Blood volume and haemodilution in extracorporeal circulation: changes during perfusion

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An adequate circulating blood volume is recognized to be important after open-heart surgery (Carr, Sloan, and Tovar, 1960). A number of post-operative studies have attempted to correlate clinical determinations of blood loss with volumes estimated by tracer techniques. Discrepancies noted have been attributed to extensive haemorrhage into traumatized tissue (Flanagan, Steinmetz, Crawford, and Merendino, 1964), internal redistribution of functional extracellular fluid by plasma exudation occurring after any form of major surgery (Berger, Boyd, and Marcus, 1964), or to the homologous blood syndrome, in which there is a shift of blood from the extracorporeal circuit into the patient during perfusion and a measurable blood deficit post-operatively (Gadboys, Slonim, and Litwak, 1962; Litwak, Slonim, Wisoff, and Gadboys, 1963). One investigation showed no discrepancy in the volumes estimated by clinical and isotope methods (Theye and Kirklin, 1963). The use of haemodilution and plasma expanders during and after cardiopulmonary bypass introduces new factors in blood volume change (Ankeney, Renner, Leverett, and Beheler, 1965) which call for measurement. The advent of a rapid, semi-automatic system (Williams and Fine, 1961) has permitted more accurate control of replacement therapy (Berger *et al.*, 1964; Flanagan *et al.*, 1964); whether the method is reliable in the presence of dextran or could be used during perfusion has not been previously determined.

An attempt has been made to evaluate these issues. It has proved possible to provide during perfusion sufficiently stable conditions of flow rate, blood loss, and distribution of blood between the patient and the extracorporeal circuit to carry

out studies of red cell and plasma volumes during bypass, particularly with low molecular weight dextran (LMWD) haemodilution. The post-operative blood volume studies on some of these patients will be reported separately.

PROTOCOL

The effect of LMWD on standard radioactive methods of red cell and plasma determinations was established in a group of patients who were recovering from thoracic surgery not involving bypass. These also served as a comparative control series for post-operative observations. All patients who underwent isotope investigations gave personal consent during the experimental period.

Changes of blood volumes during standard perfusion were studied using haemodilution with LMWD, and these were compared with the effects of perfusion using 5% dextrose solution in part, or whole blood for perfusion.

METHODS

GROUP 1 Six patients who were undergoing thoracic surgery without perfusion had initial red cell and plasma volume determinations made over 30 to 65 minutes immediately following the induction of anaesthesia. Blood loss and replacement during surgery and recovery was on a volume-for-volume basis with additional transfusion based on the clinical estimation of loss on drapes and weighed swabs. When the post-operative loss observed by pleural drainage was small, some two to four hours after return to the ward, and when the patient was considered to have been in a satisfactory blood balance state for at least two hours, 500 ml. 10% LMWD² in 0.9% sodium

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²Rheomacrodex, Pharmacia Ltd., low molecular weight dextran (Dextran 40), a.v. molecular weight 40,000

chloride was transfused over 60 to 90 minutes. Any small continuing chest drainage during infusion was matched by donor blood.

After the measurement of sample background radioactivity, a second simultaneous double-isotope injection study was carried out on completion of the LMWD infusion, and equilibration studies were made up to 140 minutes. Isotope injection was through a wide-bore indwelling needle used for transfusion; samples were withdrawn from a catheter placed in the contralateral antecubital vein.

GROUP II Plasma and red cell equilibration curves and volume changes were investigated before and during cardiopulmonary bypass in 16 patients. Initial observations were begun on a larger and unselected series of patients submitted to cardiac surgery. Clinical exigencies, unbalanced blood losses associated with cannulation or cardiectomy or occurring during blood volume studies, or obligatory changes in perfusion, necessitating exclusion from the series, resulted in a marked bias of patients with the simpler cardiac problems. First estimations followed sampling for background radioactivity and the induction of anaesthesia before thoracotomy. Isotopes were injected intravenously through a fine polyvinyl chloride catheter used for monitoring, which was then thoroughly flushed, and samples were withdrawn from a brachial artery catheter after first clearing it, usually at 10-minute intervals from 10 minutes after isotope injection. Blood loss until perfusion was measured (swab weighing and suction); any intravenous administration was restricted to dextrose 5% whenever possible (shown as 'clear fluid' in the tables). Necessary blood replacement was noted, and its red cell and plasma components were calculated after correction of a sample haematocrit for trapped plasma (Chaplin and Mollison, 1952). In three patients there was measurable bleeding after the start of perfusion and before the second blood volume studies. This also was not replaced, therapy being confined to an intentional shift of the perfusate from the machine to the patient to compensate. All losses were calculated in terms of red cell and plasma portions by correction of a sample haematocrit for trapped plasma. During perfusion all blood appearing in the cardiectomy site was rapidly returned to the heart-lung circuit to avoid mechanical sequestration. Severe blood loss before perfusion (except in case 14, in whom it was over a prolonged period and was apparently well balanced by transfusion as it occurred), un-

measurable loss on to drapes of more than a very small amount, or bleeding during the period of blood volume study called for elimination from the series. The duration of perfusion of some of the simpler cases was lengthened slightly to allow completion of the studies.

Perfusion was by means of cardiopulmonary bypass using rotating disc¹ or disposable bubble² oxygenators; the type used was randomly distributed over the patients left in the series and appeared insignificant in the study. A heat exchanger in circuit ensured normothermia, and the priming volume of the circuit ranged from 2,000 to 4,000 ml.; this was not related to the patient's weight but to the requirements of the circuit. Priming volumes, including any residual volume of solution used in preliminary calibration, and solutes used to carry medication were accurately measured. A sample haematocrit, corrected for trapped plasma, was used to determine the constituent red cell and plasma volumes. Partial bypass was commenced at about half the ultimate flow, which was in excess of 2.4 l./m.²/min., and reached in about 60 to 90 seconds. Full flow rate, once achieved, was not altered during the period of study.

