THE CAUSES AND PREVENTION OF DEFECTIVE FUNCTION OF STORED RED BLOOD CELLS AFTER TRANSFUSION.

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We have shown elsewhere that the oxygen dissociation curve of blood after storage under standard blood bank conditions in an acid citrate dextrose medium is shifted to the left progressively with storage and that the oxygen dissociation curve of the recipient of such blood is also shifted to the left (Valtis & Kennedy, 1954). Indeed, we have presented evidence that, under certain circumstances, the transfusion of stored blood to an anaemic recipient may affect adversely the oxygenation of his tissues during the few hours immediately succeeding the transfusion.

The present paper seeks to explain these abnormalities on the basis of altered pH of the stored red cells and altered electrolytic and osmotic relationships produced by storage. Secondly, it shows that the addition of sodium chloride to stored citrated blood can largely prevent the displacement of the oxygen dissociation curve which otherwise occurs *in vitro*, and that moreover, the oxygen dissociation curve of the blood of the person who receives such modified citrated stored blood does not show the undesirable shift to the left otherwise present.

MATERIALS AND METHODS.

The collection and storage of blood samples, the equilibration with the desired gas tensions, the blood gas analyses, and the plasma pH (pH_s) determinations were as described elsewhere (Valtis & Kennedy, 1954).

The pH of cells (pH_c) of the stored blood and fresh normal blood samples was calculated using the Henderson-Hasselbalch equation

$$pH_c = pK'_c + \log \frac{(BHCO_3)b}{(H_2CO_3)b}$$

where $(BHCO_3)b$ = T40 (the total CO_2 of oxygenated blood at 40 mm.Hg partial pressure of CO_2)

and $(H_2CO_3)b$ = H_2CO_3 content of blood calculated from the formula $(H_2CO_3) = a(pCO_2)$ where a is the solubility coefficient of carbon dioxide in blood (from data by Dill in the paper of Keys *et al.*, 1936 and Dill *et al.*, 1940)

pK'_c was obtained from the alignment chart and procedure given in the paper of Keys *et al.* modified in such a manner for the pK'_c to be 5.98 for normal blood (Dill *et al.*, 1937a, 1937b) giving a cell pH of 7.20-7.25. The other values for pK'_c derived from this alignment chart were changed proportionately.

The cell pH of the recipient before and after transfusion was determined by the same methods, after the blood samples, which were from anaemic individuals, had been concentrated by removal of plasma under oil, to bring the packed cell volume into the normal range.

Correction of the position of the curve according to the plasma and cell pH was carried out in curves drawn in logarithmic co-ordinates by the procedure outlined by Dill in the paper of Keys *et al.* using Fig. 1.

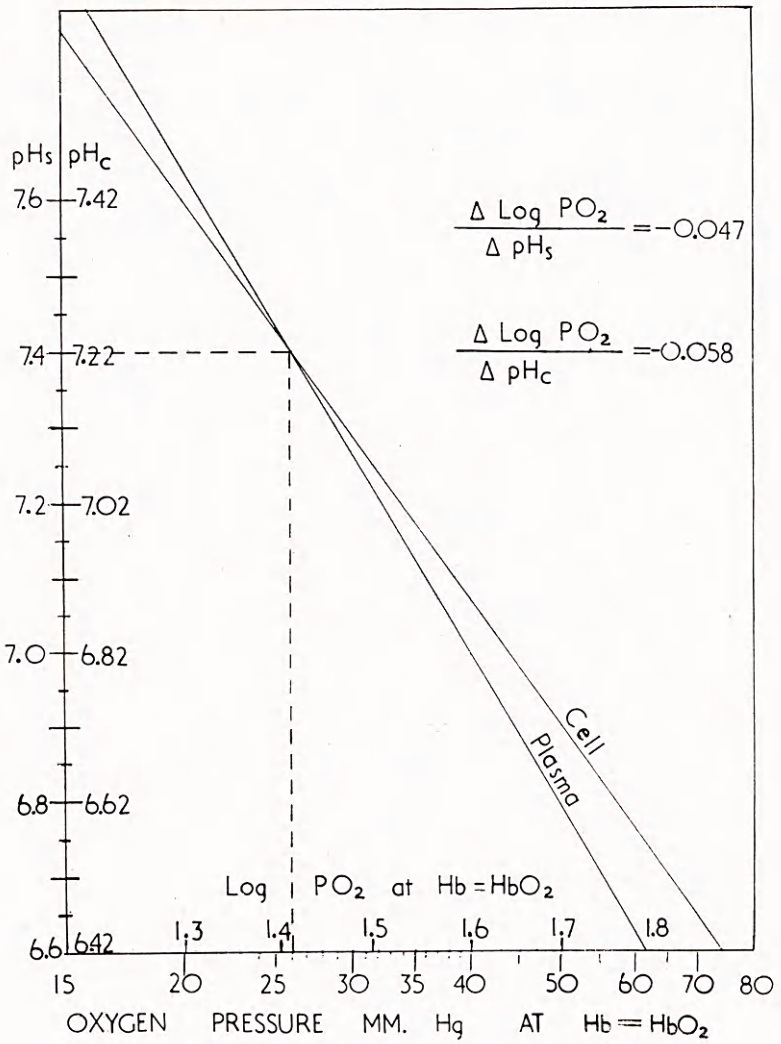

Fig. 1. The relationship between the position of the oxygen dissociation curve at $Hb = HbO_2$ and plasma pH and cell pH (modified from Dill).

RESULTS.

The results are presented in Tables 1-8*. Conventional symbols, the meanings of which are explained in the footnotes, are used for the headings of many of the columns. The position of the oxygen dissociation curve is indicated by the partial pressure of oxygen at which 50% of the haemoglobin is oxygenated (Columns H, I & J).

In Vitro Studies.

