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Hemorrhage in Normal Man: I. Distribution and Dispersal of Saline Infusions Following Acute Blood Loss:

Clinical Kinetics of Blood Volume Support

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Introduction

In the steady state there is a dynamic equilibrium between the volume of fluid in the interstitial phase * and that found in the plasma. This partition of the extracellular fluid can be expressed as the PV:IF ratio and is maintained by a number of anatomic barriers and biochemical forces. These include the capillary membranes (with their varying colloid permeability in different anatomic sites), hydrostatic pressure gradients across the capillary, and oncotic pressure of plasma proteins. The capillaries of the body include membranes of low permeability to colloid (such as those found in peripheral subcutaneous tissue), as well as some in which there is a free permeability to colloid molecules up to the size and shape of plasma albumin. Such highly permeable capillaries are found in the gut, liver, lungs and possibly the heart.

Normal flux-rates for water and ions have been estimated by kinetic analysis of isotopic disappearance curves from the plasma.[9, 10, 11, 15, 8] These analyses, together with more recent studies of capillary permeability, are summarized in the recent papers of Pappenheimer, Landis, and Renkin.[13, 23, 25] Exchange between plasma and the interstitial phase may be considered to involve three simultaneous rates: diffusion-exchange at the ionic and molecular level across the capillary; bulk filtration, with reabsorption via the distal capillary and lymph; and vesicular transport—other-

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* The term "interstitial phase" is a body compositional term indicating fluid that is outside of the plasma and also outside of cells. The term used by physiologists, "interstitial fluid," refers strictly to that small layer of fluid just outside the capillary, not including the lymph. As used in this paper, the term "interstitial phase" is used in its compositional sense, to mean the extracellular fluid volume minus the volume of plasma, and including the lymph. The *PV:IF* ratio is the ratio of plasma volume to interstitial volume; or *PV/ECW-PV*.

wise referred to as pinocytosis or cytopempsis—involving macromolecules, and probably located at the venular end of the capillary or in the postcapillary venule. When there is net movement of water or solute in either direction across the capillary, it results from a lack of balance between ingress and egress among these three different fluid-flow mechanisms.

The normal rate of plasma volume refilling has been estimated, together with the effect on the normal process of the administration of hormones, pressor drugs, norepinephrine, and angiotensin, both in man and in the dog.[17, 18, 19, 33, 14, 4] As would be predicted, this bulk movement of water, salt and protein across the capillary occurs at a very slow rate when contrasted with the calculated flux rates for diffusion or filtration-reabsorption across the capillary.

Of particular interest has been the fact that normal transcapillary refilling after hemorrhage in man does not involve any significant period of hypoalbuminemia, and the demonstration that both norepinephrine [14] and angiotensin [4] interfere with normal plasma volume refilling. Both of these pressor substances cause water and salt to leave the circulation during the period of drug injection, even if these drugs are administered during the maximal flow in the opposite direction, following hemorrhage. It would be expected that an infusion of balanced salt solution might show effects markedly contrasting to those of the pressor drugs. It would also be expected that the posthemorrhagic state might bias the dispersal of these solutions from the plasma to the interstitial phase, and their subsequent disposal via the kidneys; colloid-free solutions would be expected to dilute the plasma proteins to the same extent as the erythrocytes if no other dynamic factor is involved.

It was the purpose of this study to determine the effect, during the posthemorrhagic state, of the infusion of buffered salt solutions, particularly as regards the partition of the infused material itself between plasma and interstitial fluid, the effect of these infusions on plasma and extracellular volume, their rate of dispersal outward from the plasma, their effect on protein concentrations, their effect on renal function and the rate-constants which determine clinical effectiveness of noncolloid solutions for plasma volume support.

Materials and Methods

Ten healthy male volunteers, varying in age from 20–25 years, were admitted to the hospital. Examinations were performed to ensure physical fitness. One subject acted as a control, receiving a salt infusion but without a preliminary bleed. Three subjects were bled, but thereafter were fasted and given no infusions. In one of these (P. M.) the fasting-thirsting lasted only 8 hours. In the two other controls, not only was there no infusion given, but, in addition, the subjects were kept fasting and thirsting for a total of 40 hours, commencing 8 hours prior to the hemorrhage.* The purpose of these 3 noninfused controls was to assess the role of fluid restriction on plasma volume refill and body water distribution.

The six experimental subjects underwent a venous hemorrhage of 11 to 12.3 per cent of blood volume over a 20-minute period, followed (4 hours later) by the infusion of 2,000 ml. of lactated Ringer's solution,** administered over a period of 4 hours. No

* One of these subjects (R. F.), on attempting to stand up 8 hours after his hemorrhage, became pale and sweaty; he stated that he was going to faint although no measurable changes in blood pressure were recorded. He was, therefore, given approximately 600 ml. of water to drink over the next 3–4 hours, and then returned to fasting and thirsting state. Subject C. B. was fasted-thirsted after the bleed, and until the end of the experiment, without event.

** The lactated Ringer's solution employed for these studies contained the following ionic composition: sodium: 130 mEq./L.; potassium: 4 mEq./L.; calcium: 3 mEq./L.; chloride: 109 mEq./L.; lactate: 28 mEq./L.

oral intake of food or fluid was permitted for 8 hours, but thereafter the subjects were allowed to eat and drink normally. The experimental observations were continued for an additional 32 hours. The three who were given no saline infusion were bled 13.1–14.3 per cent of blood volume.

Measurements of the plasma volume, red cell volume, and extracellular water volume were carried out on all subjects. Plasma and red cell volumes were determined using Evans Blue dye and radiochromated erythrocytes by the standard methods of these laboratories.[20] At the outset the blood volumes were based on summation, as PV + RV = BV. Subsequent plasma volumes were sequentially determined by calculation based on the starting RV as corrected for measured withdrawals, divided by the observed large vessel hematocrit (LVH) corrected for the starting WBH:LVH ratio.[20] The validity of this method is borne out by previous comparisons based on renewed injections of dye,[14] and by the remarkably close check of the final RV with that calculated from the initial measurement minus measured withdrawals. The WBH:LVH ratio was defined as the relationship of the whole body hematocrit (RV/PV + RV) to the large vessel hematocrit (LVH) at the outset.

Extracellular water volume was based on extrapolation to zero time of the radiobromide equilibrium curve as based on plasma measurements at 60, 90 and 120 minutes after injection of a tracer dose. This figure for the initial bromide volume dilution was converted to extracellular water by correction for bromide penetration into red cells (based on the measured red cell volume at the outset) and the Donnan equilibrium, according to standard formulations previously published from these laboratories.[16] In the course of each experiment the subjects had four repeated radiobromide injections, so that each measurement of extracellular water volume could be based on a distinct and independent dilution-volume, avoiding reliance on aberrations of the bromide disappearance plateau as a basis for calculation. These four injections of radiobromide were given over a period of 48 hours without incurring an undue radiation hazard by using a small dose (10–15 μc) for the initial injection, gradually increasing the dose up to 40 or 50 μc so as to maintain the same accuracy parameters despite the gradually rising background activity.* Those subjects who acted as controls required only three such radiobromide injections.

The third radiobromide injection in the experimental subjects was given during the final 2 hours of the infusion of balanced salt solution; the tracer anion was thus presented to the extracellular fluid at a time when the latter was being expanded by infusion. One might expect a bias in the radiobromide disappearance slope under these circumstances, steady-state conditions having been distorted by hemorrhage. The plasma radiobromide slopes between 60–120 minutes were analyzed by fitting the log slopes to least-square regressions and comparing the resultant exponentials for significance.

Were the LVH to remain constant during saline infusion, dispersal rate could be assumed to be equal to the infusion rate (8.33 ml./min.); in all cases the rate of dispersal was less than this, indicating partial retention of infused saline in the plasma volume. These dispersal rates during the infusion were based on calculation of the amount of the infused volume retained in the plasma volume for each interval between serial measurements of the LVH. Six to eight interval measurements of this type were done for each patient, the results were averaged, and the final change in PV calculated from the fall in LVH.

Following sudden cessation of the infusion, LVH rose in all instances. A rough ap-

* Dosage here totalled approximately 0.361 rad, of which 0.151 rad was contributed by the radiobromine.