From recorded losses before a second blood volume determination, blood replacement, and priming volume measurements, the 'anticipated volume' of the combined patient-extracorporeal circulation was calculated on a simple addition-subtraction basis.

PRIMING In all systems there was a measured residuum of 5% dextrose after pump calibration (200 to 400 ml.) and also the water content of heparin, calcium chloride, and 8.4% sodium bicarbonate (50 to 60 mEq). The remainder of the priming volume was made up as follows:

Group II A: 20 ml./kg. 10% Rheomacrodex in 0.9% sodium chloride, the remaining necessary prime being 1,500 to 2,000 ml. cross-matched, 48-hour-old blood containing 30 ml. Edgludate per 500 ml.

Group II B: 20 ml./kg. 5% dextrose solution, the remainder being blood as in group II A.

Group II C: 48-hour-old blood with Edgludate preservative.

About 4 minutes after the start of perfusion, 2 to 3 minutes' total cardiac bypass, a sample was taken for background radioactivity. Radioactive chromium-labelled erythrocytes and iodine-labelled plasma were then simultaneously injected

¹Osborn-Bramson-Gerbode or Melrose models
²Rygg

into the venous line of the heart-lung machine, and samples were taken from the arterial line of the circuit at 5-minute intervals from 5 to 25 minutes. Since radioactivity from the first estimation was markedly diluted by mixing of the extracorporeal fluid, it was not necessary greatly to increase radioactive dosages for this estimation. Post-operative studies were made on the same patients when possible and will be subsequently reported.

In group II A (Rheomacrodex), serum LMWD concentrations were measured in eight patients and serum proteins in three patients.

In eight patients, not otherwise part of this study, variations of (peripheral) haematocrit of simultaneous samples taken from available sites during perfusion were compared (monitor lines in superior and inferior venae cavae, brachial artery, and the venous and arterial lines of the heart-lung machine) to exclude significant effects of the sampling site.

Red cell and plasma volume estimations were made from the simultaneous injection of ^{51}Cr -labelled erythrocytes and ^{131}I -labelled albumin. Red cells were tagged by the method of Mollison and Veall (1955). The patient's blood, 15 to 20 ml. in 5 to 10 ml. ACD solution, was centrifuged, the plasma removed, and radioactive sodium chromate added to the cells, which were then incubated at room temperature for 20 minutes with gentle agitation every 5 minutes. They were then washed three times in saline before final suspension in 12 to 13 ml. saline. For estimations, 10 ml. prepared cells were injected, and a standard for comparison was made by the addition of either 1 or 2 ml. of the same cells to saline to make a volume of 100 ml. It was established during preliminary work that no free plasma radioactivity was present following this method of tagging. A small quantity of plasma chromium might be anticipated from haemolysis due to perfusion trauma, but during brief perfusions, using only tagged red cells, plasma radioactivity produced only insignificant counts, the effects of which were ignored during calculations.

^{131}I -labelled human serum albumin (RISA) was diluted to approximately 0.5 to 1 $\mu\text{c.}/\text{ml.}$ A standard was prepared for a similar volume for comparison. A few drops of concentrated Edgludate were used as anticoagulant for blood samples.

The radioactivity of samples in group I was measured in an Ekco scintillation counter over 100 seconds, and in group II by means of a radiation analyser, scaler, and automatic sample-

changing device¹, for the time necessary to reach 10,000 counts. The latter were re-expressed in terms of counts/minute for samples of similar volume. Samples were measured in replicate.

Plasma volumes were estimated from the dilution factor of RISA at 'zero time', calculated by extrapolation of the plotted radioactive counts of serial samples. Iodinated plasma disappearance rates were calculated from the slope of the plotted line. Red cell equilibration curves were also plotted. Red cell mass counts were derived by subtraction of a plasma count (^{131}I) from a whole blood count ($^{131}\text{I} + ^{51}\text{Cr}$) of the same source, using volumetric correction for the sample haematocrit and for trapped plasma. Red cell volumes were determined by calculating the dilution of the mean red cell activity data. In one patient, case 11, it was necessary to calculate the red cell volume from a single 10-minute sample.

Radioactivity counts were re-charted after subtraction of background activity. For illustration, activity has been reduced by a denominator to place all curves on a common chart, and the necessary multiplicand of the ordinate is given for each sample in Table I; this observation is neces-

TABLE I
RADIOACTIVE COUNTS

Group	Curve	Multiplication Factor
I	^{131}I	Pre-infusion: cases 1 and 2×5 ; cases 3, 5, and 6×10 ; case 4×25 Post-infusion: cases 1 and 2×5 ; cases 3, 5, and 6×10 ; case 4×50
	^{51}Cr	Pre-infusion: cases 1, 2, 4, 5, and 6×5 Post-infusion: cases 1, 2, 3, 5, and 6×5 ; case 4×10
II A	^{131}I	Pre-perfusion: case 7×0.5 ; cases 1 and 2×5 Perfusion: cases 7 and 12×0.5 ; case 4×2.5 ; cases 1, 2, 5, and 6×5
	^{51}Cr	Pre-perfusion: cases 4, 5, and 6×0.5 Perfusion: cases 9 and 10×0.5 ; case 1×5 ; case 2×10
II B and C	^{131}I	Pre-perfusion: cases 13, 14, 15, and 16×0.5 ; case 8×2.5 ; case 3×5 Perfusion: case 14×0.5 ; case 15×1.5 ; cases 3 and 8×2.5
	^{51}Cr	Pre-perfusion: cases 3, 8, and 13×0.5 ; case 15×2.5 ; case 14×5 Perfusion: case 13×0.5 ; case 16×2 ; case 14×5

In order to bring all data on to common graphs, some have been scaled down or up. The radioactive count per minute should be multiplied by the factor shown for certain cases to obtain the count measured.

sary, since misconstruction may otherwise be placed on the curve variation in relation to admissible counting error; this is important when comparing this study with other authors' work on red cell equilibration.

