BLOOD VOLUME IN CLINICAL SHOCK.[1,2] I. MIXING TIME AND DISAPPEARANCE RATE OF T–1824 IN NORMAL SUBJECTS AND IN PATIENTS IN SHOCK; DETERMINATION OF PLASMA VOLUME IN MAN FROM 10-MINUTE SAMPLE

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Diminution in blood volume has long been considered the leading factor in the development of shock after trauma. Nevertheless, until recently (1 to 4) few attempts have been made to measure the effects of trauma on the blood volume and to correlate the degree of reduction with the clinical state of shock. During World War I, Keith (5) and Robertson and Bock (6) using the vital red method of Keith, Rowntree, and Geraghty (7) found that the blood volume was regularly 30 to 50 per cent below normal in soldiers suffering from shock after hemorrhage or trauma. The force of their evidence was weakened somewhat by subsequent criticism of the vital red technique and by a growing tendency during recent years to doubt the accuracy of any blood volume measurements in shock. Two seemingly valid assumptions supported this view. In the first place, it was assumed that the sluggish blood flow in shock must prevent uniform mixing of the dye with the circulating blood. Secondly, the prevalent hypothesis that shock is caused by a generalized increase in capillary permeability led to the belief that the dye escapes so rapidly from the bloodstream as to invalidate the measurement of plasma volume (8). Careful appraisal of plasma volume determinations with the dye method in dogs in which the full picture of shock was produced by trauma (9) has demonstrated that both of the above contentions are incorrect. If, as in these studies, the plasma volume is estimated from a time-concentration curve obtained on arterial samples, the volume determination is independent of the existing disappearance rate and mixing time.

The aim of the present investigation was to extend these observations to clinical cases of shock. The general plan was, (*1*) to make a systematic study of time-concentration curves of T–1824 in patients with various types of injuries and degrees of shock in order to evaluate the effect of shock on the time of mixing and disappearance rate of the dye, and to define the extent to which these factors influence the measurement of plasma volume in shock-like states; (*2*) to determine the extent and, if possible, the cause of blood volume reduction in human cases of shock. The results of the latter phase of the investigation are presented in a separate communication (10).

MATERIAL AND METHODS

The blood volume determinations on normal subjects have been drawn from unpublished data collected over a period of years. This series includes 95 determinations on 51 normal male subjects, who were either medical students or laboratory workers, ranging in age from 21 to 37 years. The measurements were made under essentially basal conditions.

The blood volume determinations on patients with injuries were done as a part of the cooperative study of the circulation in clinical cases of shock admitted to the emergency service at Bellevue Hospital, New York City. The general procedures followed and the criteria used for determining the degree of shock have been described elsewhere (3). The present reports include observations on 109 patients, 80 male and 29 female, summarized in Table I. With few exceptions, the blood volume measurements were begun before intravenous therapy was started.

Determinations of plasma volume were made with the blue dye T–1824 (11 to 13). A 19 gauge Ungar needle

[1] This investigation was carried out at Bellevue Hospital under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Columbia University with the collaboration of New York University.

[2] Generous support for these studies of blood volume in shock was provided also by the Josiah Macy, Jr. Foundation.

TABLE I

Case material from March 1942 to March 1944

Type of injury	No shock	Moderate shock	Severe shock	Total
Skeletal trauma	11	9	18	38
Hemorrhage	3	6	15	24
Abdominal injuries	3		5	8
Burns	6		15	21
Head injuries	8			8
Medical and misc.	7		3	10
Totals	38	15	56	109

equipped with a snugly-fitting obturator was first inserted into one of the femoral arteries and left in place throughout the determinations. All blood samples, the dye-free control as well as the subsequently dye-tinged samples, were drawn from this artery. For the injection of the dye, a second Ungar needle was placed in one of the antecubital veins. If blood flow in the vein was sluggish or if the vein was collapsed, it was gently forced open with saline from an infusion reservoir connected to the Ungar needle with a water-tight, 3-way stopcock. A measured amount of 0.5 per cent T–1824 [3] was injected through the stopcock with a calibrated 5 ml. syringe.[4] With this arrangement the syringe could then be rinsed with saline from the reservoir if repeated withdrawal of venous blood for this purpose was difficult.