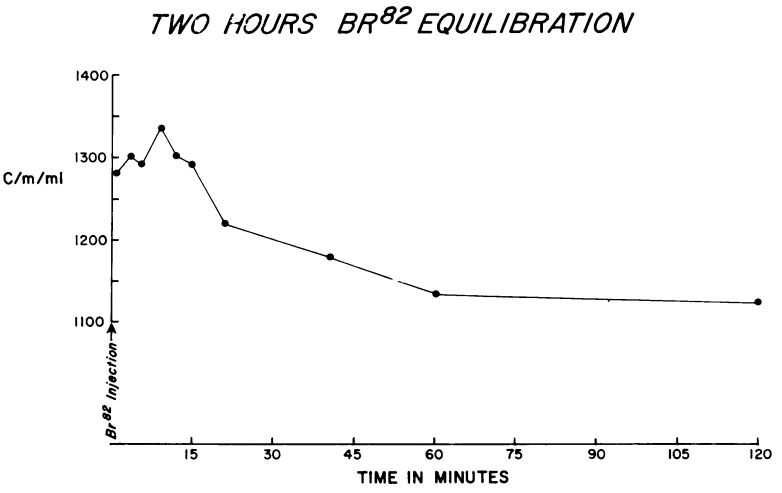

FIG. 1. Radiobromide equilibrium curve. Normal subject. After initial adjustments of concentration in radiobromide in venous blood, the slopes of which can be analyzed in terms of distributional kinetics, the radiobromide slope settles down to a very gradual washout after 60 minutes, demonstrating a single exponential decay with a biological half-time of about 16 days.

proximation of the rate of continuing dispersal or "rebound" can be calculated by the same method mentioned above. In several cases this rebound occurred at night, and almost 15 hours elapsed between serial LVH measurements at this time, making rate-calculations almost meaningless. For those few with shorter interval measurements, approximate rebound dispersal rates could be calculated.

Serum proteins and protein fractions were determined by paper electrophoresis. The total amount of protein and protein fractions in the bloodstream was calculated as the simple product of plasma volume and protein concentration; ingress rates for proteins were based on these figures.

Chemical analyses on blood and urine were carried out by standard methods previously reported.[14, 20]

Results

In Figure 1 is shown a typical 2-hour curve for radiobromide equilibrium in a normal control subject. The disappearance slope after 60 minutes takes the form of a single exponential with a biologic half-time of 16 days. The reverse extrapolation of this gradual slope to zero time yields a figure for initial dilution volume that is not significantly different from that based on the arithmetic mean of the equilibrated values.

Figures 2–5 show representative experimental results; Figure 2 is that of a typical experiment demonstrating the sharp increase in plasma volume, bromide volume and extracellular water volume resulting from the salt infusion, together with the reduction in hematocrit and the subsequent "rebound" as infused saline continues to leave the circulation following cessation of infusion. In Figure 3 is shown the effect of such an infusion on serum sodium, osmolality and protein concentration. Protein dilution is evident, as well as its tendency to normalize rapidly. In Figure 4 are shown the results on a control subject (R. F.) on whom water was restricted for about 8 hours. The dilution produced by increased oral intake of fluids at 8 hours is evident. The minor effect on osmolality is shown in Figure 5, as well as the fact that in this subject, on whom fluid intake was initially restricted, protein concentration was little altered.

Table 1 shows the resting values for these phases of body water as observed prior to hemorrhage. In Column 13 is shown the normal resting PV:IF ratio. The bottom line (*Predicted normal*) shows comparative data from the nomographic predictions of this laboratory. It is evident that phase-volumes as measured in this homogeneous group of healthy young adult males, came very close to those predicted from the for-

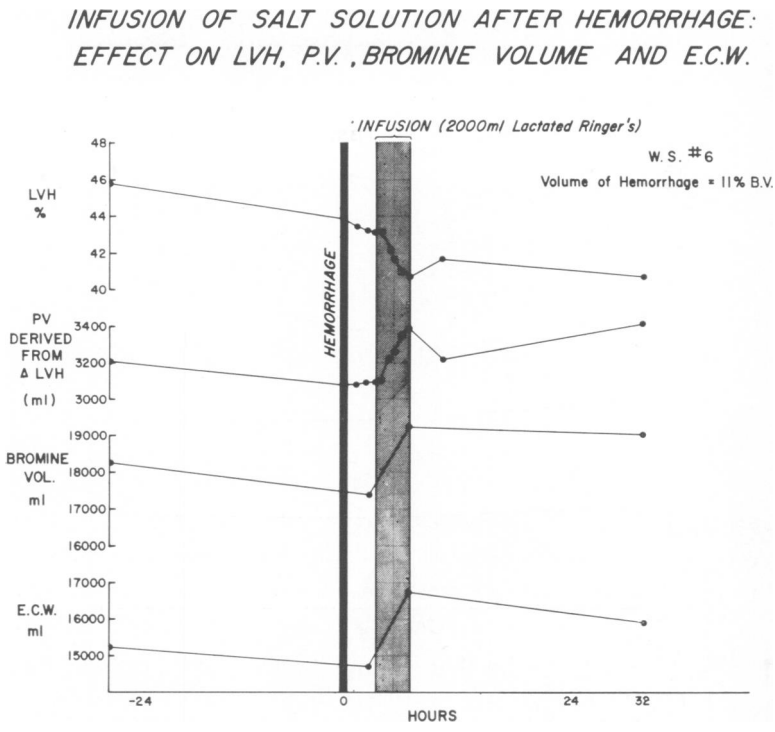

FIG. 2. Typical experiment. Subject W. S. Slight fall in large vessel hematocrit (LVH) is mirrored by a rise in plasma volume following hemorrhage. Infusion markedly accelerates this process and produces increases in uncorrected bromide volume and in extracellular fluid volume.

mulas and nomograms previously published.[5, 20] These subjects were all in excellent physical condition, and only one had an hematocrit lower than 45 at the outset. The red cell and extracellular water volumes were accordingly slightly higher than normal means predicted from a larger mixed population. The normal PV:IF ratio for this group was 0.230, indicating that slightly less than one fifth of the entire extracellular volume is to be found in the plasma when measured by these technics

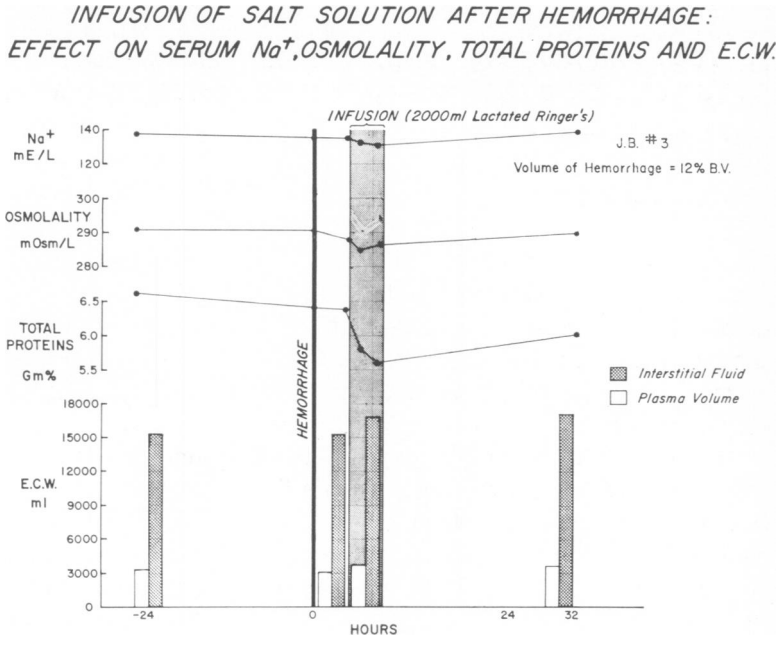

FIG. 3. Typical experiment. Subject J. B. Changes in sodium concentration and osmolality are negligible; there is a brisk dilution of protein, more marked in the globulin than in the albumin fractions (the latter not shown here).