¹Nuclear, Chicago

Haematocrits were measured in duplicate, having been centrifuged at 3,000 r.p.m. for 55 minutes at room temperature, and the mean value was used.

Serum dextran concentrations were measured by the method of Jacobsson and Hansen (1952). The clinical data of the patients studied are shown in Table II.

TABLE II
BLOOD VOLUME STUDIES IN EXTRACORPOREAL CIRCULATION

Case	Age	Diagnosis	Perfusion Time (min.)	Pump Prime
1 (B.M.)	12	V.S.D.	40	LMWD
2 (I.F.)	28	A.S.D.	24	LMWD
3 (M.H.)	12	A.S.D.; P.S.	24	Dextrose 5%
4 (D.W.)	49	Acquired V.S.D.	70	LMWD
5 (J.A.)	28	Aortic incompetence	120	LMWD
6 (J.W.)	19	A.S.D.	39	LMWD
7 (B.S.)	10	P.V.S.	26	LMWD
8 (S.O.)	18	A.S.D.	25	Dextrose 5%
9 (J.R.)	34	A.S.D.	29	LMWD
10 (J.F.)	6	A.S.D.	24	LMWD
11 (P.K.)	34	A.S.D.	24	LMWD
12 (S.C.)	7	Fallot	151	LMWD
13 (H.J.)	21	A.S.D.	24	Blood
14 (A.T.)	60	Mitral incompetence	58	Blood
15 (M.K.)	12	P.S.; corrected transposition	24	Blood
16 (B.B.)	16	V.S.D.	44	Blood

A.S.D. = atrial septal defect; V.S.D. = ventricular septal defect; P.S. = pulmonary stenosis.

RESULTS

GROUP I Red cell equilibration curves (Fig. 1) establish the acceptability of the method in the presence of dextran and during post-operative recovery. Equilibration is complete within 10 minutes, and there is no reliable evidence to suggest the presence of a slowly circulating red cell mass. LMWD post-operatively may smooth the equilibration curve of red cells.

^{131}I curves (Fig. 2) show features which are seen also in groups studied later. There is a considerable variation of the labelled plasma disappearance rate under anaesthetic conditions (first part of Fig. 2) and similarly in the perfusion cases during the initial studies (first curves in Figs 3 and 4). Excluding two perfusion cases (cases 4 and 8), the initial disappearance rate in the remaining 20 patients ranged from 0 to 19.5% per hour.

After infusion of LMWD the iodine disappearance slope was decreased, flattened, or even reversed. A strict comparison with group II is not possible, since infusion in group I was comparatively slow, and urinary excretion after operation was probably faster. The change of slope

FIG. 1. Labelled red cell equilibration after infusion of LMWD in the early post-thoracotomy patient. (Table I shows the multiplication factor to be applied to the ordinate scale for individual cases in all figures.)

FIG. 2. ^{131}I equilibration before and after infusion of LMWD after thoracotomy.

FIG. 3. Labelled plasma and red cell counts before and during perfusion using LMWD (group II A).

FIG. 4. Plasma and red cell curves before and during perfusion using 5% dextrose in prime (group II B) or whole blood (group II C).

may reflect integration between the physiological disappearance of albumin from the vascular compartment, a downward component, and shrinkage of the plasma volume as the intravascular dextran concentration falls due to absorption and urinary excretion, an upward component.

Volume changes in the post-operative state (Table III) show the plasma-expanding effect of LMWD. During dextran infusion and blood sampling from case 3 there was intrapleural haemorrhage concealed by a blocked drainage tube, shown on a subsequent radiograph, which was not compensated by blood transfusion. The unusual plasma increase, as well as the fall in the

red cell mass, reflect a physiological adjustment to haemorrhage as well as plasma expansion by dextran. In case 6 the development of a surgical haematoma in the neck may have disturbed the expected findings.

Table IV sets out the peripheral haematocrits and whole body haematocrits

$$\left(\frac{\text{measured red cell volume} \times 100}{\text{measured red cell} + \text{plasma volumes}} \right)$$

The 'correction factor', by which the peripheral haematocrit varies from the measured whole body haematocrit and for which correction must be made when calculating whole blood volumes and separate red cell and plasma volumes from a single isotope sampling method, is within the

TABLE III

PLASMA, RED CELL, AND TOTAL BLOOD VOLUME CHANGES FOLLOWING LMWD INFUSION IMMEDIATELY AFTER THORACIC SURGERY

Case No.	Blood Volume (ml.)											
	Early Operation				Post-operative (after 500 ml. LMWD infusion)				Change			
	Plasma	Red Cell	Total	P.C.V. %	Plasma	Red Cell	Total	P.C.V. %	Plasma	Red Cell	Total	
1	2,361	1,223	3,584	42	3,034	1,250	4,284	36	+673	+27	+700	
2	2,654	1,289	3,943	31.5	3,144	1,309	4,453	26.5	+490	+20	+510	
3	2,061	1,951	4,012	48.5	2,910	1,672	4,582	43	+849	-279	+570	
4	1,857	1,195	3,052	42.5	2,254	1,156	3,410	34	+507	-31	+476	
5	2,796	1,975	4,771	44.5	3,430	2,063	5,493	42	+634	+88	+722	
6	2,988	1,132	4,120	32.5	3,474	1,010	4,484	30	+486	-122	+364	

P.C.V. % = corrected peripheral haematocrit.