Fresh blood. In Table 1 is shown the relationship between the plasma pH (Column F) and cell pH (Column G) of fresh blood, fresh blood after dilution and slight haemolysis, and acidified fresh blood. The difference between the total CO_2 of plasma and of oxygenated whole blood (Column E), on which depends the difference between the plasma pH and cell pH, is decreased but proportionately to the fall of pH (Fig. 2, Curve I) and to the oxyhaemoglobin capacity. The position of the oxygen dissociation curve corrected to the standard plasma pH 7.4 (Column I) coincides with that corrected to the standard cell pH 7.22 (Column J) ($Hb=HbO_2$ at 26 mm.Hg partial pressure of oxygen). In the case of acidified fresh blood, partial restoration of the pH with fresh plasma has the same effect.

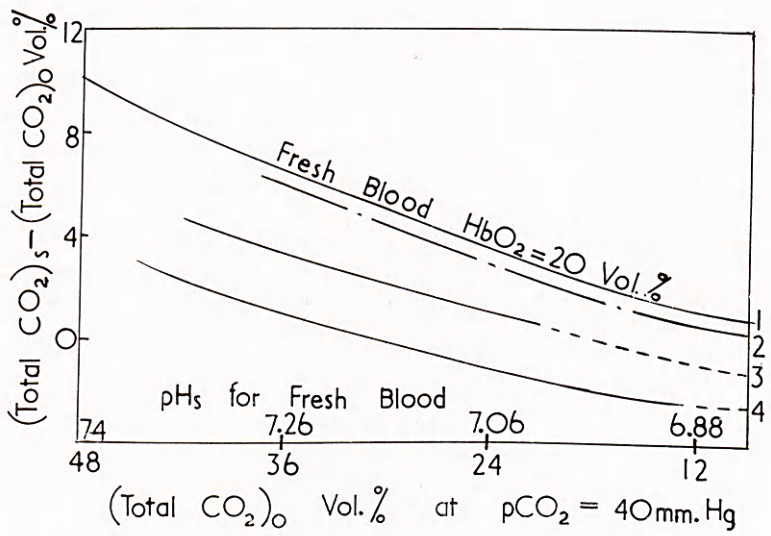

Fig. 2. The relationship between the total CO_2 of oxygenated blood at pCO_2 40 mm.Hg and the difference between the total CO_2 of plasma and oxygenated blood.

Curve 1. Fresh blood.
Curve 2. Blood stored for 20 days in A.C.D. medium after the addition of NaCl.
Curve 3. Blood stored for 24 hours in A.C.D. medium.
Curve 4. Blood stored for over 7 days in A.C.D. medium, in trisodium citrate, and in acidified heparin solution.

* The tables are grouped together at the end of the text.

Blood stored in A.C.D. medium up to 24 hours. From the results given in Table 2 it is apparent that the difference between the total CO_2 of plasma and of oxygenated whole blood is decreased to an extent which is out of proportion to the fall of pH_s (Fig. 2, Curve 3) and that the difference between the plasma pH and cell pH is likewise reduced. The oxygen dissociation curve at standard plasma pH is slightly displaced to the left ($Hb=HbO_2$ at 25 mm.Hg partial pressure of oxygen). After correction to the standard cell pH the oxygen dissociation curve lies very slightly to the right ($Hb=HbO_2$ at 27 mm.Hg partial pressure of oxygen) (Fig. 3). Partial restoration of the pH with fresh plasma does not abolish the above abnormalities.

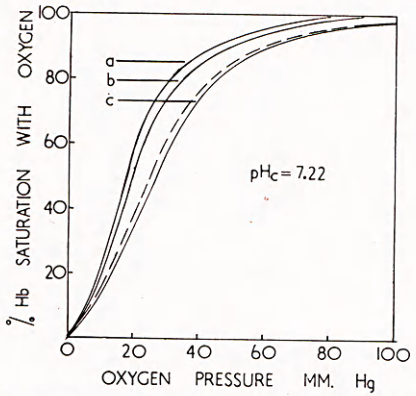

Fig. 3. Oxygen dissociation curves at cell pH 7.22
Interrupted line. Fresh normal blood.
Curve a. Blood stored for 20 days in A.C.D. medium.
Curve b. Blood stored for 7 days in A.C.D. medium.
Curve c. Blood stored for 1 day in A.C.D. medium and fresh cells plus stored citrated plasma.

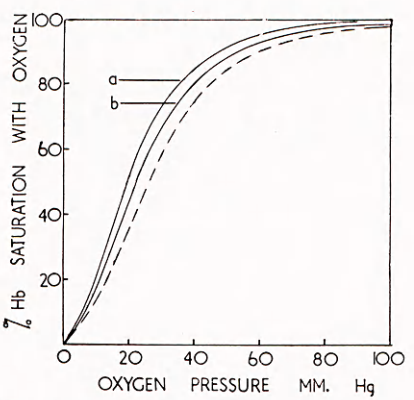

Fig. 4. Oxygen dissociation curves from blood stored for 7 days in A.C.D. medium.
Curve a. At plasma pH 7.4.
Curve b. At cell pH 7.22.
Interrupted line. Fresh normal blood.

Blood stored in various anticoagulants for 7 days. In blood samples stored in A.C.D. medium for 7 days (Table 3) the total CO_2 of plasma (Column D) is less than the total CO_2 of oxygenated blood (Column C). The difference between these CO_2 values is negative (Fig. 2, Curve 4), the plasma pH to cell pH relationship is reversed, and the cell pH is less acid than the plasma pH. The oxygen dissociation curve lies very much to the left of the expected position (Column H). Correction of the position of the curve to the standard plasma pH and cell pH makes more apparent the shift to the left and at the same time makes obvious the abnormality of the cell pH. The two curves do not coincide ; the oxygen dissociation curve corrected to the standard pH_c lies to the right of the curve corrected to the standard plasma pH, in a position intermediate between that of normal blood and that of stored citrated blood at standard plasma pH (Fig. 4). Correction, therefore, of the position of the curve according to

cell pH diminishes, but does not abolish, the abnormality. After partial restoration of the pH with fresh plasma all the abnormalities described above remain although in lesser degree. It appears that contact with fresh plasma has some effect on the restoration of the normal relationship between the total CO_2 of plasma and whole blood and the plasma pH to cell pH relationship.