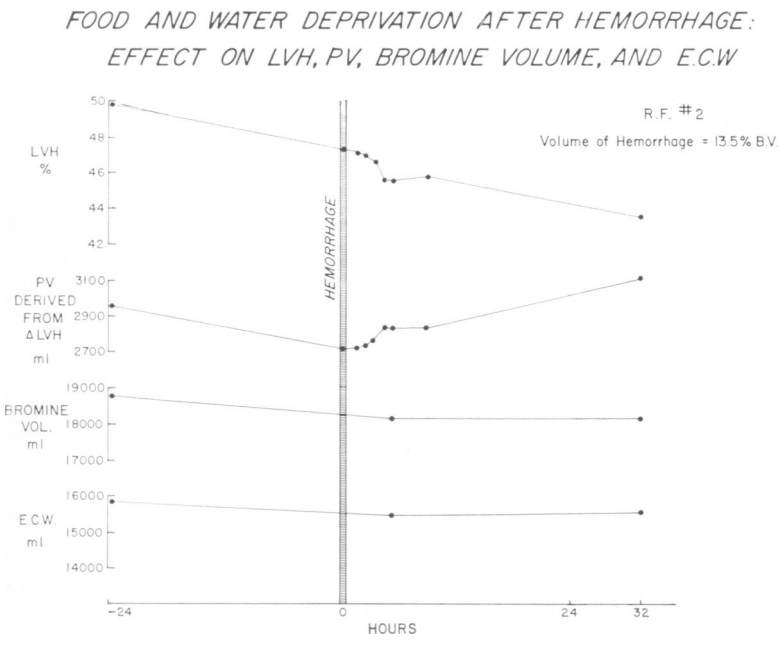

FIG. 4. Food and water deprivation for 8 hours, followed by oral intake. Subject R. F. This demonstrates the increase in plasma volume and fall in hematocrit produced by resumption of oral intake of fluid about 4 hours after hemorrhage. These are analogous to those produced by intravenous infusion, but of lesser magnitude.

in man. It is noteworthy that the one individual (P. M.) who had a PV:IF ratio higher than the others was the one who had a lower hematocrit, indicating some expansion of plasma volume with a normal blood volume prior to the experiment.

Table 2 shows the data for the blood removed by phlebotomy. These bleeds were carried out by the venous route over a period of 20 minutes. None showed any significant reduction in blood pressure (sphygmomanometer) or change in pulse rate; the subjects were supine when bled. Subject P. E. received a saline infusion, but was not bled, and therefore is not included in Table 2. The three subjects indicated below the line (P. M., R. F. and C. B.) were those who received no saline infusions although they were bled. In all tables the means for each group are shown below

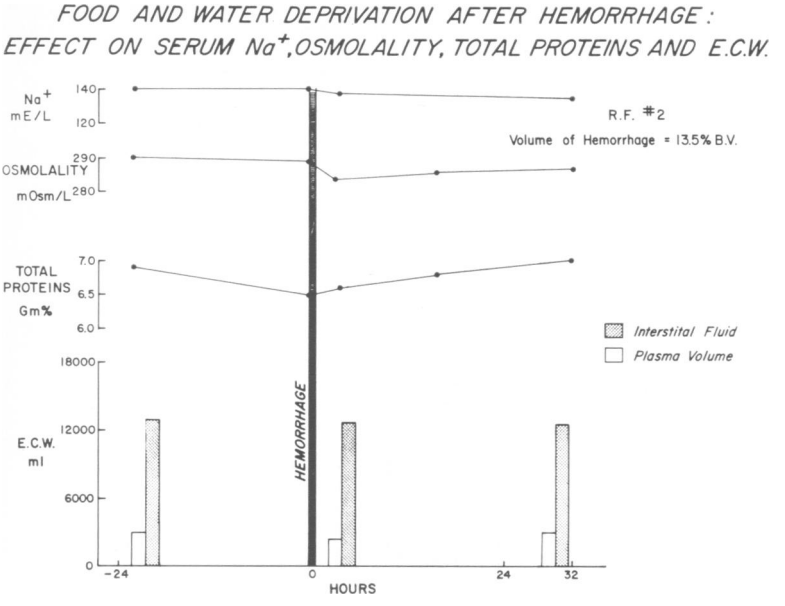

FIG. 5. Same subject as Figure 4. With oral intake of water there is a slightly greater reduction in osmolality than with intravenous infusion of isotonic solution; protein dilution in this subject was minimal. Protein concentrations show a consistent upward trend following the hemorrhage.

TABLE 1. *Basal Data, Resting*

Subj. Init.	Wt. Kg.	Plasma Volume (PV)		Red Cell Volume (RV)		Blood Volume (RV+PV=BV)		Extracellular Volume (ECV)		Hematocrit		
		ml.	%B.Wt.	ml.	%B.Wt.	ml.	%B.Wt.	l.	%B.Wt.	LVH	WBH / LVH	FV / IF
1	2	3	4	5	6	7	8	9	10	11	12	13
S. H.	71.2	2,640	3.71	2,110	2.96	4,750	6.67	17.0	23.9	49.2	0.90	0.184
G. H.	67.6	2,510	3.71	1,970	2.91	4,480	6.63	14.1	20.9	47.2	0.93	0.217
J. B.	81.4	3,350	4.12	2,740	3.37	6,090	7.48	18.6	22.8	50.2	0.90	0.220
D. H.	65.6	2,820	4.30	2,180	3.32	5,000	7.62	15.5	23.6	49.4	0.88	0.223
D. G.	89.0	3,170	3.56	2,340	2.63	5,510	6.19	17.5	19.7	47.9	0.89	0.220
W. S.	60.5	2,940	4.86	2,220	3.67	5,160	8.53	15.3	25.3	47.7	0.90	0.238
P. M.	73.8	4,000	5.42	2,160	2.93	6,160	8.35	17.4	23.6	44.0	0.80	0.298
R. F.	80.9	2,970	3.67	2,160	2.67	5,130	6.34	15.9	19.7	49.5	0.85	0.230
C. B.	73.8	3,380	4.58	2,000	2.71	5,380	7.29	15.0	20.3	45.3	0.82	0.289
P. E.	63.8	2,390	3.75	2,190	3.43	4,580	7.18	15.7	24.6	51.4	0.93	0.180
Mean	72.8	3,020	4.17	2,210	3.06	5,230	7.23	16.2	22.4	48.2	0.88	0.230
Predicted normal		3,430	4.71	2,100	2.89	5,530	7.60	17.5	24.0	43.0	0.89	0.244

each group, and the final mean for the whole table is indicated by $\bar{x}$. The blood withdrawals in the three subjects who did not receive saline infusions were slightly larger than in the others. The hematocrit of the shed blood was in all cases slightly lower than the starting LVH.

Table 3 shows the changes resulting from the hemorrhage alone, as based on measurements 4 hours after the hemorrhage and immediately prior to the salt infusions. It will be noted that plasma volume refilling has at this time commenced with a mean refill rate of 61 ml./hr. or 1.0 ml./min. The extracellular volume reductions are noted in Columns 6 and 7. In Column 9 is shown the relationship of this extracellular volume loss to the sum of plasma volume withdrawn in the hemorrhage plus urine secreted during the interval. The reduction in extracellular water volume was, on the average, well correlated with the recorded losses of plasma volume and urine (mean ratio 1.07), although there was considerable spread around this mean. In Column 10 are shown the actual differences between the change in extracellular volume and the sum of plasma withdrawn plus urine se-

TABLE 2. *Extent of Hemorrhage*

Subject Initials	Whole Blood Lost (WBL) ml.	Whole Blood Lost %BV	Plasma Volume Lost PVL ml.	Red Cells Lost RVL ml.	Hematocrit of Shed Blood LVH %
1	2	3	4	5	6
S. H.	500	10.5	270	230	46.0
G. H.	542	12.1	292	250	46.6
J. B.	725	11.9	365	360	49.8
D. H.	595	11.9	310	285	47.5
D. G.	635	11.5	340	295	46.5
W. S.	530	10.3	295	235	43.7
Mean	588	11.4	312	276	46.7
P. M.	810	13.1	480	330	40.6
R. F.	698	13.6	368	330	47.1
C. B.	770	14.3	410	360	45.5
Mean	759	13.7	419	340	44.4
$\bar{x}$	645	12.1	348	297	45.9

