THE BIOLOGICAL DECAY CURVE OF P³² TAGGED ERYTHRO-CYTES. APPLICATION TO THE STUDY OF ACUTE CHANGES IN BLOOD VOLUME [1]

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Changes in circulating blood volume occurring over prolonged periods may be estimated by serial blood volume determinations. Employing the dilution principle, plasma soluble dyes (1, 2), serum proteins tagged with I¹³¹ (3–6) or isotope labeled erythrocytes (7–10) have been found useful for this purpose. For short term studies, however, the reinjection of these agents and their resampling involve considerable effort and lead to multiplication of errors, especially when many determinations are desired over a period of several hours. Because of the difficulties entailed in such studies, rapid alterations in blood volume have been evaluated from changes in the hematocrit values, hemoglobin concentration, red cell count, protein concentration, colloid osmotic pressure, plasma specific gravity, or some combination of these. Some of these parameters do not lend themselves to very precise quantitative assay and prove unsatisfactory as measures of small fluctuations in blood volume. Furthermore, changes in protein concentration, colloid osmotic pressure, or specific gravity may be unreliable for the evaluation of plasma volume variations because of the error introduced by the relatively rapid passage of albumin into extravascular spaces in the establishment of new equilibria. In addition, non-isooncotic expansion or contraction of blood volume may lead to changes in the size of the erythrocytes (11), thus introducing an error in the hematocrit value. Lyons, Avery and Jacobson (12) have emphasized that variations in hematocrit readings and serum protein concentrations fail to reflect quantitatively changes in plasma volume.

The present report is concerned with the concentration-time curves of circulating radioactivity over periods of hours to days following the injection of P³² tagged red blood cells and the application of such curves to the continuous study of fluctuations in blood volume in certain acute clinical and experimental situations.

METHODS

Subjects were hospitalized patients of the Veterans Administration Hospital, Bronx, N. Y., who were kept at complete bed rest for the control studies except where otherwise indicated. For the experiments lasting longer than 24 hours, patients were maintained at bed rest for a week because of the progressive hemoconcentration which occurs during the first few days in bed (13). In the experiments in which the effects of diuretics and digitalis were studied the patients were maintained on constant fluid intake throughout the control and experimental periods and on a low salt diet (less than 0.20 gm. Na/day) for a week or more prior to and during the study. The patients' clinical diagnoses are given in Table I.

The methods used for the determination of the initial blood volumes and hematocrit values and for the assay of radioactivity of the whole blood samples were identical with those previously reported (6). Hemoglobin concentration was determined by the method of Wong (14) with the Leitz colorimeter. All blood samples were drawn without tourniquet stasis into syringes moistened with heparin. Venipuncture was performed in the arm contralateral to the site of injection. Sampling was begun 12 minutes after the injection to allow for adequate mixing (6).

The P³² concentrations in whole blood, corrected for physical decay ($T_{\frac{1}{2}}$ = 14.3 days), were plotted on semilogarithmic paper as a function of time and the best straight line was drawn through the points obtained during the control period. The biological half life[2] was determined directly from this line. From the extrapolation of this line into the experimental period, the expected concentration at any time could be determined.

[2] In this report the biological half life is defined as the time required for the radioactivity concentration in whole blood to fall to 50% of the initial activity after correction for physical decay.