TABLE IV
PERIPHERAL AND WHOLE BODY HAEMATOCRITS AFTER
LMWD INFUSION: GROUP I

Case	Before LMWD Infusion			After LMWD Infusion		
	P.C.V.	W. Hcrit	Correc- tion Factor	P.C.V.	W. Hcrit	Correc- tion Factor
1	42	34	0.8	36	29	0.8
2	31.5	32.7	1.04	26.5	29.4	1.1
3	48.5	48.6	1.0	43	36.4	0.85
4	42.5	39	0.91	34	34	1.0
5	44.5	41.4	0.93	42	37.5	0.9
6	32.5	27.5	0.84	30	22.5	0.75

P.C.V. = peripheral haematocrit; W. Hcrit = whole body haema-
tocrit.

normal range (0.91 is commonly used as a factor). There is little change in the ratio after Rheoma-crodex infusion.

Table V shows haematocrits of simultaneous samples from a number of sites available during perfusion in a different series of patients. Samples for blood volume studies are more easily taken from the extracorporeal sites, since it is undesirable to interrupt the continuous use of intra-vascular catheters for monitoring. There is no evidence that sampling from extracorporeal, rather than intravascular, sites during perfusion will significantly affect the volumetric calculations.

GROUP II Labelled plasma and red cell equilibra-
tion curves are shown before and during perfusion
in cases where LMWD was used (group II A,
Fig. 3) and where 5% dextrose was used (group
II B) or whole-blood priming (group II C, Fig. 4).

Red cell equilibration appears acceptable and

complete within 10 minutes in all groups before
perfusion. The method of injection during per-
fusion, into the circuit venous line, provides
conditions of early and thorough mixing within the
oxygenator, and earlier observations had suggested
that when partial heart-lung bypass at high flow
rates is established for 2 minutes, red cell mixing
is complete (Raison, 1963). This rapidity of mixing
during perfusion has been borne out by the speed
of stabilization of haematocrit during haemo-
dilution perfusion (Raison, Noronha, and Skuse,
in preparation). Although the appearances of red
cell equilibration during bypass suggest less
thorough early mixing than in the preliminary
studies, the abscissa scale and division of the
ordinate exaggerate the difference between
samples. On analysis, only two results, the radio-
active values at 25 minutes from case 5 and the
10-minute sample from case 6, differ from their
neighbours by more than the normal counting
variation of identical samples. They have been
rejected in the volumetric calculations.

Red cell equilibration curves during perfusion
appear similar whether or not a diluent is used
and suggest stabilization of mixing within 5
minutes. It might be argued that the findings in,
say, case 12 (group II A, Fig. 3) indicate incom-
plete mixing until the 10-minute sample. The
mean radioactive count at 5 minutes was 480/
minute and at 10 and 15 minutes 460/minute. The
'counting variation' around each value is 21-22,
making it impossible to deduce that there is a
significant difference, even ignoring other potential
errors of technique. For case 3 (group II B,

TABLE V
HAEMATOCRITS FROM AVAILABLE SITES DURING PERFUSION

Case	Pre-perfusion			Perfusion					Post-perfusion		
	S.V.C.	I.V.C.	Brachial Artery	S.V.C.	I.V.C.	Brachial Artery	Pump Venous Line	Pump Arterial Line	S.V.C.	I.V.C.	Brachial Artery
A	36.5	—	37.5	26.5	—	25	—	—	27.5	26.5	—
	37	—	—	25.5	—	25	—	—	28	27	—
	35	—	35.5	—	—	—	—	—	—	—	—
B	41	—	42	26.5	—	—	—	27.5	25	—	26
	—	39.5	40	26.5	—	—	—	26	—	—	—
	39.5	—	39	26	—	—	—	26	—	—	—
C	40	—	39	27	—	26.5	—	27.5	30	—	32
	41.5	—	39.5	26	—	26.5	—	26	—	—	—
	—	—	—	27	—	28	—	27.5	—	—	—
D	39	40	39	36	—	35	—	36	36	36.5	36.5
	40	—	40	35.5	—	36	—	36	—	—	—
E	36.5	37	36.5	28	—	28.5	—	28.5	34	—	33
F	—	—	—	—	28	30	30	31	—	—	—
G	—	—	—	—	28	27	28	27	—	37.5	38.5
H	37	36	—	—	29.5	29.5	—	29.5	—	—	—

I.V.C. = inferior vena cava; S.V.C. = superior vena cava.

dextrose 5% prime, Fig. 4) observations do lie outside the range of counting variation alone. The mean radioactive counts at 5, 10, and 15 minutes were 1,460, 1,400, and 1,380 respectively, a range where the variation might be 37–38. With serial samples taken under the restrictive conditions of clinical demands, it is not reliable to regard differences as small as these as significant. If the isolated findings at each of these times were taken for separate estimations of red cell volume, they would indicate values of 1,680, 1,750, and 1,780 ml. respectively. In the circumstances of perfusion it has not been thought reliable to calculate variations of this magnitude, and a mean count of 1,420/minute has been used. The possible variations of red cell volume which could be obtained from individual sample consideration are well spread in the series and would not affect the overall differences of volume change in the groups which are shown later.

The flattened ^{131}I disappearance slope during perfusion is common to all patients whether or not there is haemodilution. It has proved impossible to draw by eye a line from which decline could be calculated.

The measured components of the patients' blood volumes before perfusion are shown in the first columns of Table VI, which also sets out

any losses measured before bypass and any replacements. The measured pump-priming volumes, their haematocrits, and any losses during perfusion before the second blood volume determination are also shown.