TABLE 3. *Four Hours after Hemorrhage*

Subj. Init.	LVH 4 hr. %	Change in PV: PV_3 4 hr. ml.	Change in PV: Gain ml.	Change in PV: Gain ml./hr.	Change in ECW: ECW_3 4 hr. L.	Change in ECW: Loss ml.	ECW Data: Total Urine ml.	ECW Data: $\frac{ECW}{PVL+U}$	ECW Data: Diff. ml.	ECW Data: Diff. $\%ECW_1$	$\frac{PV}{IF}$ 4 hr.
1	2	3	4	5	6	7	8	9	10	11	12
S. H.	46.1	2,660	293	73	16.0	1,000	235	1.98	−495	2.9	0.19
G. H.	45.1	2,385	167	42	13.8	300	133	0.71	+122	0.9	0.21
J. B.	47.6	3,180	195	49	18.4	200	290	0.31	+455	2.4	0.20
D. H.	46.5	2,725	215	57	14.7	700	126	1.60	−264	1.7	0.22
D. G.	45.2	3,055	225	56	16.8	700	320	1.06	−40	0.2	0.18
W. S.	43.3	3,045	400	100	14.9	300	450	0.40	+445	2.9	0.25
Mean	45.6	2,842	249	63	15.8	533	259	1.01	+370	1.8	0.21
P. M.	38.7	4,070	550	137	16.8	600	310	0.76	+10	0.1	0.28
R. F.	45.7	2,730	128	32	15.5	300	380	0.41	+440	2.8	0.20
C. B.	43.5	2,980	20	5	13.1	1,900	385	2.40	−1100	7.3	0.31
Mean	42.6	3,260	233	58	15.1	933	358	1.19	−217	3.4	0.26
$\bar{x}$	44.6	2,981	244	61	15.6	667	292	1.07	−470	2.4	0.22

creted; only in one subject (C. B.) was there a gross discrepancy. As shown in Column 11 most of these differences are minor and range around 3 per cent of the initial measurement of extracellular water volume, a discrepancy within the error of the radiobromide method. At this 4-hour interval, the PV:IF ratio (as shown in Column 12) has been slightly reduced from normal by a hemorrhage that has occurred so recently that there has not yet been an opportunity for complete compensation by plasma volume refilling.

Table 4 shows the effects of the infused balanced salt solution as based on measurements during the third and fourth hour of these infusions. All subjects were given an infusion of 2,000 ml. over a 4-hour period. In Column 2 is shown the relationship of the infusion volume to that of the blood volume lost; these cluster around a mean dosage of 3.5 volumes of infused balanced salt solution per unit volume of blood lost. It is evident from Columns 4–7 that there has been a gross increase in plasma volume during the salt infusion, averaging 592 ml., or slightly more than one fourth of the infused material. Plasma volume has been increased 18 per cent above its starting value prior to the hemorrhage, and blood volume has been restored to normal or slightly above normal. Extracellular water volume has been grossly increased by the infusion (Column 9), and at the close of the infusion has been restored essentially to normal (Column 10). In Column 12 are shown the incremental ratios calculated as the increase in plasma volume divided by the increase in interstitial fluid volume. In five of the six bled subjects the incremental ratios are increased above the resting PV:IF ratios and are higher than the PV:IF increment in the subject who received the salt infusion without a hemorrhage (P. E.). In those subjects who had undergone blood loss, a disproportionate fraction of the infused material thus remained in the plasma volume, more than that predicted from the resting PV:IF relationship. The net effect on the PV:IF ratio (Column 13) for the group has been a slight increase from 0.23 to 0.26.

Table 5 shows dispersal rates for plasma volume during infusion. Despite variation in the final volume of infusion retained, depending on the previous hemorrhage and body weight, dispersal rates during infusion cluster around a mean of about 5.0–6.0 ml./min. With an infusion rate of 8.33 ml./min., it is thus evident that the PV retention totals about 2.3–3.3 ml./min. or slightly more than one third of the infusion rate. In Column 6 is shown the time following the start of the infusion, at which the peak out-

TABLE 4. *Effect of Saline Infusion*

Subj. Init.	Infusion 2,000 ml./ BVL	LVH	Change in PV: PV_4 ml.	Change in PV: Gain ml.	Change in PV: $\frac{PV_4}{PV_1}$	Change in PV: $\frac{BV_4}{BV_1}$	Change in ECW: ECW_4 L.	Change in ECW: Gain ml.	Change in ECW: $\frac{ECW_4}{ECW_1}$	Change in ECW: $\frac{\Delta ECW}{Inf.-U}$	$\frac{\Delta PV}{\Delta IF}$	$\frac{PV}{IF}$
1	2	3	4	5	6	7	8	9	10	11	12	13
S. H.	4.0	40.1	3,340	675	1.27	1.10	16.8	800	0.99	0.62	0.51	0.25
G. H.	3.7	39.7	2,935	550	1.17	1.04	14.5	700	1.03	0.60	0.38	0.26
J. B.	2.8	42.9	3,795	610	1.13	1.01	20.5	2,100	1.10	1.47	0.44	0.23
D. H.	3.4	41.6	3,300	575	1.17	1.04	15.9	1,200	1.03	0.84	0.40	0.26
D. G.	3.1	39.7	3,760	705	1.18	1.03	17.2	400	0.98	0.24	0.55	0.28
W. S.	3.8	40.5	3,480	435	1.18	1.06	16.2	1,300	1.06	0.91	0.28	0.28
Mean	3.5	40.8	3,435	592	1.18	1.05	16.8	1,083	1.03	0.78	0.43	0.26
P. E.		46.1	2,830	440	1.18	1.10	17.2	1,500	1.10	1.53	0.28	0.21

ward dispersal rate was observed. In general the peak rates are attained toward the end of the period of infusion, at approximately the same time that the maximum plasma volume increment is produced.

The rise in hematocrit following cessation of the infusion bespeaks the continuation of outward dispersal of fluid at a rate gradually slowing down as PV refilling again predominates, but at a much slower rate than initially; the hematocrit finally attains its ultimate value over the next 12–18 hours. Dispersal rates during the rebound phase varied from 1.34–2.2 ml./min. in those subjects in whom it could be reliably estimated, as contrasted with the rate of about 5 ml./min. during infusion. Hematocrit adjustments from the close of rebound to the end of the experiment were minor in all subjects, and the predominant event during this period was an ingress of protein, as mentioned below.

Table 6 shows the data for the whole group at the close of the experiment when final equilibrium was attained and a steady-state resumed. Hematocrit has now returned towards the figure noted immediately after the infusion, despite the intervening rebound. This has been due to continuing exchange of fluid between the plasma and interstitial volumes, now manifested in some instances by water loss, in others by water gain, and in all by some minor protein entry. In Table 6 $\bar{x}_1$ indicates the mean for the entire series save for subject P. E., while $\bar{x}_2$ indicates the mean for the entire group of ten subjects.

