7th post-operative day; this must certainly be one of the main factors in delaying the healing of some wounds and causing wound disruption.

In the post-operative state the great loss of protein as shown by the negative nitrogen balances (Cuthbertson, 1932) and the loss of weight are in need of much closer attention. Post-operative weight-gain must necessarily be slow if there is not enough available protein for the building up of muscle tissue. In the cases noted the rapid improvement and the gain in weight following the correction of blood volumes, especially after a stormy convalescence, is testimony to the efficacy of accurate replacement.

SUMMARY AND CONCLUSIONS

A simple accurate technique for the determination of blood volume using the Evans Blue dye method is described.

A series of cases is presented showing the fundamental values of blood-volume studies in the pre-operative assessment of the patient, and in the reduction of post-operative morbidity.

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