Clinically, an attempt was made in each case to maintain proper partition of the mixed volume between the patient and the heart-lung machine, securing unchanged venous pressure from that before bypass, with an acceptable arterial pressure. This is commonly thought to be the best means of maintaining an unchanged blood volume within the patient. In group II A this resulted in no shift of blood from the oxygenator, or a small apparent gain (cases 5, 11, and 12); the influence of addition to the oxygenator from temporarily emptied heart cavities was excluded. In groups II B and II C there was an observed loss of perfusate from the machine into the patient, more rapid in group II C (whole blood). Where loss of blood occurred during perfusion it was compensated by allowing a lesser volume in the machine rather than in the patient.

From these data, together with corrections for volumes withdrawn for sampling and water injected, it was possible to calculate the 'anticipated volumes' of red cells, plasma, and blood shown in Table VII, together with the volumes

TABLE VI
MEASURED VOLUMES INFLUENCING PERFUSION

Case	Pre-perfusion				Pre-perfusion				Perfusion	
	Blood Volume (ml.)			Corrected Hcrit	Blood Loss (ml.)	Blood Replacement (ml.)	Pump Hcrit	Pump Volume (ml.)	Perfusion Hcrit	Perfusion Loss before B.V. Measurement (ml.)
	Plasma	Red Cell	Total							
Group II A										
1	2,721	1,395	4,116	37	245	500 c	22.5	3,075	27.5	165
2	3,314	1,925	5,239	39	225	125 c	18	3,306	27.5	
4	3,052	1,719	4,771	45	223	250 c	23	4,000	34.5	
5	2,341	1,537	3,878	37.5	222	100 c	16.5	3,500	25.5	150
6	2,054	1,092	3,146	37	314	200	28.5	3,400	29.5	
7	1,579	609	2,188	34	90	100 c				
9	2,398	1,570	3,968	39.5	125	60 c	23	2,534	25	52
						50 c	24.5	3,514	30	
10	975	511	1,486	37	290	200	24.5	2,008	26.5	
11	2,113	990	3,103	36	100	100 c	24.5	3,514	27.5	
12	1,423	1,220	2,643	53.5	80	50 c	25.5	2,371	35.5	
Group II B										
3	1,190	933	2,123	40.5	94	70 c	28	3,463	31	
8	2,770	1,296	4,066	36.5	90	100	19.5	3,077	28.5	
						300 c				
Group II C										
13	2,253	1,578	3,831	45	80	110 c	28.5	3,395	38	
14	2,860	1,177	4,037	35.5	545	100 c	33	3,590	34	
						700				
15	1,337	810	2,147	40.5	70	50 c	31.5	3,060	38.5	
						50				
16	2,382	1,156	3,538	37.5	85	300 c	35	3,783	36	

c = clear fluid.

TABLE VII
VOLUME CHANGES OBSERVED IN PERFUSION

Case	Anticipated Volume (ml.)			Measured Volume (ml.)			Change		
	Plasma	Red Cell	T.V.	Plasma	Red Cell	T.V.	Plasma	Red Cell	T.V.
Group II A									
1	5,331	1,950	7,281	5,696	1,974	7,670	+365	+24	+389
2	6,013	2,432	8,445	6,312	2,396	8,708	+299	-36	+263
4	6,259	2,539	8,798	6,346	2,759	9,105	+87	+220	+307
5	5,111	1,995	7,106	6,384	2,006	8,390	+1,273	+11	+1,284
6	4,508	2,024	6,532	5,186	2,047	7,233	+678	+23	+701
7	3,492	1,148	4,640	3,613	1,165	4,778	+121	+17	+138
9	5,146	2,461	7,607	5,305	2,437	7,742	+159	-24	+135
10	2,508	896	3,404	2,606	919	3,525	+98	+23	+121
11	4,762	1,855	6,617	5,260	1,983	7,243	+498	+128	+626
12	3,202	1,782	4,984	4,100	1,880	5,990	+908	+98	+1,006
Group II B									
3	3,753	1,908	5,661	3,719	1,728	5,447	-34	-180	-214
8	5,489	1,864	7,353	5,417	1,718	7,135	-72	-146	-218
Group II C									
13	4,747	2,509	7,256	3,897	2,407	6,304	-850	-102	-952
14	5,431	2,446	7,877	5,006	2,260	7,266	-425	-186	-611
					2,315	7,321		131	-556
15	3,471	1,766	5,237	3,241	1,616	4,857	-230	-150	-380
16	5,088	2,448	7,536	4,642	2,628	7,270	-446	+180	-266

T.V. = total circulatory volume.

measured and the discrepancies between them, shown as 'change'.

There is an unexplained chance occurrence of greater loss before bypass in the LMWD group. Neither this, haemorrhage during bypass before the second volume estimations, nor a slow, severe loss (545 ml.) contemporarily replaced (700 ml.) in case 14, appear to have influenced the overall results.

In spite of clinical limitations the results are amenable to statistical analysis. The 'anticipated volumes' of cases perfused with LMWD (group II A) were first compared with the combined groups II B and C (dextrose 5% and whole blood). The difference between the means for plasma volume is $4,633.2 - 4,663.2 = 30.0$ ml., which is negligible, whereas that between red cell volumes is $1,908.2 - 2,156.8 = 248.6$ ml., which, although not significant, is more than 10%. This can be accounted for largely by the different prime in groups II B and C. This analysis indicates that the two groups were initially comparable.

As there is considerable variation between individual readings, it is necessary to take the base line of each into account in calculation. Calculating the proportion measured/anticipated volume for each component in each patient yields highly significant differences.

Rheomacrodex priming produces a notable increase of plasma volume compared with a reduction when either of the other primes was used ($P < 0.001$). There is also a difference in the red cell volumes, there being a possible slight gain in the LMWD group and a definite loss in

the other two groups ($P < 0.01$). The resultant total blood volume increases in group II A and disappears in groups II B and II C are also significantly different ($P < 0.001$).