From Table 6, it is evident that the extent of the final plasma volume refill (Column 8) has resulted in an increase of 8 per cent in plasma volume (Column 7) with the restoration of blood volume essentially to normal (Column 10), as indicated

TABLE 5. *Dispersal Rates during Infusion (ml./min.)*

Subject Initials	Cumulative Rate	Interval Rates: Range	Interval Rates: Mean	Interval Rates: S.D.	Peak Rate Time (min.)
1	2	3	4	5	6
P. E.	6.46	3.0–9.5	6.22	±3.18	170
S. H.	5.37	4.5–6.3	5.12	±0.60	240
G. H.	6.32	−0.5–9.8	5.90	±3.47	165
J. B.	5.69	−1.6–7.0	4.81	±3.62	225
D. H.	6.35	4.1–8.7	6.64	±2.32	85
D. G.	6.50	2.0–8.3	5.82	±2.95	220
W. S.	6.84	4.4–8.4	6.34	±1.91	240
Mean	6.21	2.3–8.3	5.84		192

TABLE 6. *Final Equilibrium at 32 Hours*

Subj. Init.	LVH Final %	Red Cell Volume: Virtual ml.	Red Cell Volume: Measured RV_5 ml.	Red Cell Volume: $\frac{V}{M}$	Plasma Volume: PV_5 ml.	Plasma Volume: $\frac{PV_5}{PV_1}$	Plasma Volume: Gain ml.	Blood Volume: BV_5 ml.	Blood Volume: $\frac{BV_5}{BV_1}$	Extracellular Volume: ECW_5 L.	Extracellular Volume: $\frac{ECW_5}{ECW_1}$	Extracellular Volume: Gain or Loss ml.	$\frac{PV}{IF}$
1	2	3	4	5	6	7	8	9	10	11	12	13	14
S. H.	42.2	1,776	1,800	0.99	2,940	1.11	702	4,740	1.00	17.3	1.02	+300	0.20
G. H.	43.8	1,617	1,740	0.93	2,560	1.02	469	4,300	0.96	14.5	1.03	+400	0.21
J. B.	45.6	2,274	2,270	1.00	3,275	0.98	410	5,550	0.91	20.6	1.09	+2,000	0.19
D. H.	44.2	1,795	1,950	0.92	3,050	1.08	659	5,000	1.00	15.8	1.03	+400	0.24
D. G.	38.2	1,963	1,930	1.01	3,805	1.20	1,099	5,770	1.04	17.2	0.98	−300	0.29
W. S.	43.2	1,888	1,920	0.98	3,000	1.02	479	4,920	0.95	15.0	0.99	−200	0.25
Mean	42.9	1,886	1,935	0.96	3,105	1.07	636	5,047	0.98	16.7	1.02	+433	0.23
C. B.	42.3	1,560	1,560	1.00	2,940	0.87	76	4,500	0.84	13.4	0.89	−1,600	0.28
P. M.	35.7	1,742	1,740	1.00	4,360	1.09	972	6,100	0.99	19.0	1.09	+1,600	0.30
R. F.	38.9	1,749	1,600	1.09	3,700	1.25	1,210	5,300	1.03	15.5	0.98	−300	0.31
Mean	39.0	1,684	1,633	1.03	3,667	1.07	753	5,300	0.95	16.0	0.99	−100	0.30
$\bar{x}_1$	41.6	1,818	1,834	0.98	3,292	1.07	675	5,131	0.97	16.5	1.01	+256	0.25
P. E.	47.6	2,032	2,140	0.95	2,690	1.13	471	4,830	1.05	16.4	1.04	+700	0.20
$\bar{x}_2$	42.2	1,840	1,865	0.98	3,232	1.08	654	5,101	0.98	16.5	1.01	+300	0.25

by a ratio of 0.98 for starting volume to final volume. This ratio is slightly lower (0.95) for those who were fasted and thirsted, and is lowest (0.84) for the subject (C. B.) who was totally fasted and thirsted throughout the experiment.

Columns 3–5 of Table 6 show the data for red cell volume. The "Virtual Red Cell Volume" (Column 3) is that predicted from the initial measured red cell volume minus the measured withdrawals. If the method for measurement of red cell volume in such experiments is accurate, it is to be expected that the final red cell volume (Column 4) will be close to or identical with the virtual red cell volume, as the period of 32 hours is negligible for significant red cell resynthesis. It is evident from Column 5 that the method here is remarkably accurate, yielding a mean of 0.98 (range 0.92–1.09) for the group.

The data shown in Columns 11–13 of Table 6 have analogous significance as regards the accuracy and reproducibility of the extracellular fluid volume measurements. The infused saline solution has now been dispersed, and extracellular volume has been allowed 32 hours in which to equilibrate in all subjects except those three who were fasted and thirsted. As shown in Column 12 the final volume of the extracellular fluid (as based on the fourth injection of radiobromide) is 1.01 the initial value, as a mean for the entire series. The very minor overall gains and losses are shown in Column 13. Only in subjects C. B. and P. M. is there a continuing discrepancy, both of these being subjects who were fasted. In subject C. B. who was fasted and thirsted throughout, there is a continuing unrepaired deficit of 1,600 ml. In subject P. M. who received his extra fluid by mouth, there is a continuing excess. Column 14 of Table 6 shows the final PV:IF ratios. In those subjects receiving the salt infusions, this value has returned precisely to its starting norm, at 0.230. In those who were fasted and thirsted, the value is still high, at 0.30, indicating the failure of these subjects to restore interstitial fluid volume after its drain into the plasma.

As mentioned above, subject C. B. shows continuing deficiencies in all the measured volumes, with final plasma volume at 0.87, blood volume 0.84 and extracellular volume 0.89 times the respective starting values.

Table 7 shows the average serum protein concentrations in these subjects. Pro-

TABLE 7. *Serum Protein Changes in Gm/100 ml (Averages)*

	Total Protein	Total Albumin	Globulins				
			Total Globulin	α1	α2	β	γ
	1	2	3	4	5	6	7
Pre-hemorrhage	6.53	3.97	2.55	0.32	0.50	0.78	0.95
Post-hemorrhage	6.49	3.87	2.56	0.35	0.55	0.76	0.92
All pre-infusion	6.51	3.93	2.55	0.33	0.52	0.77	0.95
Post-infusion	6.00	3.77	2.31	0.29	0.49	0.70	0.83
32 hr. (infusions given)	6.38	4.03	2.32	0.33	0.48	0.78	0.87
Post-hemorrhage (6 hr.) (no infusion)	6.33	3.54	2.78	0.33	0.47	0.86	1.12
32 hr. (fast)	6.72	3.74	3.02	0.40	0.57	0.90	1.14
32 hr. (total fast) (C. B.)	6.90	3.74	3.12	0.40	0.57	0.97	1.11

tein levels show an inverse relationship to albumin and globulin changes with respect to all three conditions imposed: hemorrhage alone, hemorrhage followed by infusion and hemorrhage followed by fasting. During the 4 hours after hemorrhage there was no significant change in total protein concentration or in any of its components. Then, following salt infusion, total protein concentration fell about 0.5 Gm.% or about 8 per cent of the starting value. Total globulins fell 9 per cent of their starting value, while albumin concentrations fell only 4 per cent. Among the globulin fractions the fall was most marked in the beta and gamma components, the latter falling a full 12 per cent of their starting values. Finally, at 32 hours, total protein concentrations returned to normal, associated with the return of albumin concentrations to normal. Total globulin and gamma globulin remained low throughout, once they had been diluted by the infusion—behavior reminiscent of erythrocyte concentration. In those three subjects in whom infusions and oral fluids were withheld after hemorrhage, there was clear evidence of an increased concentration of total proteins also most marked in the globulin fractions; total globulin concentrations increased 22 per cent, beta globulins 26 per cent and gamma globulins 17 per cent of starting values. In Subject C. B., who underwent total fasting and thirsting, the highest total protein concentrations were observed and likewise the highest globulin concentrations. The change in albumin concentration due to fasting and thirsting was negligible, but these fasting subjects ended the experiment with lower albumin concentrations than those who had received infusions.

As an average for all subjects, the plasma volume gain of 654 ml. involved the entry into the circulation of fluid at an average albumin concentration of 4 Gm.%, indicating that the posthemorrhagic refill has involved the movement of approximately 26.5 Gm. of albumin into the bloodstream from extravascular sites. With complete fasting and thirsting, plasma volume refill is less complete and the final albumin concentration is slightly lower.