TABLE I

Summary of data on blood volume determinations in 18 subjects

Experimental condition	Case No.	Initial volumes (ml.)			Duration control period (hrs.)	Biological half life (hrs.)	Hours following onset of experimental condition	New volumes (ml.)			Absolute (ml.) and % change			Diagnosis and comments
		T.B.V.	RBC V.	P.V.				T.B.V.	RBC V.	P.V.	T.B.V.	RBC V.	P.V.	
Bed rest—control	1	6,050	3,190	2,860	70	36.4								Polycythemia vera. Three 500 ml. phlebotomies during week prior to study
	2	4,700	1,185	3,515	70	23.5								Pernicious anemia. Intravenous vitamin B12 given on first day of observations
I.v. saline infusion, 500 ml.	3	5,590	1,560	4,030	4.9	12.6	0–1.6	no detectable change						Chronic glomerulonephritis, nephrotic stage
I.v. human serum albumin, 50 gm.	4	6,690	2,430	4,260	23.5	25.2	4	8,390	hct. not obtained		+1,700 +25.4%			Laennec's cirrhosis, compensated. 80 ml. RBC removed during study
							26	6,950	2,380	4,570	+310 +4.65%	−50 +1.2%*	+325 +7.5%	
							reinjection study 26.3	6,800	2,330	4,470				
Phlebotomy, 650 ml.	5	4,800	2,310	2,490	3.5	21.8	1.7	5,130	2,310	2,820	+330 +6.9%	0 +14.5%*	+330 +13.2%	Cor pulmonale, 335 ml. RBC removed in phlebotomy
Phlebotomy, 625 ml.	6	7,860	4,200	3,660	23.5	19.3	1.5	8,380	hct. not obtained		+520 +6.6%			Polycythemia vera
Paracentesis	7	5,700	1,620	4,080	3.0	19.0	1.5	5,250	1,560	3,690	−450 −7.9%	−60 −3.7%	−390 −9.6%	Laennec's cirrhosis, decompensated
I.v. mercurial diuretic (Mercupurin)	8	4,880	1,920	2,960	2.8	16.9	5.0	4,450	1,850	2,600	−430 −8.8%	−70 −3.7%	−360 −12.2%	Hypertensive and arteriosclerotic heart disease; congestive heart failure. 5 hr. urine excretion, 640 ml. in excess of similar control period
							12.0	4,240	hct. not obtained		−640 −13.1%			
	9	6,310	3,280	3,030	2.5	22.5	22.0	4,760	hct. not obtained		−1,550 −24.6%			Hypertensive and arteriosclerotic heart disease; congestive heart failure. 22 hr. urine excretion, 4,100 ml. in excess of similar control period

* Volume of RBC withdrawn taken into account.
T.B.V.—total blood volume.
RBC V.—red blood cell volume.
P.V.—plasma volume.

TABLE I—*Continued*

Experimental condition	Case No.	Initial volumes (ml.)			Duration control period (hrs.)	Biological half life (hrs.)	Hours following onset of experimental condition	New volumes (ml.)			Absolute (ml.) and % change			Diagnosis and comments	
		T.B.V.	RBCV.	P.V.				T.B.V.	RBCV.	P.V.	T.B.V.	RBCV.	P.V.		
Effect of ambulation and recumbency	10	7,430	2,860	4,570	23.5	23.0	amb. 3.0	7,070	2,840	4,230	−360 −4.8%	−20 −0.7%	−340 −7.4%	Hypertensive and arteriosclerotic heart disease; congestive heart failure	Control period at bed rest. Followed by ambulation. Followed by return to bed
							bed 1.6	8,350	3,200	5,150	+920 +12.4%	+340 +11.9%	+580 +12.7%		
	11	4,340	1,610	2,730	3.0	25.0	amb. 3.0	4,140	1,590	2,550	−200 −4.7%	−20 −1.2%	−180 −6.6%		
							bed 2.0	4,850	1,650	3,200	+510 +11.8%	+40 +2.5%	+470 +17.1%		
	12	6,550	3,110	3,440	3.0	25.6	amb. 2.0	6,450	3,040	3,410	−100 −1.5%	−70 −2.3%	−30 −0.9%		
							bed 2.0	6,970	3,220	3,750	+420 +6.3%	+110 +3.5%	+310 +9.0%		
	13	5,680	2,240	3,440	23.0	21.2	amb. 3.8	4,740	2,040	2,700	−940 −16.5%	−200 −8.9%	−740 −21.5%	Convalescent rheumatic arthritis. No heart disease	
							bed 2.0	5,000	2,120	2,880	−680 −12.0%	−120 −5.4%	−560 −16.3%		
	14	7,000	3,060	3,940	30.0	17.0	amb. 2.0 bed 2.0	no detectable change						Glaucoma. No heart disease	
Effect of recumbency	15	5,700	2,800	2,900	2.5	39.0	bed 1.0	6,400	3,070	3,330	+700 +12.3%	+270 +9.6%	+430 +14.8%	Hypertensive and arteriosclerotic heart disease; congestive heart failure	Control period during ambulation. Followed by return to bed
	16	5,530	2,130	3,400	2.5	12.0	bed 1.0	5,950	2,050	3,900	+420 +7.6%	−80 −3.8%	+500 +14.7%		
Effect of digitalis in heart failure	17	4,720	2,060	2,660	26.0	34.2	20	4,120	1,880	2,240	−600 −12.7%	−180 −8.7%	−420 −15.8%	Hypertensive and arteriosclerotic heart disease, congestive heart failure; 20 hr. urine excretion, 1,500 ml. in excess of similar control period	
	18	6,650	2,540	4,110	22.0	21.0	22	5,640	2,230	3,410	−1,010 −15.2%	−320 −12.6%	−700 −17%	Hypertensive and arteriosclerotic heart disease; congestive heart failure. 36 hr. urine excretion, 1,070 ml. in excess of similar control period	
							34†	5,200	2,120	3,080	−1,450 −21.8%	−420 −16.6%	−1,030 −25.1%		

