CHANGES IN HAEMOGLOBIN CONCENTRATION AND PLASMA SPECIFIC GRAVITY FOLLOWING PLASMA TRANSFUSIONS

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The reduction in the haemoglobin concentration of the blood following the rapid transfusion of a known quantity of plasma has been used as a means of calculating the pre-transfusion blood volume (Robertson and Bock, 1919; Scholander, 1938; and Bushby, Kekwick, and Whitby, 1940). This method makes several assumptions: (1) that complete mixing of the plasma with *all* the circulating blood takes place; (2) that the number of red cells in circulation during transfusion and mixing remains constant; and (3) that the plasma volume after transfusion and mixing is equal to the volume of plasma before transfusion plus the volume of plasma transfused. If one or more of these assumptions is incorrect, the blood volumes estimated may differ considerably from the true blood volume. Again, it has been assumed (Hayward, 1942) that when the haemoglobin concentration returns to its pre-transfusion level the volume of plasma transfused has been eliminated from the blood stream. This assumption implies that the only factor which causes an increase in haemoglobin concentration is the movement of fluid from the blood. It is important, therefore, to examine any evidence which throws light on the validity or otherwise of these fundamental assumptions.

The following observations, made during an investigation into the physical and chemical changes in the blood after transfusion of plasma, concern variations in haemoglobin concentration and plasma protein content of the blood found in animals during the first hour after rapid plasma transfusions. Their bearing on the above assumptions will be discussed.

Methods

Cats anaesthetized with nembutal injected intraperitoneally (44 mg. per kg. of body weight) were used. No plasma injection was given until 4 hours after induction of anaesthesia. This was to allow for the disappearance of an increase in plasma volume (10%) which follows nembutal anaesthesia (Hamlin and Gregersen, 1939). Blood samples were taken from a femoral artery, and plasma was injected through a femoral vein. The animals were heparinized during the experiment and plasma from heparinized animals was employed for transfusion. In 2 experiments (Nos. 94 and 95) the plasma was diluted with Ringer-Locke solution. Blood samples did not

exceed 1 c.cm. each. Haemoglobin estimations were made by the alkaline haematin method, using a photo-electric colorimeter. Plasma proteins were estimated by the specific gravity method of Barbour and Hamilton (1926) and controlled by direct determinations by the biuret method of Robinson and Hogden (1940). Plasma protein values were calculated from the plasma S.G. by the formula of Weech, Reeves, and Goettsch (1936). Such values were within $\pm 1.5\%$ of those obtained by the biuret method. The plasma S.G. value was accurate to ± 1 in the fourth place—i.e., a value of 1.0279 might be 1.0278 or 1.0280, which gives an error of $\pm 0.5\%$ in terms of plasma protein.

Plasma volumes as determined are accurate to within $\pm 3\%$ (dye method), and the blood volumes were calculated from these by multiplying by 100 and dividing by the plasma percentage (haematocrit) determined from a femoral artery blood sample.

Plasma Specific Gravity

Thirteen normal animals were each given a single plasma transfusion and the changes in plasma specific gravity were then observed (Table I). If the transfused plasma mixed completely with the animal's plasma and no fluid or plasma protein entered or left the blood stream, the S.G. after mixing can be calculated from the following formula:

$$PV_1 \times SG_1 + PV_2 \times SG_2 = (PV_1 + PV_2)SG_3$$

where PV_1 is the plasma volume before transfusion, PV_2 the volume of plasma transfused, SG_1 the S.G. of the animal's plasma before transfusion, SG_2 the S.G. of the transfused plasma, and SG_3 the S.G. of the animal's plasma after mixing has taken place. This calculated value (SG_3) is to be found in the last column of Table I. Of the 53 observations, 10 were below the calculated value, 4 were the same as this value, and 39 were above it. The 10 observations below the calculated value were distributed as follows: 8 were less than 5 points below the expected value, and 2 were between 6 and 9 points below. In contrast, the 39 observations above the calculated value showed much greater discrepancies: 9 were 1 to 5 points above the expected value, 9 were 6 to 10 points above, 12 were 11 to 15 points above, 8 were 16 to 20 points above, and 1 was 21 points above.