Table 8, shows the data for the increases in concentration of total circulating protein and albumin during the infusion. Columns 2–6 show the total protein calculated as the product of volume and concentration, prior to the infusion. Columns 7–11 show the data at the end of the infusion. The differences are shown in Columns 12 and 13. It will be noted that approximately 17.2 Gm. of albumin have entered the blood during the infusion over a 4-hour period, for an average of slightly more than 4 Gm./hr. The average concentration of protein in the plasma volume increment during the saline infusion is 3.15 Gm.% for total protein and

TABLE 8. *Protein Ingress during Infusion (4 Hours)*

Subj. Init.	Pre-Infusion PV ml.	Total Protein Gm.%	Albumin Gm.%	Protein Gm.	Albumin Gm.	Post-Infusion PV ml.	Total Protein Gm.%	Albumin Gm.%	Protein Gm.	Albumin Gm.	Gains Protein Gm.	Albumin Gm.
1	2	3	4	5	6	7	8	9	10	11	12	13
S. H.	2,660	6.7	4.4	178	106	3,340	6.0	3.7	200	123	22	17
G. H.	2,385	6.7	3.9	153	93	2,935	6.3	4.1	185	120	32	27
J. B.	3,180	6.4	3.6	206	114	3,795	5.8	3.6	220	136	14	22
D. H.	2,725	6,5	4.5	177	122	3,300	6.0	3.9	196	130	19	8
D. G.	3,055	6.8	4.3	208	130	3,760	6.2	4.0	233	150	25	20
W. S.	3.045	6.9	3.8	210	114	3,480	6.0	3.5	209	123	—	9
Mean	2,840	6.7	4.1	189	113	3,435	6.0	3.8	207	130	18.7	17.2

2.90 Gm.% for albumin. The infusion is obviously free of protein.

Table 9 summarizes data on the radiobromide disappearance slopes. Four injections of radiobromide were given each subject. Therefore, four slopes were analyzed for each subject. The ranges and means are shown in the table. The hemorrhage itself reduces the rate of disappearance of bromide from the plasma volume, while the infusion restores it to normal. At the end of the experiment, even though transcapillary refilling of the plasma volume has virtually ceased, a lesser slope of bromide disappearance is again noted. Statistical comparison for significance between these slopes shows only that the second and fourth slopes are significantly less steep than the first and third.

Urinary secretion data are not shown in detail in this paper. Comparative charts, contrasting these flows with those in similar experiments employing mannitol, are reported elsewhere.[6] In brief, the effect of the saline infusion was to increase urinary flow to about twice the pre-infusion post-hemorrhage values; the flows attained, however, did not exceed approximately 2.0 ml./min. even at the peak of the infusion. The rate of total solute excretion does not follow this pattern of change, as hemorrhage alone did not reduce solute excretion, nor did infusion increase it; sodium excretion rate was little altered by the hemorrhage alone, and doubled (to approximately 200 μEq./min.) by the infusion. Those individuals who were bled showed a much less brisk natriuresis following the infusion than did the unbled control. Potassium excretion showed no increase in absolute rate with hemorrhage alone, but with the infusion of the balanced salt solution, there was an increase of potassium excretion almost twofold, to approximately 75 μEq./min., and this rate was greatest in those who were both bled and infused. Taking averages for the prebleed and postbleed period, there was only about 20 mEq. of potassium appearing in the urine above the basal excretion rate; there was no clear reduction in the urinary sodium:potassium ratio in any of these subjects at any time.

Discussion

Critique of Methods

All of the methods employed in this study have been described extensively from these laboratories previously and require no additional comment. Any method for measurement of extracellular fluid volume is in a sense arbitrary. Any tracer-dilution based on an established equilibrium at 2 hours or less is to be considered as measuring a phase of body water that is "functional" or "available." There is no difference (either theoretical or demonstrable) in the "functional" quality of fluid outside of the plasma as quantified by the radioactive ions (sodium, bromide, sulfate, thiosulfate) versus the crystalloids (inulin, sucrose or mannitol). The latter yield lower values for volume, and PV:IF ratios based

TABLE 9. *Radiobromide Slope Analysis* $\left(\frac{dR}{dT} \times 10^{-4}\right)$

	BrV_1 Pre-Hemorrhage	BrV_2 Post-Hemorrhage	BrV_3 Post-Infusion	BrV_4 32 Hours
Range	1.35–5.40	0.22–3.30	1.80–4.00	0.05–2.90
Mean	3.50	1.55	2.65	1.10

on crystalloid dilution will, therefore, be higher (approximating 0.30). The bromide volume dilution is quite readily reproducible as shown by these experiments. If the method is standardized and used consistently, it is valid to correct the raw data for erythrocyte and Donnan equilibrium effects and express them as "extracellular water volume."

Data on radiobromide slopes are sparse in the literature. It is of interest that during normal transcapillary refill the bromide slopes are less steep, indicating some alteration in the biologic half-time, in plasma, of this halogen. The differences seen here are small and had to be validated by statistical methods; they are not evident by visual inspection. It was not expected that salt infusion would restore this slope to normal. Although not shown in these tables —in the interests of brevity—calculations both for volume and slope have been carried out using the arithmetic means of the equilibrated dilution volumes without extrapolation or any additional corrections. The same results and interpretations obtain, but with somewhat higher extracellular volumes.

Normal Values

Prediction of blood volume data for normal subjects depends here upon the use of the summation method; in the literature data are available to permit the employment of these nomograms in patients. A recent review[15] shows the wide differences which result from the differing methods for blood volume prediction, particularly in individuals of abnormal body habitus. The point is emphasized that each laboratory should study its own normals so as to permit reliable prediction by the methods currently in use in that laboratory; this particular group of ten young men was a homogeneous one, and it is not remarkable that their values should be close to those predicted from this laboratory.

Effects of Hemorrhage at 4 Hours

During the initial 4 hours following hemorrhage plasma volume refilling has proceeded at a rate of approximately 61 ml./hr. with extremes from 5–137 ml./hr. In previously reported[14] work, normal transcapillary refill without any therapy has a mean rate of 25–35 ml./hr. for the first 24–40 hours, depending on the extent of the initial bleed. It is evident that the plasma volume refills at a rate described by a curve with a slope convex upwards and of decreasing inflection. The first 4 hours display the most rapid rates. Most of these early rate figures are clustered closely around the mean, there being only two exceptions, one of these (P. M.) being a subject with a large bleed and a very rapid refill. We have no explanation for the fact that Subject C. B. had the slowest rate of refill in this initial phase, since all of the subjects were fasting and thirsting for this initial period.

When whole blood is withdrawn acutely, the PV:IF ratio is abruptly decreased by the loss of plasma. Here, at 4 hours, it is seen still to be lower than normal (0.21). These were small hemorrhages; with larger bleeds the magnitude of the acute reduction of the PV:IF ratio would be more marked. All subjects showed some loss of extracellular volume after the hemorrhage, but this loss, when corrected for the urine secreted during the initial 4-hour periods,

is seen to be negligible. In none of these subjects was there an unexplained or disproportionate reduction in extracellular volume reminiscent of those reported by Shires [30] in shock.

Effect of Infusions; Outward Dispersal Rates During Protein Entry

The infusion of 2,000 ml. in 4 hours involves the rate of 500 ml./hr. or 8.33 ml./min. This is not to be regarded as an excessive rate of infusion or "flooding" of the circulation. The net result of such infusion has been to increase the plasma volume to a level far above normal, thus returning the blood volume to normal, and by outward dispersal to increase the extracellular volume to normal with a mild diuresis.

The effect of hemorrhage has been to bias the short-term distribution of the infused saline solution toward the plasma volume. Although the resting PV:IF ratio was 0.23, the incremental ratios at the fourth hour averaged 0.43, approximately 590 ml. of the 2,000 ml. infused remaining in the plasma at this time in those who were bled prior to infusion.

These findings are of both theoretical and practical significance. They demonstrate (in contrast to the findings in Subject P. E. who received an infusion with no preliminary hemorrhage) that the posthemorrhagic state, while producing a net fluid flow from interestitial to plasma phases of body water, likewise biases the distribution of an infused balanced salt solution toward the plasma volume, reducing its rate of dispersal outward across the capillary. A disproportionate increase in plasma volume is thereby produced. Some inkling that this might be the case had been provided by a previous experience, in unpublished work, during which we observed an excessive reduction in hematocrit in a bled subject who took water by mouth.

The dispersal of the infused saline solution out of the plasma at a mean rate of 5 ml./min. represents the net vector of fluid exchange in both directions, as indicated by the fact that albumin is entering the plasma at the same time. While 1,200–1,500 ml. of saline solution was leaving the circulation, approximately 15–17 Gm. of protein (mostly albumin) was entering the plasma. The saline infusion, rather than producing a "washout" of protein, evidently restored interstitial volume sufficiently so that protein flow into the plasma could be maintained. It is conceivable that much larger saline infusions, by producing a more drastic protein dilution, might mask this effect almost completely, leading to the erroneous interpretation of "washout."