Blood samples stored with trisodium citrate or an acidified heparin solution show the same abnormalities but to a less degree. Storage of blood with a heparin-fluoride anticoagulant, which does not lower the pH, does not affect the position of the oxygen dissociation curve or the relationship between the total CO_2 of plasma and whole blood and therefore the plasma pH to cell pH relationship.

Blood stored in A.C.D. medium for 20 *days.* In citrated blood stored for 20 days the same abnormalities exist as described for 7-day stored citrated blood but they are more marked (Table 4). Despite the fact that the pH has fallen from approximately 6.8 to approximately 6.6 the oxygen dissociation curve is shifted further to the left (Fig. 3). The difference between the total CO_2 of plasma and whole blood (Fig. 2, Curve 4) between the plasma pH and the cell pH, and between the position of the curves after correction to the standard plasma pH and cell pH, are not basically different from the values for 7-day stored citrated blood samples (Fig. 5). After partial restoration of the pH with fresh plasma the difference between the total CO_2 of plasma and of oxygenated whole blood, and so the difference between the plasma pH and cell pH becomes smaller. The result of this is to bring the curves at standard plasma pH

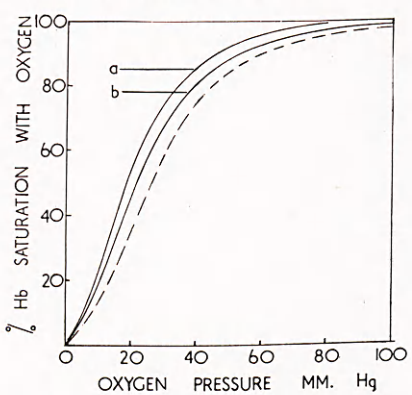

Fig. 5. Oxygen dissociation curves from blood stored for 20 days in A.C.D. medium.
Curve a. At plasma pH 7.4.
Curve b. At cell pH 7.22.
Interrupted line. Fresh normal blood.

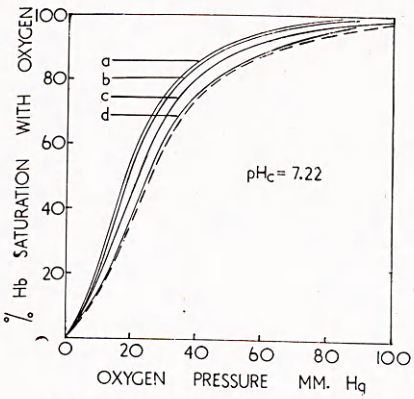

Fig. 6. Oxygen dissociation curves at cell pH 7.22.
Curve a. Blood stored for 20 days in A.C.D. medium.
Curve b. Blood stored for 20 days in A.C.D. medium after plasma replacement.
Curve c. Blood stored for 20 days in A.C.D. medium after addition of phosphate buffer.
Curve d. Blood stored for 20 days in A.C.D. medium after addition of NaCl.
Interrupted line. Fresh normal blood.

and standard cell pH slightly closer together mainly because of the slight shift to the right of the curve after correction to standard cell pH (Fig. 6).

Fresh red cells to which has been added citrated plasma derived from 20-day stored blood show the same abnormalities as fresh citrated blood (Fig. 3).

Blood stored in A.C.D. medium for 2 months. After correction for the amount of carboxyhaemoglobin present (Table 5, Columns B, K, L. M) the position of the oxygen dissociation curve at standard plasma pH and cell pH is only slightly further to the left of the position of the curves from 20-day stored samples.

Comments on in vitro results. It is apparent from the results given above that the alteration in cell pH is the most important factor in the production of the shift of the oxygen dissociation curve to the left. Yet after 7 days of storage there is no further alteration in the difference between the total CO_2 of plasma and of oxygenated whole blood and in the difference between the plasma pH and cell pH although the shift to the left of the oxygen dissociation curve, corrected to the standard cell pH, progresses with further storage (Fig. 3). A further interesting point is that in citrated blood samples a definite alteration in the difference between the CO_2 of plasma and of oxygenated whole blood and in the relationship between plasma pH and cell pH occurs within a few hours of storage yet at this stage the oxygen dissociation curve is not shifted to the left.

Effect of electrolytes in vitro. When attempting to correct the plasma pH of stored blood to 7.3 or 7.4 by the addition of a molecular solution of disodium phosphate it was noted that the position of the oxygen curve was not that expected from the results found with correction of the plasma pH by fresh plasma or the position given by calculated correction to the plasma pH of 7.4. This observation prompted us to investigate the effects of other electrolytes with the following results. The addition, in a proportion of 10%, of molecular phosphate buffer (disodium phosphate plus monopotassium phosphate) of the same pH as the plasma pH of the stored blood sample, produces a shift towards the right of the oxygen dissociation curve which, however, is still abnormal in position, although the plasma pH remains unchanged. On the other hand, the addition of a molecular solution of sodium chloride to stored blood of low plasma pH (20-day storage), in a proportion of 10 per cent produces a complete return to the normal position of the oxygen dissociation curve although the plasma pH is not altered. These findings are presented in Table 6 and Fig. 6.

The difference between the total CO_2 of plasma and of oxygenated blood and the difference between the plasma pH and cell pH are considerably smaller after the addition of phosphate buffer. The addition of sodium chloride has a more marked effect and restores these relationships

to normal (Fig. 2, Curve 2). In 20-day stored citrated blood, after rotation in a tonometer for 1 hour at 37°C and arterial gas tensions, the normal relationship between cell and plasma chloride anion is reversed (Table 7). The addition of sodium chloride restores to normal this relationship. A further noticeable effect of the addition of sodium chloride to citrated blood is the diminution of spontaneous haemolysis in blood samples stored over prolonged periods.

In Vivo Studies.