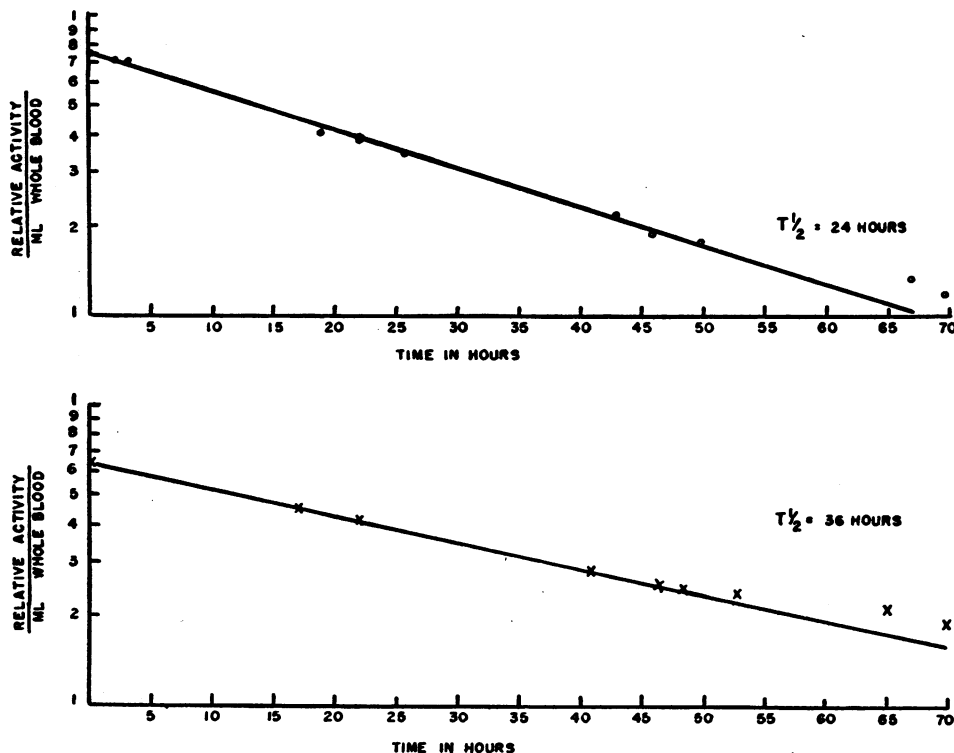† Values probably quantitatively unreliable for reasons discussed in text.

FIG. 1. SEMILOGARITHMIC PLOTS OF RELATIVE ACTIVITY/Ml. WHOLE BLOOD AS A FUNCTION OF TIME IN SUBJECTS AT BED REST

Lower curve, case 1; upper curve, case 2.

The following formulae were used in calculating the initial volumes:

$$RBC\ volume_{initial} = \frac{Injected\ activity}{Average\ activity\ of\ 12\text{-}15}$$

min. whole blood samples

$$\times\ observed\ hct.\ \times\ 0.98.^{3}\quad (1)$$

The mean value of the ratio average body hematocrit/peripheral vessel hematocrit has been found to be 0.924 (6). Therefore

$$Av.\ body\ hct. = 0.924 \times observed\ hct. \times 0.98,\quad (2)$$

$$Total\ blood\ volume_{initial} = \frac{RBC\ volume}{Av.\ body\ hct.},\quad (3)$$

$$Plasma\ volume_{initial} = Total\ blood\ volume_{initial}$$
$$-\ RBC\ volume_{initial}.\quad (4)$$

Following hemodilution or hemoconcentration, the new volumes were calculated as follows:

$$Total\ blood\ volume_{new} = Total\ blood\ volume_{initial}$$
$$\times \frac{expected\ activity}{observed\ activity}.\quad (5)$$

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[3] The factor 0.98 is used to correct for plasma trapped in the erythrocyte column which under the conditions of centrifugation used in this study has been found to be 2% (15).