The evidence gathered by Pappenheimer [13, 23] and summarized by Renkin [25] suggests that the transport of molecular species, having a weight greater than 10,000, across the capillary wall is by pinocytosis or cytopempsis (the latter term being used to mean vesicular transport across a membrane between two extracellular phases, as distinct from pinocytosis or vesicular transport into or out of the cell). The movement of water, ions and low-molecular-weight crystalloids is generally considered to occupy the main portion of the capillary wall itself with filtration on the arterial end and reabsorption on the venous end, molecular diffusion occurring throughout, and the entire relationship obeying Starling's Law. By contrast, the cytopempsis is considered to involve a site somewhat more distal in the capillary, possibly even in the postcapillary venule, and to involve spaced openings observed through the microscope to be at distinct points along the capillary. The findings in these experiments support such a "two-channel" theory of transcapillary movement of fluid and solutes, since we have here produced a situation in which net flow of water and ions is outward across the capillary while the movement of albumin is inward. Were the albumin entry entirely via the thoracic duct, it could likewise account

for these findings, but the rate of thoracic duct flow and the protein concentration therein would both have to be very high to explain the findings.

The dynamic nature of blood volume kinetics after hemorrhage and during saline infusion is well demonstrated by the coexistence of these three rates: a spontaneous plasma volume refill of 0.5–2.0 ml./min., replaced during infusion by an outward dispersal of infused saline at 5.0 ml./min. (with a retention in the plasma volume of saline at 3.3 ml./min.), and a continued ingress of albumin at approximately 4.0 Gm./hr. The final result, attained in terms of plasma volume, colloid osmotic pressure and circulatory maintenance, is a product of these three rates and the biological half-times of the processes involved.

The rebound rate, or continued dispersal of plasma after abrupt cessation of the infusion, is much slower than the dispersal during the infusion, as one would expect. In those in whom the measurement was at all reliable, the acute dispersal rate during infusion of 5.0 ml./min. was reduced to a mean loss of 1.3–1.2 ml./min. during rebound. By about 2 hours it was evident that this rebound process had ceased and that plasma volume refill had resumed at its normal slow and constantly decreasing rate.

Over the short term, blood and extracellular volume are restored at the expense of erythrocyte and globulin concentration, while albumin maintenance is remarkably well maintained despite the threat of dilution.

The rate of synthesis of albumin in the liver is so slow that one must assume the majority of this "new" albumin (newly entering the plasma volume) to be preformed albumin previously present in some other area of the circulation, possibly the hepatosplanchnic lymphatics. We postulate that the initial albumin entry into the circulation after hemorrhage involves the translocation of this albumin into the plasma volume; whereas the long slow increase in total circulating albumin, from about the 4th to the 24th hour after the hemorrhage, represents new albumin synthesis in the liver, possibly at an increased rate, and under endocrine control. Further study will be essential to corroborate such a postulate.

Final Equilibrium

During the final 24 hours of the experiment there was a gradual readjustment of the plasma volume and the plasma protein concentrations to the final values shown in the tables. Gains and losses of plasma volume varied from a maximum further loss of 350 ml. to a maximum further gain of 480 ml.; most subjects experienced only minor changes during this final readjustment period.

In all, however, there was a further gain in protein concentration most noticeable in the albumin fraction, indicating that colloid readjustments were occurring quite rapidly, regardless of plasma volume changes, to compensate for the transient hypoproteinemia produced by the infusion. This is a manifestation of the dynamic equilibrium of flow across the capillary after hemorrhage. In one or two subjects these late protein changes involved a considerable further ingress of albumin, as, for example, in Subject B. G. who gained 480 ml. of plasma volume while albumin concentration was increased from 3.98 to 4.33 Gm.%. The simultaneous exchange of water and protein during this time indicates the net ingress of 40 Gm. of albumin in 480 ml. of fluid, a mean concentration of 8.2 Gm.%. This seemingly impossible feat is, of course, due to albumin ingress at or below isooncotic concentrations followed by the dispersal of protein-free water. In one or two subjects the loss of water almost perfectly accounts for the increase in albumin concentration noted. For example, in Subject W. S., 145 ml. of plasma volume was lost during this late readjustment, while albumin concentration increased from 3.53 to

3.99 Gm.% and total protein from 6.0 to 6.40 Gm.%. Calculation of total circulating albumin and protein in this subject showed almost perfect constancy in the face of these losses of water. In the group as a whole, total protein increased an average of 0.35 Gm.% and total albumin 0.27 Gm.% during this final 24-hour interval, a significant compensation for the slight hypoproteinemia produced by dilution.

Of particular interest is the fact that those who had been given the infusion returned to a perfectly normal PV:IF ratio of 0.23 at the end of the experiment, as interstitial fluid volume had been restored by the infusion following an initial loss into the plasma for refilling purposes. By contrast, the three subjects who were fasting and thirsting ended the experiment with an upward distortion of the PV:IF ratio to 0.30, indicating that the depletion of interstitial fluid volume, involved in the restoration of plasma volume, had not itself been restored to normal.

Despite the suggestion of Stewart and Rourke[31] that there was potassium diuresis after hemorrhage, in previous studies in the dog we failed to demonstrate any gross participation of cell water in restoration of interstitial fluid volume toward normal, following spontaneous plasma volume refilling. In the experiments reported by McNeill *et al.*[17, 18, 19] there was little evidence for participation of cellular water in the process of refilling plasma volume; only when aldosterone was injected in the adrenalectomized animal could one note any significant movement of new fluid into the dilution-volume of radiobromide. Total potassium excretion rates, both in these experiments and in the dog, were increased only very slightly and insignificantly by the hemorrhage alone. Much larger hemorrhages, or a state of prolonged flow-deficiency with peripheral ischemia, hypoxia and acidosis would alter potassium balance drastically.

Enrichment of the plasma volume refill-fluid with albumin is quite evident in these experiments. There was no significant period of posthemorrhagic hypoalbuminemia in any subject. Nonetheless, it was somewhat unexpected to note that albumin concentrations were better maintained in those subjects receiving infusion of protein-free saline than in those who were fasted and thirsted. In addition, we did not expect to find evidence for continuing albumin ingress during the same period of time when saline fluids were being actively dispersed out of the circulation. It also appeared to be significant—and unexpected—that globulin was diluted by infusion to a greater extent than albumin, and that mild fasting and thirsting would result in increased globulin concentration. Calculation of total circulating globulin in the fasted and thirsted subjects shows that in two of the three, there was a true increase in total circulating globulin.

Compositional Interpretation — Kinetic Model

It was originally conceived, as based on the studies of Flexner and others[9, 10, 11, 15] when isotopes first became available, that the early rapid component in sodium, chloride and water decay slopes was due to events at the capillary wall. Flexner and coworkers calculated that about 105 per cent of plasma water exchanged across the capillary per minute, with equally large amounts of sodium.

These ideas required extensive revision because of the findings of Edelman.[8] He showed by an ingenious set of experiments, based both on plasma slope-analysis and tissue washout, that the early fast components seen by Flexner were actually due to exchange into an area of body cells, and that the capillary effect was not visible at all in the early plasma curves. Indeed, the dilution volume for deuterium at the end of 1 minute was greater than that for the entire extracellular volume; deuterium was equilibrated into red cell water in less than

1 minute, and for as long as 20 minutes after injection there was a significant arteriovenous difference, indicating continued cellular penetration.

These bits of information suggest that resting exchange rates for water and salt across the capillary are extremely rapid, probably even more rapid than calculated by Flexner, and that each minute the body capillaries exchange, in the steady state, amounts of water and salt grossly in excess of those found in the entire plasma volume.

More recent work, based on studies of the isolated hind limb of the cat [13, 23] and analysis of lymph protein concentrations [25] yielded more quantitative data for the total volume of fluid exchange across the capillary. The absolute magnitudes, however, are not much different from those earlier isotopic estimates, with diffusion exchange across the capillary of approximately 2–3 L. of fluid per minute; in essence, the plasma volume exchanging all of its water in the capillary bed with each pass of a cardiac output unit. Rates for filtration and reabsorption are, of course, much slower, estimated by Pappenheimer and Landis [13] at about 600 ml./hr., or approximately 20 L./day. Of this, approximately 2–4 L./day is returned to the blood via the lymph flow, principally the thoracic duct.