The subjects on whom the following observations were made were suffering from severe anaemia and the oxygen dissociation curves of many of them were shifted to the right (Table 8). After correction of the position of the oxygen dissociation curve to the standard cell pH the abnormality of position largely, but not completely, disappears (Kennedy & Valtis, 1953).

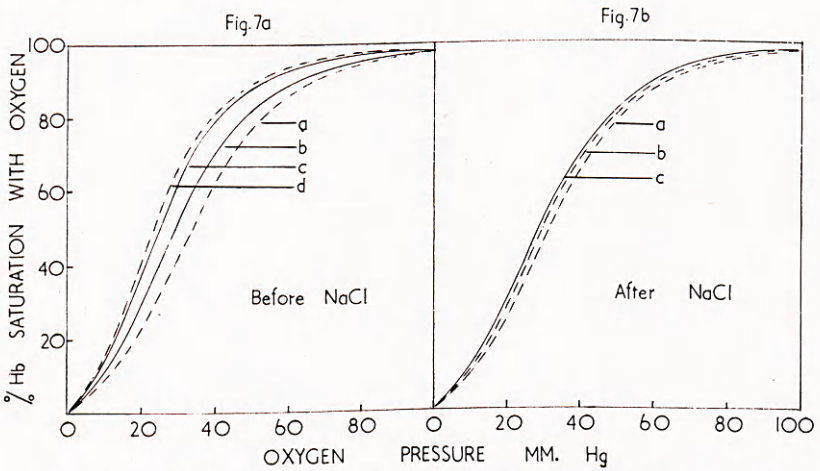

Fig. 7a. The oxygen dissociation curve of the recipient of blood stored in A.C.D. medium.
Curve a. Before transfusion at plasma pH 7.4.
Curve b. Before transfusion at cell pH 7.22.
Curve c. Immediately after transfusion at cell pH 7.22.
Curve d. Immediately after transfusion at plasma pH 7.4.

Fig. 7b. The oxygen dissociation curve of the recipient of saline modified stored citrated blood.
Curve a. Before transfusion at plasma pH 7.4.
Curve b. After transfusion at plasma pH 7.4.
Curve c. Before and after transfusion at cell pH 7.22.

Transfusion of plasma derived from 20-day stored citrated blood does not produce a shift to the left of the oxygen dissociation curve of the anaemic recipient (Case 1). Transfusion of 20-hour stored citrated blood produces a small shift to the left of the recipient's oxygen dissociation curve but, after correction to standard cell pH, this shift is no longer present, suggesting that the shift of the curve is due to alteration in the cell pH of the recipient (Case 2). Transfusion of citrated blood of 7 or more days' storage produces a significant shift to the left of the oxygen

dissociation curve of the recipient (Cases 3, 4, 5, 6). After correction of the pre- and post-transfusion curves to standard cell pH a large part, but not all, of the shift is no longer present (Fig. 7a). This indicates that factors other than altered cell pH play a part in the production of the shift of the curve.

Effect of addition of sodium chloride to stored blood. If to stored citrated blood is added before transfusion a molecular solution of sodium chloride in a proportion of 10 per cent, the marked shift to the left of the recipient's oxygen dissociation curve no longer occurs (Table 8). A small shift to the left is present which is very slightly augmented by correction of the slightly lowered plasma pH to the standard plasma pH. After correction to standard cell pH the pre- and post-transfusion oxygen disssociation curves almost coincide (Fig. 7b), suggesting that sodium chloride not only affects the corpuscular pH but also the other factors concerned in the production of the shift to the left of the oxygen dissociation curve.

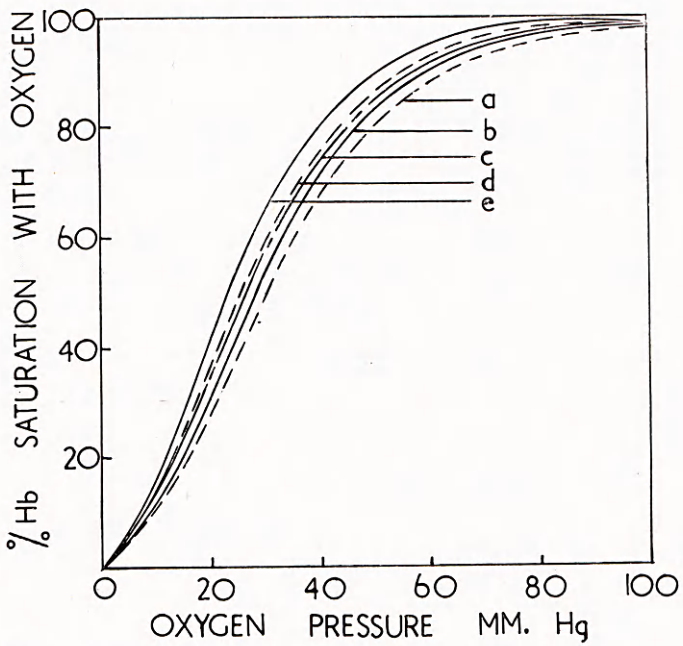

Fig. 8. Effect of ammonium chloride on the oxygen dissociation curve.
Curve c. Before NH_4Cl.
Curve a. After NH_4Cl at blood plasma pH and cell pH.
Curve b. After NH_4Cl at standard plasma pH and cell pH.
Curve d. After transfusion of stored citrated blood at plasma pH and cell pH.

Effect of administration of ammonium chloride to recipient. The oral administration of 12 grams of ammonium chloride (2 gm. 4-hourly) produces a marked shift to the right of the oxygen dissociation curve, the shift being proportional to the fall in the recipient's plasma pH

(Case 9). After transfusion of 2 pints of stored citrated blood the oxygen dissociation curve is considerably to the left of the position after ammonium chloride but only slightly to the left of the normal position. Correction of the position of the curves to standard plasma pH or cell pH reveals that a marked shift has occurred this shift being masked by the ammonium chloride effect on plasma pH (Fig. 8).

DISCUSSION.