In spite of the small number of cases, the differences are similarly significant when comparing the plasma expansion of LMWD priming (II A) and plasma loss with whole blood (II C), and when comparing the preservation of red cell volumes in group II A with erythrocyte disappearance in group II C.

Comparing dextrose 5% (II B) with blood (II C) primes, there is a difference in the smaller plasma volume reduction of the former. It is not statistically significant, probably only due to the small number of cases, and is certainly very open to suspicion.

Cases 4 and 16 appear not to conform to the usual pattern. Case 4 came to operation in profound cardiac failure and body water derangement associated with intensive diuretic therapy, but there were no unusual clinical features in case 16. Only the red cell volume of case 4 could affect the analysis, and a further analysis after exclusion of this datum shows no alteration in significance. The inclusion of these two cases does not therefore affect the observations.

Serum concentrations of LMWD and proteins are shown for a number of cases in Table VIII. Although the volume of Rheomacrodex used is weight-related to each patient, the relatively fixed-volume demands of the extracorporeal prime tend to obliterate this ratio (Raison, 1962) and there is no apparent relation between the dextran con-

TABLE VIII
LOW MOLECULAR WEIGHT DEXTRAN IN CARDIOPULMONARY BYPASS

Group II A	Wt. (kg.)	Surface Area (m. ²)	Volume Rheomacrodex 10% Used (ml.)	Total Pump Vol. (ml.)	LMWD (g./kg.)	Perfusion Time (min.)	LMWD Concentrations (mg./100 ml.)	Albumin (g./100 ml.)	Globulin (g./100 ml.)	Total Protein (g./100 ml.)
Case 2	67.5	1.72	1,000	3,806	1.5	5	1,000			
						10	670			
						15	610			
						20	800			
4	54	1.66	500	3,350	0.93	5	545			
						10	520			
						15	645			
						20	575			
						25	575			
						30	580			
5	74	1.84	1,000	3,500	1.35	5	375			
						10				
						15	390			
						20				
6	49	1.45	500	3,400	1.02	5	980			
						10	830			
						15	830			
						20	740			
7	35	1.14	500	2,534	1.43	5	1,220			
						10	1,400			
						15	1,400			
						20	1,290			
9	58	1.6	1,000	3,514	1.73	5	2,640	2.35	2.22	4.57
						10	2,200	2.35	2.15	4.50
						15	2,100	2.35	2.22	4.57
						20	1,820	2.35	2.22	4.57
10	22	0.85	450	2,008	2.04	5	1,900	1.98	2.22	4.20
						10	1,600	2.23	1.97	4.20
						15	1,240	2.23	2.10	4.33
						20	2,280	2.18	1.72	3.90
11	52	1.58	500	3,514	1.97	5	2,200	2.18	2.07	4.25
						10	2,200	2.10	2.15	4.25
						15	2,380			

TABLE IX
PERIPHERAL: WHOLE BODY HAEMATOCRIT RATIO IN GROUP II

Case	Pre-perfusion			Perfusion		
	P.C.V.	W. Hcrit	Correc- tion Factor	P.C.V.	W. Hcrit	Correc- tion Factor
Group II A						
1	37	33.8	0.91	27.5	25.7	0.93
2	39	36.7	0.94	27.5	27.5	1.0
4	45	36	0.80	34.5	30.3	0.88
5	37.5	39.8	1.06	25.5	23.9	0.93
6	37	34.7	0.93	29.5	28.3	0.96
7	34	27.8	0.827	25	24.4	0.98
9	39.5	39.5	1.0	30	31.4	1.4
10	37	34.3	0.92	26.5	26	0.98
11	36	31.2	0.87	27.5	27.4	0.99
12	53.5	45.8	0.85	35.5	31.3	0.88
Group II B						
3	40.5	44	1.08	31	31.7	1.0
8	36.5	31.9	0.87	28.5	24	0.84
Group II C						
13	45	41.2	0.91	38	38	1.0
14	35.5	29	0.82	34	31	0.91
15	40.5	37.7	0.93	38.5	33.3	0.86
16	37.5	32.6	0.87	36	32.5	0.9

P.C.V. = corrected peripheral haematocrit; W. Hcrit = whole body haematocrit.

centration and either the pump priming volume or the combined circulating volume ('anticipated volume'). Some levels are below those regarded as minimal for effective prevention of micro-

circulatory red cell aggregation (1,200 to 2,000 mg./100 ml.) or those found by Long, Sanchez, Varco, and Lillehei (1961) during perfusion (1,400 to 1,600 mg./100 ml.). Nor are serum concentrations directly related to the plasma volume expansion found. They are moderately well maintained over the short periods investigated. Serum proteins, once diluted by LMWD, are stable. A number of subsequent observations have confirmed this. In the early period studied, there do not appear to be variations of the major oncotic influences during perfusion.

The haematocrit ratio changes are shown in Table IX. The pre-perfusion mean correction factor for all three groups is 0.91 (S.D. ± 0.054). During bypass, the mean correction factor for the combined groups is 0.96 (S.D. ± 0.55), that for group II A alone 0.99 (± 0.45), and for group II C (whole blood, the least haemodilution) 0.92 ± 0.34 . These figures are not significant, having regard to the small numbers and to the small variations of peripheral haematocrit found at different sites in perfusion (Table V).

DISCUSSION

It is possible to carry out blood volume measurements during perfusion; previous estimations have been based on changes in venous pressure

(Kaplan, Edwards, Helmsworth, and Clark, 1960). The notable clinical advantages of Rheomacrodex (Raison, 1965) determined that the investigation was principally to assess the effect of this haemodiluent. Since the study was of stable conditions during early perfusion, the major hazard to such estimations, that of considerable oozing and unmeasurable loss in drapes, was not of consequence in this series.