Haemoglobin Concentrations

The haemoglobin concentrations were followed in 10 of the 13 animals. The values were expressed as percentages of the control value before transfusion began. If it be assumed (1) that

TABLE I

No.	Plasma Vol. before 1st Injection (c.cm.)	Plasma S.G. before 1st Injection	Volume of Plasma Injected (c.cm.)	S.G. of Plasma Injected	S.G. of Plasma after Injection										Calculated Plasma S.G.
					S.G.	Min. after	S.G.	Min. after	S.G.	Min. after	S.G.	Min. after	S.G.	Min. after	
70	144	1.0290	20	1.0287	1.0297	3	1.0288	5	1.0302	20	1.0305	30	1.0304	45	1.0289
72	168	1.0276	16	1.0293	1.0279	9	1.0277	24	1.0282	39	—	—	—	—	1.0277
73	92	1.0297	20	1.0266	1.0302	10	1.0307	25	1.0302	40	1.0302	55	—	—	1.0291
74	123	1.0299	20	1.0244	1.0302	8	1.0291	23	1.0293	38	1.0291	120	—	—	1.0291
90	117	1.0284	19	1.0284	1.0282	8	1.0286	13	1.0291	28	1.0293	43	—	—	1.0284
91	87	1.0313	10	1.0270	1.0323	9	1.0316	14	1.0306	28	—	—	—	—	1.0309
92	195	1.0265	20	1.0247	1.0281	10	1.0282	16	1.0279	26	1.0273	41	—	—	1.0263
93	104	1.0321	15	1.0279	1.0316	10	1.0307	16	1.0308	26	1.0319	47	—	—	1.0316
94	214	1.0304	30	1.0210	1.0297	10	1.0289	15	1.0293	22	1.0289	39	1.0289	54	1.0292
95	252	1.0304	50	1.0222	1.0301	11	1.0296	16	1.0300	30	1.0296	45	1.0292	60	1.0290
96	111	1.0232	16	1.0270	1.0255	10	1.0250	15	1.0248	27	1.0248	42	1.0246	59	1.0237
100	151	1.0295	20	1.0253	—	—	1.0311	25	1.0309	40	1.0308	55	—	—	1.0290
106	124	1.0283	20	1.0259	1.0292	10	1.0277	25	1.0280	40	1.0278	55	—	—	1.0279

to a shift of fluid in c.cm. per 100 c.cm. of plasma and plotted against the discrepancies between the observed and the calculated haemoglobin values, no correlation between these sets of variables is apparent (see Graph above). Differences between observed and calculated haemoglobin concentrations cannot, therefore, be accounted for on the basis of a shift of protein-free fluid from the blood stream.

It has been pointed out above that if blood volumes are calculated from the observed haemoglobin values after plasma transfusion, the results are of little value owing to the high values obtained. Hayward (1942), in a series of 8 cases of peripheral vascular disease, studied the effects of serum transfusion on, among other observations, the haemoglobin concentration and packed cell volume (haematocrit). In his Table I he gave the values of these two estimations before and after transfusion and stated that by following the changes in them he was able to observe the alterations in blood volume. By using the formula for estimating blood volumes mentioned above, the values of the blood volumes can be obtained. These are given in Table V. It will be noted that out of the 8 values obtained

TABLE V

Case No.	Blood Volume from Hb Values (c.cm.)	Blood Volume from Haematocrit Values (c.cm.)	Case No.	Blood Volume from Hb Values (c.cm.)	Blood Volume from Haematocrit Values (c.cm.)
1	3,792	6,300	6	7,345	4,089
2	10,286	7,733	8	6,800	5,354
4	5,784	6,000	9	2,400	—
5	4,800	3,160	13	4,350	—

from the haemoglobin determinations, 4 and probably 5 are higher than the normal human blood volume. Three—probably 4—obtained from haematocrit readings are higher than the normal value.

In the experimental series listed in Table III all the blood volumes but one were much higher than those calculated by a direct method. It seems not unreasonable to suppose that the same factors which are operating to produce a discrepancy between the expected and observed haemoglobin values in the animals may also operate in man. If the discrepancy cannot be explained in the animals by supposing a shift of protein-free fluid, it is unlikely that such a shift is sufficient to explain the discrepancies in the blood-volume estimations calculated from Hayward's data.