The expected activity was obtained by extrapolation of the control decay curve to the same time as the observed activity.

$$RBC\ volume_{new} = Total\ blood\ volume_{new}$$
$$\times\ av.\ body\ hct._{new},\quad (6)$$

$$Plasma\ volume_{new} = Total\ blood\ volume_{new}$$
$$-\ RBC\ volume_{new}.\quad (7)$$

It is appreciated that the figure 0.924 represents only an average value for the ratio of average body hematocrit/peripheral vessel hematocrit and further, that this ratio may change during the course of the experiment. However, the use of this value represents the best compromise short of doing repeated erythrocyte and plasma volume determinations at every point.

RESULTS

I. *Biological decay curves of circulating radioactivity following injections of P³² tagged red blood cells in subjects in the "steady" state*

In two subjects at bed rest the curves were followed for 70 hours or for two and three half lives, respectively (Figure 1). When the radioactivity concentrations were plotted on semi-logarithmic

paper as a function of time, a straight line could be drawn through the points obtained during the first 50 hours in both cases. Subsequently the points fell above the straight line with gradually increasing differences. This departure from the simple exponential relationship after several days is in part explained by the increasing significance of the small amount of P³² in the plasma compared to the diminishing activity still remaining in the cells and the activity which has returned to the blood stream in newly formed cells. The two cases selected for these studies were chosen to exaggerate this latter factor (polycythemia vera recently subjected to repeated phlebotomy and pernicious anemia under treatment with vitamin B₁₂).

Table I gives the biological half lives observed in 18 subjects. It also includes the data of experiments in which acute changes in blood volume were induced. In these 18 experiments the biological half life varied from 12.0 to 39.0 hours.

The reasons for this variation have not been investigated but may be related to the differences in the rate of phosphate transfer. In nine cases, the control observations were continued for 22 hours or longer and in the other nine cases for 2.5 to 4.9 hours. In all of these cases the observed points deviated from the straight line by no more than 2–3%. The longer the desired period of experimental observations, the longer is the required control period. This is essential in order to minimize the effect of slight errors in the slope of the exponential decay line and to increase the significance of deviations of the experimental points from the expected values.

II. Application of the biological decay curves to the evaluation of changes in blood volume

Figure 2 (A)–(G) shows the changes in blood volume occurring in a number of different situations. In each case the extrapolation of the control curve as a discontinuous line into the experi-
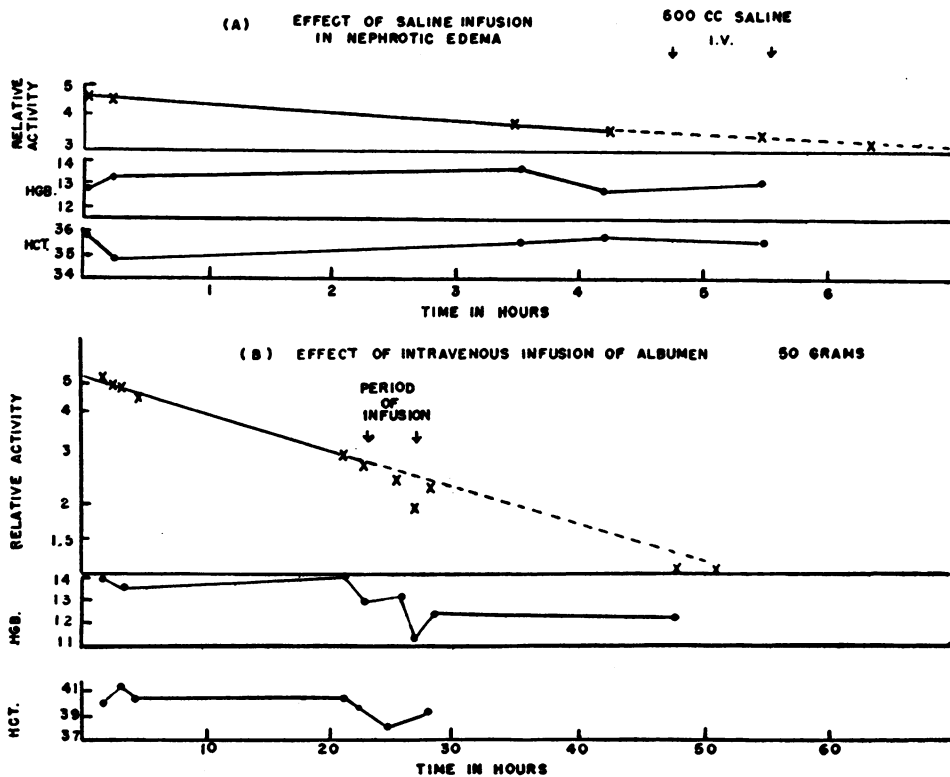

FIG. 2(A)–(G). SEMILOGARITHMIC PLOTS OF RELATIVE ACTIVITY/Ml. WHOLE BLOOD AS A FUNCTION OF TIME

The points joined by the solid lines were obtained during the control period.

mental period is used to calculate the magnitude of the changes resulting. Where points fall above the line, hemoconcentration and decrease in total blood volume have occurred. The reverse is true for points falling below the line.