In two of the LMWD group, plasma expansion was unexpectedly large. Case 5 was an obese young woman with some unexplained ankle oedema. Cellular osmolality may be a factor in determining expansion. None of the volume determinations was available to the clinician; the expansion in case 12 might have been of practical significance. This was a small patient with cyanotic tetralogy of Fallot whose haematocrit fall during bypass was regarded as satisfactory. Venous pressure was maintained at its pre-perfusion value, and although it was appreciated that there was some increase of volume within the oxygenator, the retrospective clinical impression was that it was by no means as great as 1,000 ml. The patient was presumably obliged to accommodate a significant part of this expansion. Almost identical observations were made in another small patient with tetralogy of Fallot before the present studies were started, and the findings were then regarded as chance, being then isolated. 10% Rheomacrodex has initially a water-binding capacity of approximately its own volume on rapid infusion (Davies, Ricketts, and Williams, 1963). The plasma volume expansion from the patient on perfusion may be considerably less than twice the volume of LMWD used, if there is available water in the perfusate, whether or not this contains readily diffusible solutes, since the LMWD effect is on the oncotic rather than merely on the total osmotic characteristics of blood. An expansion greater than twice the LMWD used is, however, unusual and requires further investigation. If such expansion is a feature of polycythaemic blood mixing with LMWD, it may be of considerable importance to be aware of the change, since one of the factors which influences the clinician's assessment of blood balance at the end of perfusion, when drape loss may be large and unreliably estimated, is the volume of blood remaining in the extracorporeal circuit. Both cases of tetralogy died within 18 hours, of myocardial failure, and inadvertent early overtransfusion may have been an unrecognized factor. Evidence from this study suggests that the semi-automatic method of blood volume measurement during perfusion would be

reliable and may have clinical importance, perhaps in small children when the oxygenator volume is comparatively large.

Clinical determinations are most easily performed using RISA. Opinion favours extrapolation of the radioactive count slope to a theoretical zero, but methods avoiding repeated sampling have been devised. A ^{131}I disappearance rate of 10% per hour (Pritchard, Moir, MacIntyre, and Inkley, 1955) has been used by some, while others use 4% per hour, which Baker and Wycoff (1961) describe for the later part of the disappearance curve, noting more rapid falls in the first 15 to 20 minutes in normal conditions.

Swan, Montgomery, Jenkins, and Marchioro (1960) demonstrated a two-part curve, predictable under a given set of stable conditions. More recently, the usefulness and validity of serial determinations and extrapolation have been challenged with the use of a semi-automatic technique (Williams and Fine, 1961). Our results indicate that there may be considerable variation of the disappearance rate in anaesthetized and post-operative patients, although the differences due to this are small, below the level of clinical significance using a 10-minute sample. During cardiopulmonary bypass the ^{131}I disappearance rate is so slow that it produces an almost horizontal line which may enhance the accuracy of single sample estimations, since the computing system used does not include a slope factor. Since this flattening of the ^{131}I disappearance slope is common to all three perfusates studied, it does not seem that the previously assumed explanation for the flattening of the ^{131}I slope in the presence of LMWD—that diminution of plasma volume due to dextran leaving the vascular compartment reduces the otherwise anticipated rate of fall of ^{131}I radioactivity—can be a major factor in perfusion, especially since the serum dextran concentration does not seem to fall rapidly in the special conditions of total bypass. Although the complexly shifting inter-relationship between the two oncotic factors, protein and dextran, at the capillary membrane must be an influence on ^{131}I disappearance in the presence of dextran, this effect is masked during perfusion by another change, common to at least these three perfusates. It may be that, in spite of widely different and large plasma gains or losses observed with different perfusates, there is also a common factor, a slower plasma volume loss similar in all three groups, which almost exactly balances the otherwise-to-be-expected normal disappearance rate of radioactive albumin. Equally, and perhaps more plausibly, the mere institution of (almost non-

pulsatile) extracorporeal perfusion leads to a change in rate of albumin transudation from the vascular compartment, or to a reduction in the capillary area through which such escape occurs. The full extent of a change in rate of protein transfer can be masked if there is a compensatory change in the rate of total protein transfer through the lymphatics during perfusion, and there need be no change in serum protein concentration.

Erythrocyte volume determinations using the semi-automatic method¹ are based on sample haematocrit and do not usually take account of variations due for trapped plasma and for peripheral:whole body haematocrit ratio. The latter appears to change during perfusion in an upward direction (Table VII), though not significantly. It is possible that the proportion of trapped plasma is altered in haemodilution, especially by LMWD with its property of altering red cell suspension stability. This matter has not yet been investigated. The factor of alteration in haematocrit due to red cell swelling with pH change (Carrier, Douglas, and Crowell, 1964) has been ignored. Such corrections are, however, small, and calculations of blood volume and red cell mass during perfusion, based solely on plasma activity counts and a formally corrected haematocrit ($\times 0.91$), are extremely close to those obtained by the double-isotope technique, except in case 4, where the unexplained high red cell volume disturbs the relationship. It is therefore possible to use the Volemetron method during perfusion.