When the steady state of these processes (which normally maintain a perfect balance of fluid loss and fluid restoration) is upset by hemorrhage, the bulk movement of water, salt and protein which results is very slow as contrasted with that comprising overall transcapillary flux. Our data suggest that in the first hour plasma volume refill proceeds as rapidly as 60–120 ml./hr. (1–2 ml./min.), and that this tapers off to about 48 ml./hr. at the fourth hour. The average for the first 24–40 hours is about 25–35 ml./hr. The largest variable is the initial rapid rate, and it appears on the basis of inadequate evidence that this is determined by the magnitude of the bleed.

Ebert and coworkers [7] studied large hemorrhages in 6 normal human volunteers, the blood losses ranging from 760–1,220 ml. and occurring more rapidly than in these studies, namely in 6–13 minutes. Recalculation of their data for refill rate shows an average of 64.2 ml./hr., or about 1 ml./min. maintained for the first 24 hours. The total protein ingress recalculated from their data totalled 47 Gm. in 2 hours, an average of 23.5 Gm./hr. acutely; for the first 24 hours the total plasma protein increment was 91 Gm., an average of 3.8 Gm./hr.

If we assume that the rate of plasma volume refill is a function of the volume remaining to be refilled, it may be possible, when more data are available, to derive a mathematical expression to indicate the rate-constant for bulk movement of water and solute across the capillary in response to hemorrhage.

In the clinical consideration and bedside prescription of colloid-free solutions for replacement following loss of whole blood, the rates of refill and dispersal, as demonstrated herein, become controlling. No single "formula" will serve satisfactorily for more than a few minutes. Here, an amount of fluid approximately thrice the volume of whole blood lost maintained volume relationships satisfactorily for a few hours. If such restoration following massive blood loss could not be strengthened by the infusion of colloid solution within 6 hours, then saline infusion would have to be repeated to maintain volume.

Previous studies in this general area have been conducted. Anomalous bulk movement of extracellular water in or out of the body cell mass must be regarded with suspicion when invoked as an explanation for physiologic happenings in this setting. The studies of Warren *et al.*[34] involved infusions of very large amounts of salt solutions in dogs, amounts almost equal to the total body weight in 6 hours, and demonstrated that a significant portion of these

massive infusions remained in the plasma volume. They also noted that the proteins originally circulating in the plasma volume were still to be found circulating in the dog, but a slightly larger fraction of them was outside of the plasma volume, pursuant to this massive infusion with colloid-free solutions. No evidence for permeation of these fluids into body cells was found. If the infused solutions are isotonic with respect to extracellular ion concentrations and total osmolality, fluid shifts across the cell membrane do not appear to be prominent. If concentrated low-molecular-weight crystalloids are infused in ion-free water, then cell water is quickly mobilized. It is noteworthy that in body compositional studies carried out on a wide variety of very ill patients [14, 21] anomalous movement of water without solute across the cell membrane was rarely observed. Even in a recent study [32] of very drastic potassium loss, involving grossly abnormal ion fluxes across the cell membrane, there was very little evidence for bulk movement of water so long as the total cellular mass remained constant.

The relationship of colloid osmotic pressure to saline infusion has been studied by a number of workers, including Scott and Worth,[26] Coller and coworkers [2] and Hamilton *et al.*[12] The studies of Shires and his group [1, 3, 27–30] have focussed attention on the fact that many patients who have a prolonged low-flow state with arterial hypotension exhibit deficits of extracellular fluid volume. Many patients have gone for several hours with continuing losses of fluid by lungs and skin, together with some continuing urine secretion. When blood volume is finally restored some underlying dehydration and desalting will often be observed. This need not be interpreted as indicating anomalous movement of water into body cells.

In acute hemorrhage, plasma is lost, and some extracellular water deficit is produced and readily demonstrated, as indicated in the experiments herein reported. When these "deficits" are considered in the light of the actual volume of plasma withdrawn and the urine formed in the interval, they are found to be no more than those predicted from the losses. Anomalous movement of water into cells is not required for interpretation.

In only a few of the aforementioned studies have observations been made in man, and in none of those involving controlled hemorrhage was the experiment so designed as to indicate whether or not protein ingress continues during saline infusion and dispersal. Other data on saline infusions following bleeding in dogs were obtained in studies of Parkins [24] and Morrison.[22]

Summary and Conclusions

A study of hemorrhage has been conducted in 10 normal male volunteer subjects. Blood loss by phlebotomy over a 20-minute period totalled 10.5–14.3 per cent of blood volume. After a 4-hour period without treatment, six of the subjects received saline infusions totalling approximately 3.5 ml. per ml. of blood lost. This infusion, 2,000 ml., was given over a 4-hour period.

Measurement of phase volumes was carried out, including those for extracellular water, by repeated injections of radiobromide. Three of the subjects were fasted, rather than being treated by infusion; one of these three maintained a total fast and thirst throughout the experiment.

The resting ratio of plasma volume to interstitial water was 0.230 for the group, with a range of 0.180–0.298. Hemorrhage lowers the PV:IF ratio acutely through bulk removal of plasma. Plasma volume refill raises this ratio to a value above normal (0.30), where it remains for as long as 40 hours if no fluids are given to restore interstitial fluid volume to normal. Continued losses of water and salt by skin, lungs and urine contribute further to the reduction in interstitial fluid volume.

The infusion of balanced salt solution over a period of 4 hours resulted in a transient restoration of plasma volume above normal; interstitial fluid volume and blood volume both returned to normal. The PV:IF ratio was restored to a normal value. The incremental PV:IF ratio in the bled subjects had a mean value of 0.43, indicating a retention of the infused solution to an extent greater than that predicted from the resting PV:IF ratio of 0.23.

Administration of fluids by mouth has a similar effect.

Subjects who remained fasting and thirsting demonstrated a prolonged rise of PV:IF, as interstitial fluid volume remained low and was not restored by any route. The one subject who was kept totally fasting and thirsting, and whose blood loss was 14.3 per cent of blood volume, at the end of the study still had a PV:IF ratio of 0.28, with a plasma volume 13 per cent below normal, a blood volume 15 per cent below normal and an extracellular fluid volume 11 per cent below normal with an unrepaired deficit of 1,600 ml. This deficit was accountable largely on the basis of plasma volume removal plus continued losses by kidney, lungs, and skin.

Changes in extracellular fluid volume were entirely consistent with the remainder of the observations. There were no anomalous changes observed whose interpretation required one to invoke bizarre mechanisms such as the movement of isotonic solute across cell walls. Discrepancy between the observed changes in extracellular water volume and the sum of plasma volume and urine losses averaged about 370 ml., or less than 2 per cent of the initial extracellular fluid volume—discrepancies that are well within the range of error of the method.

Albumin concentrations were not significantly lowered during the 4 hours following hemorrhage, and the effect of the saline infusion was to lower globulin concentrations and hematocrit more than albumin. Dynamic entry of albumin was not inhibited by the infusion, and in fact continued during the infusion at a rate of approximately 4 Gm./hr. In those subjects receiving infusions, final albumin concentrations were normal, and were higher than those observed in the subjects who were kept fasting and thirsting. Globulin, by contrast, was extensively diluted by the infusions, and in those who were kept fasting and thirsting there was evidence for hyperglobulinemia at the end. These data provide indirect support for the concept that separate anatomic sites are involved for the flow of water and ions, as contrasted with albumin.

Alterations in renal function were not remarkable, either as a result of the hemorrhage alone or the subsequent infusion. The infusion produced a brisk increase in sodium excretion rate and some tendency to retain potassium.

The exponential slope of radiobromide disappearance following equilibrium showed a diminution during the posthemorrhagic state, with restoration to normal by this salt infusion.

The posthemorrhagic state must be regarded as a dynamic one, subject to kinetic analysis. Dynamic factors include rate-constants for spontaneous plasma volume refill, dispersal of infused saline, ingress of protein and renal excretion of water and salt. Balance between these determines plasma volume at any point in time. Understanding of these rate-factors is essential to the treatment of hemorrhage and in the use of salt solutions for blood volume replacement.

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