It is known that the position of the oxygen dissociation curve is dependent basically on the cell pH and we have shown that an alteration in this pH relative to the plasma pH occurs in citrated blood with storage. As far as we are aware, direct determinations of the pH of cells stored in A.C.D. medium have been performed only by Maizels (1943) who found that the cell pH became greater than the plasma pH. Thus, after 1 week of storage the cell pH was 7.13 and the plasma pH 7.07 and after 4 weeks the values were 6.97 and 6.82 respectively. Maizels, however, gives his results with reservation in view of the inherent difficulties of the methods employed. In addition, it should be remembered that the pH studies, and indeed all the published electrolyte studies in stored blood, were performed on blood samples under conditions far removed from those obtaining in the body. The only exception to this is the work of Harris (1941) and Maizels (1949) on potassium levels in stored blood after incubation at 37°C. Of at least equal importance, in our opinion, to the incubation at body temperature is the equilibration of the blood samples with arterial blood gas tensions.

We have determined the cell pH from the data given by Dill *et al.* (1937a, b), after equilibration of the blood samples with arterial gas tensions at body temperature. These data were derived from fresh blood studies and are not wholly applicable to our studies on stored citrated blood which has a very low pH and a different CO_2 solubility coefficient. The effect produced by these differences is, however, slight and, more important is constant (Valtis & Kennedy, 1954). Nevertheless, the results we give for cell pH should be considered relatively rather than absolutely. Our results show that the cell pH of citrated blood becomes greater than the plasma pH with storage. In citrated blood of 7 or more days storage, if we correct the position of the oxygen dissociation curves according to standard cell pH we find that the abnormality in the position of the oxygen dissociation curve is partially corrected (Figs. 4 & 5). The cell pH of the anaemic individual before and after transfusion was determined by the same indirect methods. If these methods are applied to anaemic blood where the cell phase is small, there is the disadvantage that experimental errors are multiplied. To avoid this disadvantage we have determined the cell pH after concentrating the anaemic blood samples to bring the packed cell volume

into the normal range. This manipulation, in our opinion, permits more accurate comparative study. We have shown elsewhere that concentration does not alter the oxygen dissociation curve (Kennedy & Valtis, 1953). Calculation of the cell pH of the recipient before and after transfusion shows that there is a pronounced alteration of the cell pH to the alkaline side after transfusion, which is responsible for the major part of the displacement to the left of the oxygen dissociation curve (Fig. 7a). There is, however, a degree of displacement of the oxygen dissociation curve, both in the *in vitro* and the *in vivo* studies, which is not explicable on the basis of altered cell pH and for which some other explanation must be sought.

While the position of the oxygen dissociation curve is dependent largely upon the cell pH it is also significantly influenced by the ionic and osmotic status of the red cell, which in turn are dependent on the electrolyte equilibria between cell and plasma and the condition of the cell membrane. During storage swelling of red cells occurs (Drew *et al.*, 1939 ; Crosbie & Scarborough, 1941) and this increases the water content and decreases the haemoglobin concentration within the red cell ; the Donnan ratio ' r ' as developed by Van Slyke therefore increases. The effect of this is to produce a shift to the left of the oxygen dissociation curve. After the addition of molecular phosphate buffer to stored citrated blood the shift to the left of the oxygen dissociation curve becomes less marked and the packed cell volume returns to normal. It is probable that this is due to the reversal of the red cell swelling. The addition of the same volume of molecular sodium chloride produces a more marked effect on the position of the oxygen dissociation curve than does phosphate buffer although the phosphate buffer is more hypertonic and causes a greater reduction of packed cell volume. It is apparent, therefore, that in addition to the effect on the plasma to cell pH relationship and the water content of the cells, a further factor is involved in the abnormality of the oxygen dissociation curve and its reversal by sodium chloride.

We have shown that the total CO_2 content of stored citrated blood is higher than the total CO_2 content of the plasma from such blood. The chloride ion, which freely penetrates the cell membrane, obeying Donnan's law moves in accordance with the relationship between the total CO_2 of cell and plasma. Our results show that the addition of sodium chloride restores the relationship of CO_2 and chloride between cell and plasma to normal. It is possible that this movement of chloride and carbon dioxide is accompanied by a similar redistribution of other ions the final effect being the restoration of the normal relationship between cell and plasma electrolytes, cell and plasma pH, and cell and plasma water content.

Fig. 7 clearly demonstrates the beneficial effect upon the recipient's post-transfusion oxygen dissociation curve of adding sodium chloride to the stored citrated blood prior to its administration. The greater part of the sodium chloride effect is due to the osmotic and electrolytic redistribution discussed above. That the lowering of the plasma pH of the recipient plays only a small part can be judged from the very slight further shift in the position of the curve after correction to standard plasma pH and cell pH.

The oral administration of ammonium chloride before and during the transfusion of stored citrated blood also prevents a shift to the left of the normal position of the individual's oxygen dissociation curve but it does this by lowering the plasma pH of the recipient. Correction of the position of the curve to standard plasma pH and cell pH reveals that in fact a marked shift to the left does occur.

In our studies the amount of sodium chloride added to the stored citrated blood produced a final concentration of approximately 0.45%. Since the addition of sodium chloride to stored blood immediately before transfusion significantly improves the functional capacity of the stored red cell, as is shown by its effect on the oxygen dissociation curve, it is logical to expect that it will have a beneficial effect upon the survival of the transfused cells. An investigation to prove this is beyond the scope of the present communication but it may be noted that we have observed that the addition of sodium chloride to stored citrated blood diminishes the degree of spontaneous haemolysis which occurs with long storage and that Gibson *et al.* (1947) found a slightly hypertonic solution of sodium chloride to be the best diluent to add to packed red cells before transfusion.

SUMMARY.

The abnormality of the oxygen dissociation curve of stored citrated blood and the undesirable shift to the left of the oxygen dissociation curve of the recipient of such blood are shown to be due to altered cell pH and altered electrolytic and osmotic relationships between the cells and plasma of the transfused blood.