Hahn *et al.* (1942) have shown that the red cell volume as determined by the plasma volume-haematocrit method gives values 10% to 40% in excess of the true red cell volume. The reasons for this disparity were pointed out by Fahraeus (1929) and emphasized by Stead and Ebert (1941). If the plasma volume as determined by the dye method is regarded as accurate to within $\pm 3\%$ (after allowance has been made to ensure that the two phases in the mixing of the dye with the plasma have been completed; Hahn *et al.*, 1942), then the true blood volume in the experiments in the present series must be less than that actually determined by the plasma volume-haematocrit method. The calculated haemoglobin value after plasma transfusion must therefore be lower than the figure given, and hence the disparity between the observed and the calculated haemoglobin values should be much greater than those recorded here.

If it be assumed that a rapid transfusion of plasma requires an increase in the capacity of the capillary bed to accommodate the increase in blood volume, then each of these newly opened capillaries will be lined with a film of cell-free plasma moving sluggishly in comparison with the axial stream of corpuscles and plasma. The quantity of plasma required to form this film has been estimated by Hahn *et al.* (1942) as 21% of the plasma volume. Therefore, of the plasma transfused, about 79% only will be available to mix with the axial streams and so to "dilute" the circulating blood. Modifying the original formula for calculating the expected haemoglobin concentration to

$$\frac{BV}{BV + \frac{79V}{100}} = \frac{Hb_2}{Hb_1}$$

and calculating the expected haemoglobin concentration, the values in Table VI are obtained. The observed values used for

TABLE VI

No.	Expected Hb Conc. (100% V) A	Expected Hb Conc. (79% V) B	Observed Hb Conc. C	Discrepancy between C and B
72	94.4	95.7	95.2	-0.5
73	88.5	90.7	92.5	+1.8
90	101.3	103.2	104.3	+1.1
91	91.9	93.1	97.8	+4.7
92	95.8	97.0	95.9	-1.1
93	94.0	95.8	99.8	+4.0
94	94.8	96.4	97.9	+1.5
95	87.0	89.3	99.2	+9.9
100	91.9	93.5	93.7	+0.2
106	91.5	93.2	92.4	-0.8

comparison are those recorded between 8 and 11 minutes after the end of the transfusion. The discrepancies in 7 out of 10 are less than 2%, and 3 are between 4 and 10%. It would appear, therefore, that the immobilization of plasma to form such peripheral films is a factor of some importance. If loss of protein-free fluid from the blood stream is the other factor operating to produce the large discrepancies still remaining in Nos. 91, 93, and 95, then there ought to be some parallelism between the calculated fluid loss (Table IV) and the haemoglobin discrepancies. A comparison of these values shows that a movement of protein-free fluid alone cannot be the explanation.

One is forced to the conclusion that the assumption that only protein-free fluid moves into or out of the blood stream during the period of observation is untenable. As Whipple and his school have demonstrated the ease with which plasma protein can be translocated from plasma to cells and from cells to plasma, it seems probable that movement of substantial amounts of fluid containing plasma proteins does take place after plasma transfusions. Plasma transfusions, therefore, in normal animals, and probably also in man, cannot be used to estimate blood volumes, because, first, fluid leaves the blood stream in substantial amounts over short periods of time, and, secondly, all the plasma transfused is not used to "dilute" the circulating blood. A return of the haemoglobin concentration to its pre-transfusion value could take place with a total plasma volume still in excess of the pre-transfusion volume. Plasma can remain immobilized as a surface film without being concerned in the ratio of cells to plasma in rapid circulation.

Summary

Intravenous transfusions of plasma into anaesthetized animals are followed by changes in haemoglobin concentration and plasma protein content which cannot be explained on the basis of a simple mechanical mixing of the transfused plasma with the circulating blood. The formation of surface films of plasma in capillaries opened up to receive the transfused plasma reduces the amount of plasma available for dilution of the circulating blood and leads to high haemoglobin values with consequent high estimates of blood volume. Movement of protein-free fluid from the blood stream cannot account for the observed changes in plasma S.G. and haemoglobin concentration. Movement of plasma proteins probably occurs simultaneously with this fluid shift.

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