(A) *The effect of a 500 ml. intravenous saline infusion in a patient with marked nephrotic edema* (*Figure 2A*). The absence of detectable hemodilution demonstrates the rapidity with which the saline solution left the intravascular circulation. As a conservative estimate, this patient had 30 liters of extracellular fluid. Thus, if proportional distribution of the infused saline had taken place in plasma, interstitial, and edema fluid, a change in total blood volume of less than 1% would have been expected.

(B) *The effect of an intravenous infusion of 50 gm. of salt-free albumin in 200 ml. of solution in a patient with compensated cirrhosis of the liver* (*Figure 2B*). The marked hemodilution due to augmentation of plasma volume as well as the rapid, though only partial, restoration is clearly evident. By the following morning the total blood volume was only 4.6% (310 ml.) greater than that prior to the administration of the albumin. A re-

injection study was performed at this time and the results agreed well with the calculated values (Table I). The equilibration of intravascular with extravascular albumin is dealt with elsewhere (16).

(C) The effect of phlebotomy (*Figure 2C*). Two cases have been studied in this manner. Both showed identical changes. The curve of one of these (case 5) is shown. Rapid hemodilution which resulted in a total blood volume exceeding the original occurred within one and one-half hours following the phlebotomy in both cases.

(D) *Effect of paracentesis in a patient with decompensated cirrhosis of the liver* (*Figure 2D*). The rapid hemoconcentration, followed by a tendency toward restoration of the original volume, is suggestive of a markedly accelerated loss of plasma into the peritoneal cavity. This loss apparently progressed more rapidly at first than the ability of the extravascular fluid accumulation to restore plasma volume. When more prolonged, this reduction of plasma volume may be a significant factor in the relative oliguria frequently noted on the day of paracentesis in this patient and in others.
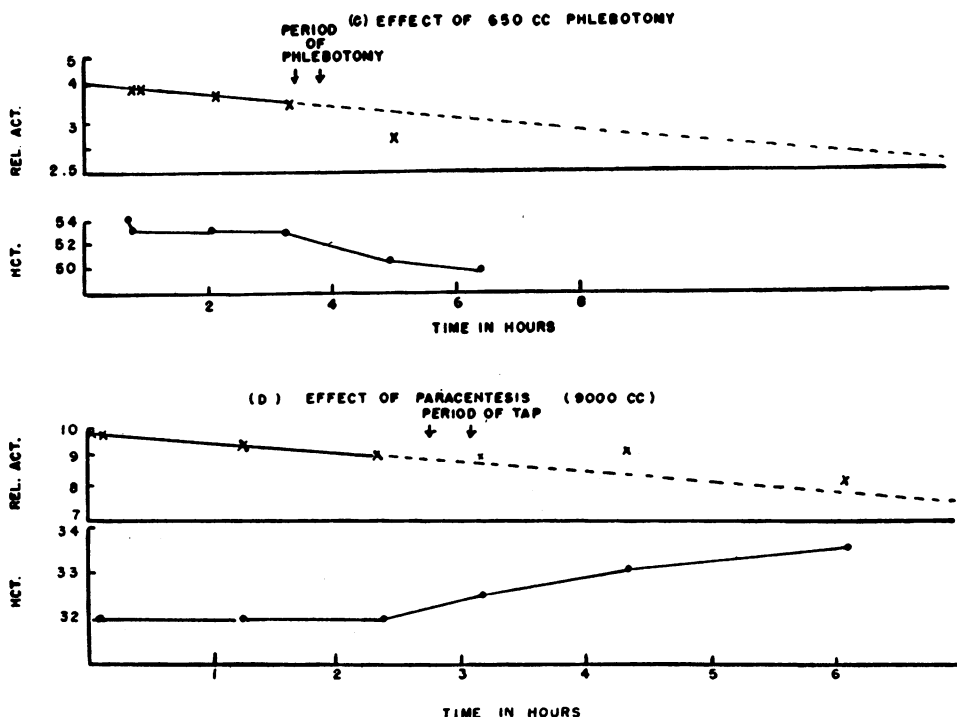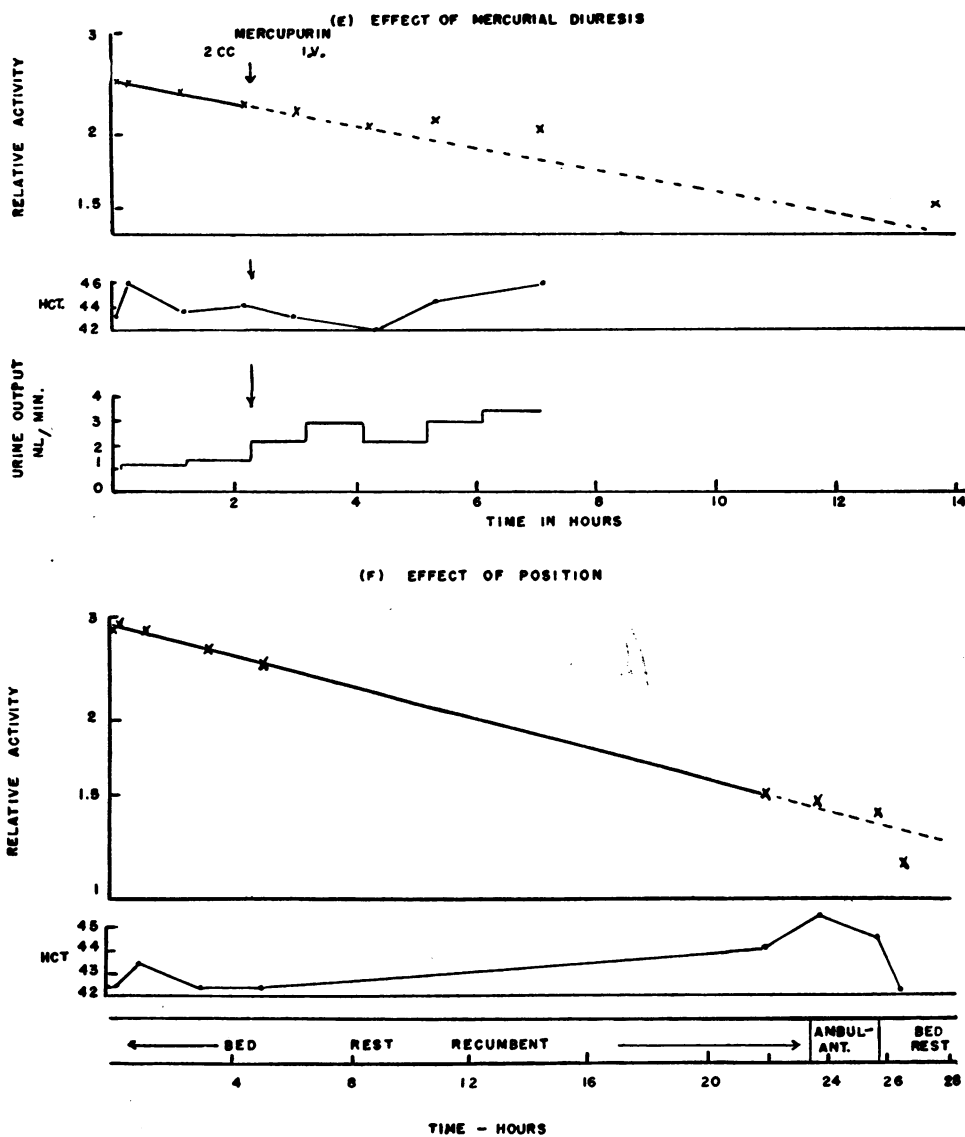

FIG. 2. (*Continued.*)

FIG. 2. (Continued.)

(E) Effect of mercurial diuresis (Figure 2E). Two patients with marked edema due to heart failure were studied following the intravenous administration of 2 ml. of Mercupurin. In one (case 8, Figure 2E), there appeared to be an insignificant fall in the hematocrit shortly after onset of diuresis. In both cases the P^{32} curves demonstrated progressive hemoconcentration beginning within a few hours after the onset of diuresis. This evidence militates against any hemodilution prior to diuresis. Here too, then, the ability of extracellular fluid stores to restore plasma volume

lagged behind the loss of plasma water into the urine. The plasma volume remained appreciably reduced even by 12 and 22 hours, respectively, following the injections.