The amount of 0.5 per cent dye injected for each determination was either 4 or 5 ml. depending upon the weight of the patient (approximate average dose, 0.3 mgm. per kgm. body weight). Arterial blood samples (3 to 4 ml.) were collected at 5-minute intervals during the first half-hour after the dye injection and at 10-minute intervals thereafter. These were placed in oiled tubes, permitted to clot, and centrifuged. The serum was pipetted off and centrifuged a *second time* to get rid of all red cells and oil droplets. The concentration of dye in each sample was determined with a König-Martens visual spectrophotometer (13) and the results plotted and analyzed as described below.

The plasma volume was calculated from the equation:

$$\text{Plasma volume in ml.} = \frac{V_1 \times K \times 250}{C_0}$$

where V_1 = ml. of 0.5 per cent T–1824 injected,

K = optical density of 0.5 per cent T–1824 diluted 1 : 250 in plasma or serum,[5]

C_0 = initial concentration of dye expressed as optical density and derived by extrapolation of the disappearance curve to the time of injection (see below).

Changes in the hematocrit value and serum protein concentration were followed closely during the period covered by the dye curve. Hematocrit determinations were usually made only on every other blood sample (1 ml. Wintrobe tubes, heparin, and centrifugation for 40 minutes at 3000 r.p.m.), whereas every sample was analyzed for serum protein content with an Abbe refractometer. The percentage of protein was calculated from the refractive index according to the equation:

$$\text{grams per cent protein} = \frac{\text{R.I.}_{\text{serum}} - (\text{R.I.}_{H_2O} + 0.0022)}{0.00198}$$

in which 0.0022 is the non-protein refractive index and 0.00198 the conversion factor as determined by Neuhausen and Rioch (14). Our purpose in using the refractometer was not primarily to determine the absolute level of serum protein, but to measure accurately the relative changes in protein content. As explained below, these are essential for the final analysis and interpretation of the time-concentration curves of T–1824.

The serum protein content of some of the samples was measured routinely with the falling drop method of Barbour and Hamilton (15) using the formula of Weech, Reeves, and Goettsch (16) for conversion of serum specific gravity to serum protein content.[6] Insofar as relative changes were concerned, the results with the falling drop method and the refractometer agreed precisely. However, values with the refractometric method on the patients in shock varied + 0.4 to − 0.5 grams per cent from those obtained with the falling drop method. Nitrogen determinations (few cases) gave values that usually fell in between these two.

The total blood volume has been calculated from the formula:

$$\frac{\text{plasma volume in ml.} \times 100}{100 - \text{hematocrit}} = \text{total blood volume in ml.}$$

The fact that the total volume so calculated is probably slightly higher than the true blood volume is of little significance in the present study since the results are compared with average normal values calculated in the same manner. Strictly speaking, a small correction should be applied to the hematocrit value. Gregersen and Schiro

[3] Supplied in 5 ml. ampules by the Warner Institute for Therapeutic Research, New York City. Spectrophotometric analysis of the T–1824, prepared by the Warner Institute, has shown that it is of greater purity than T–1824 obtained from other sources.

[4] Inasmuch as the dye solution obscures the end of the plunger, a line is etched on it about ¼ inch from the end to permit accurate filling of the syringe. If the full capacity of the syringe is to be used, another line is also etched on the barrel about ¼ inch above the 5 ml. mark. The syringe must be calibrated for the volume it *contains* at various settings of the plunger and *not* for delivery. This follows from the fact that the syringe is rinsed after the dye is injected, otherwise a variable amount of dye solution would remain in the needle and stopcock.

[5] The dilution of the dye in serum or plasma for the purposes of standardization is accurately and conveniently carried out with the Scholander micrometer pipette (Science, 1942, **95**, 177).

[6] The serum protein values included in the tables of the general report (3) were obtained with the falling drop method on the first arterial sample drawn from the patient after admission.

(17) and Shohl and Hunter (18) found that the relative cell volume determined by centrifugation for 30 minutes at 3000 r.p.m. is about 4 per cent higher than the true erythrocyte volume because of plasma trapped in the packed cell layer of the hematocrit tube. Chapin and Ross (19) find that the difference is somewhat greater (8.5 per cent). The question of unequal distribution of erythrocytes in the circulation and its possible effect on the estimation of total blood volume will be considered in the following communication.