The addition of sodium chloride to the stored citrated blood before transfusion, to give a final proportion of 0.4 to 0.5 per cent, corrects the abnormality of the oxygen dissociation curve. The manner of action of the sodium chloride is discussed.

TABLE 1.

In vitro results with fresh blood samples and acidified fresh blood samples.

Sample No.	HbO_2 capacity vol.%	CO content vol.%	(Total CO_2)o vol.%	(Total CO_2)s vol.%	$\triangle CO_2$(s−o) vol.%	Blood * pHs	Blood pHc	PO_2 for Hb = HbO_2		
								Blood pHs and pHc	pHs = 7.4	pHc = 7.22
	A	B	C	D	E	F	G	H	I	J
Fresh Blood										
1	21	0.5	52	62.5	10.5	7.43	7.26	25	26	26
2	18.5	0.7	48	56.5	8.5	7.39	7.22	27	27	27
3	20	0.5	42	50	8	7.33	7.17	28	25.5	26
4	20	0.25	48	58.5	10.5	7.40	7.23	26	26	26
5	18	0.5	47	56.5	9.5	7.38	7.21	26	25.5	26
6	19	0.5	50	61	11	7.42	7.24	26	26.5	25.5
Mean	19.5	0.5	48	57.5	9.5	7.39	7.21	26.5	26	26
Effects of Dilution and Haemolysis										
7	15	0.5	53	61	8	7.42	7.23	25.5	26	25.5
8	14	0.75	47.5	59	7.5	7.38	7.16	25.5	25	25
9	15	0.25	55	62	7	7.43	7.25	25	26	26
Mean	14.7	0.5	51.5	59	7	7.41	7.215	25.5	26	25.5

TABLE I (cont'd.)

Sample No.	HbO_2 capacity vol.%	CO content vol.%	(Total CO_2)o vol.%	(Total CO_2)s vol.%	$\triangle CO_2$(s−o)	Blood * pHs	Blood pHc	PO_2 for Hb=HbO_2		
								Blood pHs and pHc	pHs=7.4	pHc=7.22
	A	B	C	D	E	F	G	H	I	J
Acidified Fresh Blood										
1	19	0.7	14.5	16.2	1.7	6.8	6.72	50	26	26.5
2	18	0.3	24	28	4	7.07	6.94	37	26	26
3	16	0.3	19	21	2	6.93	6.83	41	25	25.5
4	20	0.5	30.5	37	6.5	7.20	7.06	32	26.5	26.5
5	17	0.3	25	30	5	7.10	6.96	36	26	26
6	18	0.5	22.5	26	3.5	7.03	6.90	39	26	26
Partial Restoration of pH with Fresh Plasma										
7	18	0	44	52	8	7.35	7.18	27.5	26	25.5
8	19	0.2	35.5	41	5.5	7.25	7.10	30	26	26
9	16	0.2	37	43	6	7.28	7.12	30	26.5	26

Column C (Total CO_2)o = the total carbon dioxide of oxygenated blood at a CO_2 partial pressure of 40 mm.Hg.
Column D (Total CO_2)s = the total carbon dioxide of true plasma from oxygentated blood at a CO_2 partial pressure of 40 mm.Hg.
Column E $\triangle CO_2$(s−o) = the difference between (Total CO_2)s and (Total CO_2)o.
Columns H, I & J. PO_2 for Hb=HbO_2 = the partial pressure of oxygen at which oxyhaemoglobin-saturation is 50% at the plasma and cell pH of the recipient (Column H), at standard plasma pH (Column I) and at standard cell pH (Column J).
* Determined in true plasma from blood saturated with oxygen when PCO_2=40 mm.Hg.

TABLE 2.

In vitro results with blood samples stored in A.C.D. medium up to 24 hours.

Sample No.	Time of Storage	HbO_2 capacity vol.%	CO content vol.%	(Total CO_2)o vol.%	(Total CO_2)s vol.%	$\triangle CO_2$(s−o) vol.%	Blood pHs	Blood pHc	PO_2 for Hb = HbO_2		
									Blood pHs and pHc	pHs = 7.4	pHc = 7.22
		A	B	C	D	E	F	G	H	I	J
1	30 minutes	16	0.3	26	29	3	7.09	6.98	35	25.5	26
2	1 hour	17	0.5	24	26	2	7.03	6.94	38	25	27
3	16 hours	15.5	0.3	23	25	2	7.03	6.93	39	25.5	27
4	24 hours	14.5	0.7	25	26	1	7.03	6.97	37	25.5	26.5
5	4 hours	16.5	0.5	25	26	1	7.07	7	37	26	27.5
6	20 hours	17	0.7	21	22	1	6.94	6.88	40	25.5	26.5
7	22 hours	15	0.5	28	29	1	7.09	7.01	35	25.5	27
8	20 hours	17	0.7	22.5	23.5	1	6.97	6.90	39	25	26
Partial restoration of pH with fresh plasma											
9		16	0.1	35	40	5	7.24	7.10	30	25	26.5
10		15	0.1	38	41	3	7.25	7.14	31	26	28
11		16	0.2	34	37	3	7.20	7.09	30	24	26

TABLE 3.

In vitro results with blood samples stored in various anticoagulants for 7 days.

Sample No.	HbO_2 capacity vol.%	CO content vol.%	(Total CO_2)o vol.%	(Total CO_2)s vol.%	$\triangle CO_2$(s−o) vol.%	Blood pHs	Blood pHc	PO_2 for Hb = HbO_2		
								Blood pHs and pHc	pHs = 7.4	pHc = 7.22
	A	B	C	D	E	F	G	H	I	J
Blood stored in A.C.D. Medium										
1	15.5	0.5	17	15	−2	6.76	6.78	40	19.5	22.5
2	16	0.7	19.3	17	−2.3	6.81	6.85	38	20	23.5
3	15	0.5	17	15.5	−1.5	6.78	6.78	39	20	22
4	17	0.2	17.8	16	−1.2	6.78	6.80	38	19	22
5	16	0.5	20.5	18.3	−2.2	6.84	6.88	35	20	23.5
6	18	0.5	21	18.8	−2.2	6.85	6.90	36	20	24
7	14	0.2	15.5	13	−2.5	6.68	6.74	42	19.5	23
Partial restoration of pH with fresh plasma										
8	15	0.5	28.5	30	1.5	7.1	7	29	21	22.5
9	16	0.7	39	41	2	7.25	7.14	25	21.5	22.5
10	15	0.5	30	31.2	1.2	7.12	7.02	28	20.5	22
11	17	0.7	43	45.5	2.5	7.29	7.17	24	21.5	22.5

[*continued overleaf.*

TABLE 3 (cont'd.)