(F) Effect of position (Figure 2F). Three patients with slight dependent edema and suffering from attacks of paroxysmal nocturnal dyspnea due to heart failure, and two subjects with no evidence of heart disease were studied following control observations at bed rest. A typical curve of the cardiac cases is shown in Figure 2F. In all of the cardiac patients there was noted some de-
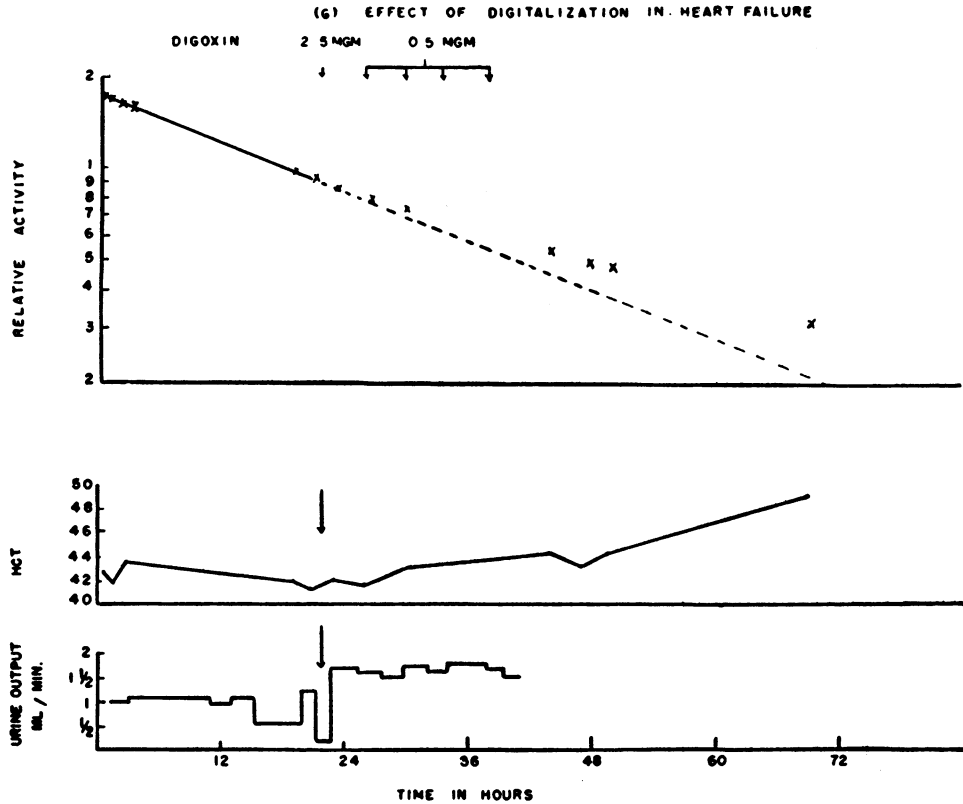
FIG. 2. (Continued.)

gree of hemoconcentration during the period of ambulation following bed rest. The hemodilution
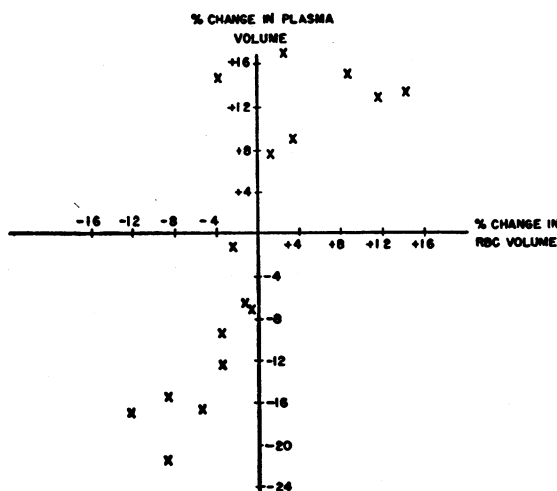

FIG. 3. THE EFFECT OF CHANGES IN PLASMA VOLUME (RESULTING FROM CONCENTRATION OR DILUTION) ON ERYTHROCYTE SIZE, AS DETERMINED BY CHANGES IN TOTAL RBC VOLUME

on return to recumbency was in excess of that required to restore the original volume. However, in both the noncardiac cases, this latter phenomenon was not observed even though, in one, significant hemoconcentration had taken place during the ambulant period. In two other patients with heart failure (cases 15 and 16), the control curve was obtained during ambulation. Assumption of the recumbent position led to significant hemodilution.