In two (cases 14 and 15) of the four cases perfused with 'whole blood', the recorded red cell and plasma disappearances are in the proportion of the haematocrit. Even in this group there is some dilution of perfusate from water retained after calibration, from medication, and from the Edgludate solution present. These elements may provide the relatively higher plasma loss seen in the other two cases (cases 13 and 16). These findings support the clinical observation of the disappearance of blood into the patient during perfusion by homologous blood (Gadboys *et al.*, 1962; Litwak *et al.*, 1963) noted by others, including us (Raison, 1962). It is surprising that, in the only two cases which received dextrose 5%, there should be a disappearance of red cells of the same order as in group II C, while the loss of plasma is small. The loss in circulating volume observed clinically with whole blood perfusion occurs within the first few minutes. Volu-

metric calculations suggest that 5% dextrose with whole blood results in only a small loss of plasma during brief perfusion. The number of cases studied, however, is too small to comment reliably on the rate at which the total circulating volume reduces with dextrose 5%, as the water, unheld by colloid, disappears into the tissues and is more rapidly excreted during perfusion due to the much greater osmotic diuresis induced by dextrose 5% compared with Rheomacrodex. The perfusate shrinkage is, however, seen clinically and occurs more slowly than with whole blood. When dextrose alone is used to prime the circuit, further solution has usually to be added to maintain apparent volume, usually within the first 10 to 20 minutes.

This study provides evidence of the sequestration of blood during homologous blood perfusion and of red cells with dextrose perfusion, and demonstrates the protective effect of Rheomacrodex (not merely by plasma expansion but by preventing this sequestration). It fails to demonstrate the existence of a slowly circulating red cell mass. The clinically improved peripheral perfusion seen with LMWD and its prevention of sequestration suggested a link with the work of Shoemaker and Iida (1962) and Susuki and Shoemaker (1964), who found that LMWD eliminated the two-compartment vascular system sometimes found in shock when part of the red cell mass equilibrates slowly or not at all. Hypovolaemia may be common to shock and extracorporeal circulation, as well as poor tissue perfusion. Recently, Tanaka, Bennett, Sherman, Brown, and Maloney (1965) have used a ⁵¹Cr technique to show that substances, including LMWD, reduce the delayed equilibration of erythrocytes caused by perfusion of homologous blood in dogs; their studies were made, however, in more stable conditions after bypass and they describe the technique as not a sufficiently accurate one for surgical application. Dagher and Moore (1964) have been unable to repeat the findings of Shoemaker and Iida (1962).

The order of variation in red cell radioactivity plots in our study must be regarded as within experimental limits and not identifiable with the steadily declining and much larger changes (22% in 50 minutes) ascribed by Shoemaker to the presence of a slowly circulating red cell mass. During the brief period available for studies in perfusion, one cannot distinguish the red cell equilibration curves of LMWD from those of dextrose and whole blood priming, even though there is a volumetric sequestration measurable

¹Volemetron, Ames Atomium Ltd.

with the latter two. Any slow equilibration must be very long delayed, if present. Shoemaker also found the existence of a non-circulating volume, however, directly comparable with our observed losses of whole blood in homologous blood perfusions and of red cells in dextrose-blood perfusions.

This study cannot identify the site of such sequestration. Possible sites are the lung fields during total cardiopulmonary bypass, the leg in which the femoral artery has been perfused in a retrograde direction, and undefined areas of the vascular tree in which sludging of blood may have occurred. Pulmonary sequestration might be purely physical, arising from arrest within that circulation during total bypass, and might be expected to occur in the LMWD group as much as in the other two groups, so that it would not be the site of the non-circulating volumes found in the whole blood or dextrose-blood groups. In addition, since complete mixing of the circulation is obtained within 2 minutes on partial perfusion (Raison, 1963), plasma volume expansion from LMWD will also be complete during partial bypass. Isotope injection and sampling during perfusion were made after the institution of total bypass, so that the large measured plasma volume increases in the LMWD series would not include a further volume supposedly sequestered in the lungs. This would require an altogether excessive total plasma expansion and, since such sequestration would be of plasma and red cells, a total increase of red cells on initial mixing greater than the small increase actually noted (which is probably insignificant). If there is 'partial sequestration' (the presence of a slowly circulating volume) in any of these areas, it should lead to a slope in the ^{51}Cr equilibration curves during perfusion, if significant, and there might be a difference in the slopes of groups II B and II C compared with group II A if, in the latter, LMWD increased the rate of equilibration from these body masses. The sequestration observed with whole blood and dextrose-and-blood is total (non-circulating volume).

It appears, therefore, that whereas a simple solution may improve the clinical features of perfusion by mere haemodilution, the advantage of LMWD is that it can fix the haemodilution over a period of time and also prevent red cell or whole blood sequestration. The volume lost from the circulation, and the proportion of the vascular bed rendered inactive by this loss, during blood or blood-dextrose perfusions may be small and tolerable in brief perfusions but of greater significance with the demand for long-term circula-

tory support in conditions where peripheral stagnation is already probable. The effects of adding albumin to dextrose dilution or of using plasma have not been investigated. Albumin is not readily available in Britain, plasma may bear additional dangers, and both would cost more than LMWD. Whether LMWD should be used as a 10% solution, when it may take up water from the tissues, or in such dilution as will render the perfusate isoncotic (Roe, Swenson, Hepps, and Bruns, 1964) has yet to be established, and this must be correlated with the serum concentration necessary to prevent intravascular sludging and sequestration.

SUMMARY

Simultaneous, separate red cell and plasma volume measurements have been carried out in a limited number of clinical cases during controlled conditions of cardiopulmonary bypass.

The effects of low molecular weight dextran as part of the extracorporeal circuit prime have been compared with those of haemodilution with 5% dextrose and those of whole-blood priming.

Low molecular weight dextran causes plasma volume expansion and maintenance of the red cell volume.

Haemodilution by dextrose 5% is confined to that of its own volume during brief perfusion and is associated with red cell disappearance.

Whole-blood perfusion is associated with a reduction of both red cell and plasma masses below those anticipated.

The technique has failed to establish the occurrence of delayed red cell equilibration in the period studied, but suggests the presence of a non-circulating blood volume when whole blood or haemodilution with dextrose 5% are used, and demonstrates prevention of this sequestration by LMWD.

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