Sample No.	HbO_2 capacity vol.%	CO content vol.%	(Total CO_2)o vol.%	(Total CO_2)s vol.%	$\triangle CO_2$(s−o) vol.%	Blood pHs	Blood pHc	PO_2 for $Hb = HbO_2$		
								Blood pHs and pHc	pHs = 7.4	pHc = 7.22
	A	B	C	D	E	F	G	H	I	J
Blood stored in Trisodium Citrate										
12	17	0.5	38	41	3	7.25	7.14	27	23	24
13	16	0.5	35	38	3	7.22	7.10	27.5	22.5	23.5
Blood stored in Heparin + Lactic Acid										
14	18	0.7	25	25.5	0.5	7.01	6.96	36	23.5	25
15	19	1	20.5	20.5	0	6.90	6.87	40	23	25
16	20	0.7	30	30.7	0.7	7.10	7.04	33	23.5	26
Partial restoration of pH with fresh plasma										
17	18	0.7	40.5	46.5	6	7.3	7.15	26.5	24	24
18	19	1	30	33	3	7.15	7.02	31	24	25
Blood stored in Heparin + Sodium Fluoride										
19	20	0.7	50	58.5	8.5	7.41	7.25	25	25.5	26
20	21	1	45.5	54.5	9	7.38	7.20	26	25.5	25.5
21	19.5	0.7	45.5	53 5	8	7.37	7.20	26	25	25.5

TABLE 4.

In vitro results with blood samples stored in A.C.D. medium for 20 days.

Sample No.	HbO_2 capacity vol.%	CO content vol.%	(Total CO_2)o vol.%	(Total CO_2)s vol.%	$\triangle CO_2$(s−o) vol.%	Blood pHs	Blood pHc	PO_2 for Hb = HbO_2		
								Blood pHs and pHc	pHs = 7.4	pHc = 7.22
	A	B	C	D	E	F	G	H	I	J
1	15.5	1	15	12.5	−2.5	6.65	6.73	40	18.3	21
2	16	0.7	14	12	−2	6.63	6.69	41	18.5	21.5
3	14.5	0.7	15	12.5	−2.5	6.64	6.73	41	18.3	21.5
4	17	0.5	13	11	−2	6.58	6.65	42.5	18	20
5	16.5	1	14.5	12.5	−2	6.65	6.71	41	19	21
6	15	0.7	14	12.5	−2.5	6.65	6.69	40	18.5	21
Partial restoration of pH with fresh plasma										
7	15	0.5	28	28.7	0.7	7.08	7	28	20	21
8	12.5	0.7	30	30.5	0.5	7.11	7.02	26	19	20.5
9	16	1	38	40	2	7.24	7.11	23	19.5	20.5
10	15	0.7	40	41.5	1.5	7.26	7.14	22	19.5	20.5
Plasma from 20 days stored citrated blood + fresh red cells										
11	17	0.3	21.5	23	1.5	6.97	6.87	41	26	26
12	18	0.5	20	21	1	6.93	6.82	42	25.5	25
Partial restoration of pH with fresh plasma										
13	16	0.3	35	40	5	7.24	7.10	30	25.5	26

TABLE 5.

In vitro results with blood samples stored in A.C.D. medium for 2 months.

Sample No.	HbO_2 vol.%	CO content vol.%	(Total CO_2)o vol.%	(Total CO_2)s vol.%	$\triangle CO_2$(s−o) vol.%	Blood pHs	Blood pHc	PO_2 for $Hb = HbO_2$					
								Blood pHs and pHc	pHs = 7.4	pHc = 7.22	Correction for CO		
											Blood pHs	pHs = 7.4	pHc = 7.22
	A	B	C	D	E	F	G	H	I	J	K	L	M
1	14	2	11	9.5	−1.5	6.5	6.57	41	15	18.5	47	17.5	20.5
2	15.5	2	12	9	−3	6.47	6.59	40	15.5	18	46	18	20.5
3	14.5	2.5	11	8.5	−2.5	6.42	6.57	40	16	18.5	48	17.5	21

Columns K, L & M. PO_2 for $Hb = HbO_2$ = the partial pressure of oxygen at which oxyhaemoglobin-saturation is 50% after correction for carbon monoxide at the plasma and cell pH of the recipient (Column K), at standard plasma pH (Column L) and standard cell pH (Column M).

TABLE 6.

Effect of addition of different electrolytes *in vitro*.