(G) Effect of digitalis in heart failure (hypertensive and arteriosclerotic disease with normal sinus rhythm) (Figure 2G). The findings were identical in both cases given Digoxin. There was no detectable change in blood volume until several hours after the onset of diuresis. After this time there occurred progressive hemoconcentration resembling the pattern observed following mercurial diuresis. The position of the 72 hour point in case 18 (Figure 2G) is undoubtedly too high, in light of the observations presented in Figure 1. The accompanying change in hem-

atocrit, however, leaves little doubt as to the marked hemoconcentration which had occurred at this time.

Figure 3 shows the effect of changes in plasma volume on the total red blood cell volume. It is of interest to note that increase and decrease in total erythrocyte mass associated with hemodilution and hemoconcentration, respectively, were observed in all cases but one (case 16) where hematocrit values were available for calculation. In general these changes were more marked when plasma volume alterations were greatest. In many cases, however, these changes were so slight as to be within the range of technical error.

DISCUSSION

Significant mobilizable blood depots, restricted from the general circulation, have not been demonstrated in man (17). Acute variations in blood volume, exclusive of those due to hemorrhage or transfusion, therefore, have been considered chiefly dependent on changes in the size of the plasma compartment. In the "steady" state at bed rest, the plasma volume appears to remain remarkably constant, aside from the slow and gradual hemoconcentration which occurs during the first few days as demonstrated by Widdowson and McCance (13). Therefore, experiments requiring observations over more than 24 hours should be preceded by three to four days of bed rest.

Metcalf (11) has observed, following serum transfusions, a striking correlation between the per cent increase in protein concentration and per cent decrease in the size of the red blood cells, leading to marked changes in hematocrit values independent of the effect of plasma volume shifts. However, consistent changes in the size of the red cells have not been observed by another group (18) after the infusion of hyperoncotic albumin solutions. In the present study, hemodilution and hemoconcentration appeared to exert slight effects on the size of the red cells, assuming that erythrocytes did not leave or enter the active circulation during the period of the observations. On the assumption that total circulating hemoglobin remains unchanged, relative changes in plasma volume may be evaluated by calculation from simultaneously observed changes in hematocrit and hemoglobin concentration (18, 19), regardless of

effects on erythrocyte size. For evaluation of absolute changes this method requires, in addition, a plasma volume determination. The method presented here allows for more precise quantitation of these changes in a simple manner, and its validity is not affected by mobilization of red blood cells from depots if such does occur.

While a thorough consideration of the physiological basis of the changes observed in the experiments reported here is not within the scope of this report, certain of the results deserve brief comment.

Calvin, Decherd, and Herrmann (20) and Stewart (21) noted early increases in plasma volume following administration of digitalis to patients in heart failure. The late effects were in reverse. In the two cases studied here, hemodilution was not noted at any time and diuresis was followed by hemoconcentration. Eichna and his associates (22) also failed to note significant changes in plasma volume prior to the diuresis produced by Digoxin.

In the cases of mercurial administration, the observation that diuresis occurs first and is followed by hemoconcentration is in accord with reports by others (23, 24, 12). The discordant finding of de Vries (25) that these changes occur only in nonedematous subjects is not borne out by the observations presented here. The absence of any change in the cases studied by Blumgart and his coworkers (26) may be discounted since in five of the 10 experiments, the excess urine loss amounted to less than 250 ml., and in no case did it exceed 1,000 ml. The present study gives no support to the concept promoted by some authors (20, 27) that a tissue effect causing a shift of fluid into the vascular system, with resulting hemodilution, precedes the diuresis produced by mercurial diuretics.

The effect of recumbency in increasing plasma volume in patients with congestive heart failure confirms the findings of Perera and Berliner (28). However, the overdilution, on return to bed, in excess of the previous hemoconcentration produced by ambulation, which was observed in all cardiac cases, has not been previously noted.

The rapid decrease of the plasma volume following its initial augmentation resulting from intravenous albumin administration is evidence of

the rapid disappearance of much of the added albumin from the circulation. Further observations and considerations of this problem are to be presented elsewhere (16).

SUMMARY AND CONCLUSIONS

1. Following intravenous administration of P^{32} labeled erythrocytes, a semilogarithmic plot of the radioactivity per unit volume of whole blood as a function of time is a straight line for at least 48 hours in subjects with unchanging blood volumes. The biological half lives in 18 experiments ranged from 12 to 39 hours.

2. This exponential relationship permits the determination of serial whole blood and plasma volumes with an error of 2–3%. It may therefore be used to quantitate rapid changes in these volumes without requiring reinjection techniques.

3. The observed alterations in blood volume produced by digitalis, mercurial diuretics, positional changes and human serum albumin infusion are briefly discussed.

ACKNOWLEDGMENTS

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