Sample No.		Packed cell volume	HbO_2 capacity vol.%	(Total CO_2)o vol.%	(Total CO_2)s vol.%	$\triangle CO_2$(s−o) vol.%	Blood pHs	Blood pHc	PO_2 for $Hb = HbO_2$		
									Blood pHs and pHc	pHs = 7.4	pHc = 7.22
		A	B	C	D	E	F	G	H	I	J
1	Blood stored for 20 days	41	15.5	15	12.5	−2.5	6.65	6.73	40	18.3	21
	Restoration of pH with fresh plasma		15	40	41.5	1.5	7.26	7.14	22	19.5	20.5
	Addition of M/Disodium Phosphate (until pH = 7.3)		13	38	40.5	2.5	7.25	7.12	25	21	22
	Addition of M/Disodium Phosphate (until pH = 7.4)		12.5	54	58	4	7.4	7.26	22	22	
	Addition of M/Phosphate Buffer pH = 6.6	32	14	13	12.5	−0.5	6.65	6.65	47	21	22
	Addition of M/NaCl	33	14	12	12.5	0.5	6.65	6.62	55	25	25
2	Blood stored for 20 days	46	17	13	11	−2	6.58	6.65	42.5	18	20
	Addition of Phosphate Buffer pH = 6.6	36	15	12	12	0	6.63	6.62	49	21.5	22.5
	Addition of M/NaCl	34	15	11	12	1	6.63	6.58	57	25	25.5
3	Blood stored for 20 days	40	14.5	15	12.5	−2.5	6.64	6.73	41	18	20
	Addition of Phosphate Buffer pH = 6.6	31	13	14	13	−1	6.65	6.65	49	22	23
	Addition of M/NaCl	32	13	11.5	12	0.5	6.63	6.60	57	25	25

TABLE 7.

Chloride content in mg.% of whole citrated blood and true plasma before and after addition of sodium chloride.

Whole Blood	True Plasma
Before sodium chloride	
328	294
325	289
After sodium chloride	
780	877
791	877

The effect of transfusion of stored citrated blood on the oxygen dissociation curve of the recipient and the effect of sodium chloride and ammonium chloride.

Case No.	Transfused patient	Time of estimation in relation to transfusion	Amount of transfused blood	Time of storage of transfused blood	P.C.V.	HbO_2 capacity vol.%	CO content vol.%	(Total CO_2)o vol.%	(Total CO_2)o of concentrated blood	(Total CO_2)s vol.%	$\triangle CO_2$(s−Con) vol.%	Blood pHs	Blood pHc	PO_2 for Hb=HbO_2		
														Blood pHs and pHc	pHs=7.4	pHc=7.22
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	Male aged 68	Immediately before			30	14	0.5	53		61		7.42		29	30	
		Immediately after	Plasma 600 c.c.	20 days	29	13.5	0.5	54		62		7.44		29	30.5	
2	Male aged 73	Immediately before				9	0.2	48.5	41	56	15	7.39	7.15	32	31.5	28.5
		Immediately after	2 pints	20 hours		10.5	0.2	50	43	55	12	7.38	7.18	30.5	30	28.5
3	Male aged 16	Immediately before			20	9	0.2	48.5	43	53	10	7.37	7.17	28	27	26
		25 minutes after	2 pints	7 days	28	12	0.2	52	49	56	7	7.39	7.25	23	22.5	24
		24 hours after	,,	,,	28	12.5	0.5	47	43	53	10	7.36	7.18	27.5	26	26
4	Female aged 30	Immediately before			10.5	6	0	50	41	57	16	7.4	7.14	32	32	28.5
		Immediately after	2 pints	7 days	19	9.5	0.3	54	49	58	9	7.41	7.25	24	24	26.5
		6 hours after	,,	,,	20.5	10	0.3	52	46	56	10	7.39	7.20	28	28	27
5	Male aged 68	Immediately before			21	8	0.5	51	42	57.5	15.5	7.4	7.16	29	29	27
		Immediately after	2 pints	14 days	27	11	0.7	53	48	56	8	7.39	7.24	24	24	25
		24 hours after	,,	,,		11	0.5	52	46	59	13	7.41	7.20	28	28.5	27

[*continued overleaf.*

TABLE 8 (cont'd.)

Case No.	Transfused patient	Time of estimation in relation to transfusion	Amount of transfused blood	Time of storage of transfused blood	P.C.V.	HbO_2 capacity vol.%	CO content vol%.	(Total CO_2)o vol.%	(Total CO_2)o of concentrated blood	(Total CO_2)s vol.%	$\triangle CO_2$(s−Con) vol.%	Blood pHs	Blood pHc	PO_2 for Hb = HbO_2		
														Blood pHs and pHc	pHs = 7.4	pHc = 7.22
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
6	Female aged 53	Immediately before			18	7	0.2	53	40	62	22	7.43	7.12	33	34	28.5
		Immediately after	3 pints	7-14 days	27	11	0.7	56	49	60	11	7.41	7.25	24	24.5	25
		4 hours after	,,	,,	27.5	11.5	0.5	55	47	60	13	7.41	7.22	26	26.5	26
		24 hours after	,,	,,	27	11	0.5	51	43	58	15	7.4	7.18	30	30	27.5
		Effects of addition M/Solution of NaCl in a proportion 10% to the transfused blood														
7	Female aged 56	Immediately before			22	10	0.2	52	42	59	17	7.41	7.16	29	29	26
		Immediately after	2 pints	14 days	28	13.5	0.5	49	43	55	12	7.37	7.17	28	27	26
8	Female aged 53	Immediately before			16	7	0.5	55	43	59.5	16.5	7.42	7.17	30	31	28
		Immediately after	2 pints	15 days	21	10	0.7	50	43	57	11	7.39	7.17	29	29	27
		Effects of administration of NH_4Cl														
9	Male aged 16	Before NH_4Cl				8.5	0	54	47.5	58.5	11	7.41	7.23	26	26	26
		Immediately before transf.			19	8		42	41	46	5	7.30	7.15	30	27	27
		Immediately after	2 pints	14 days	16	11	0.5	46	44	50	6	7.34	7.20	25	23	23

Column J: $\triangle CO_2$(s−Con) = Difference between the (total CO_2) of plasma and of concentrated oxygenated blood at CO_2 partial pressure 40 mm.Hg.

ACKNOWLEDGEMENTS.

We wish to express our thanks to Professor L. J. Davis for his helpful advice and criticism throughout this study, to Dr. J. Wallace of the West of Scotland Blood Transfusion Service for his co-operation, and to Dr. J. C. Eaton who performed the chloride estimations. One of us (D. J. V.) was enabled to undertake this work by scholarships from the E. C. A. for Greece and from the University of Thessaloniki. Much of the cost of apparatus and materials was provided by the Rankin Fund of the University of Glasgow.

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