MIXING TIME AND DISAPPEARANCE RATE OF T–1824

Estimates of the mixing times and disappearance rates from the time-concentration curve of a dye depend upon the assumption made regarding the exact form of the disappearance curve. Recent evidence indicates that the loss of T–1824 from the blood stream is determined mainly by the rate at which albumin itself escapes (20, 21). The function of dye-loss with time should therefore be exponential and best represented by a straight line on a semi-log plot (logarithm of the plasma dye concentration plotted against time). This conclusion has been substantiated, during the present investigation, by examination of a large number of dye curves obtained on normal human subjects and on patients with various forms of injury and degrees of shock. The semi-log plot was therefore adopted as the basis for estimating time of mixing and rate of disappearance and for extrapolation of the disappearance curve to determine the initial dye concentration, C_0.

The term disappearance rate as used here is defined as the percentage of dye lost from the circulating plasma during the first hour; that is,

$$\text{Disappearance Rate} = \frac{(C_0 - C_{60})}{C_0} \times 100$$

where C_0 is the initial dye concentration as given by extrapolation, and C_{60} is the plasma dye concentration 60 minutes after the injection.

The question as to whether or not the dye disappears from the circulation at the same rate after the second dye injection as after the first appears to be a controversial one (22 to 24). We have found no difference, but contradictory data on this point may depend upon the particular dye employed and upon the type of instrument used to measure the dye density. Instruments employing the photoelectric cell principle do not as a rule give a straight line calibration between optical density and dye concentration over a wide range. Tests in this laboratory have shown that, when the control sample contains residual dye, the values obtained with photoelectric instruments lie *not* along the original calibration curve, but along one of a family of curves each of which is determined by the amount of dye in the control sample. The result is that differences in the density of the blank, such as occur with repeated injections of dye, may introduce a considerable error. This difficulty is automatically eliminated with instruments such as the König Martens spectrophotometer, or the new Nickerson Decade Photometer, in which the difference in density between the control and the dye-tinged sample is read directly.

Correction of the time-concentration curve for fluid shifts. Rapid changes in plasma volume during the determination distort the dye curve and unless these are clearly recognized and the appropriate corrections are made for such changes, the estimates of the initial dye concentration, rate of disappearance and the time of mixing may be quite erroneous. If the blood samples are withdrawn from the veins, the possibilities of local changes created by stasis must also be considered. Changes in the circulation as a whole are eliminated so far as possible by keeping the subject at rest and quiet for some time before and throughout the period of measurement.

It soon became evident, however, in the study on injured patients that the maintenance of a steady state for any considerable period was generally impossible. Restlessness, changes in breathing, short periods of respiratory obstruction, retching and vomiting cause irregularities in the dye curve. Another major problem arose as a result of the intravenous infusions of variable amounts of saline and other solutions required for the cardiac output and renal clearance studies that were being carried out by other members of the shock team. These complications, however, provided excellent illustrations of the various ways in which fluid shifts may modify or distort the dye curve and demonstrated the necessity of making corrections for these irregularities in the original data.

The method of correcting for these irregularities is based on the assumption that during the short

period covered by the dye curve the amount of total circulating protein remains unchanged and that, therefore, changes in the plasma volume can be derived from the changes in serum protein concentration. Changes in the hematocrit value are useful as confirmatory evidence. The correction is obtained by applying the following formula:

$$D_t \times \frac{P_0}{P_t} = D_c$$

in which

D_t = observed dye concentration (expressed as optical density) at time t after the injection of dye.

P_t = serum protein concentration at time t.

P_0 = serum protein concentration in the control sample drawn just before the injection of dye.

D_c = theoretical dye concentration if plasma volume had remained unchanged.

A brief consideration of the dye curves presented in Figure 1 will show how important this treatment of the data may be for a correct interpretation of the results.

Curve 1 illustrates a case in which 300 to 350 ml. of normal saline were introduced while the auricular catheter was being inserted immediately following withdrawal of the first dye sample. The diluting effect of the saline may be seen by the sharp fall in both the hematocrit value and in the serum protein concentration. A change of the same magnitude is evident in the dye concentration between the first and second dye samples. When the above correction for the drop in serum protein is applied to the dye values, they fall on the same line. It may be noted that the plasma volumes calculated from the original and corrected dye-curve differ by 200 ml. Furthermore, from the uncorrected data it would be erroneously assumed that mixing was not complete at the time of first sample (8 minutes). However, in this instance the disappearance rate would have been correctly estimated because no further fluid shift occurred after the initial dilution.

In curve 2 (J. H.) temporary dilution with saline occurred in the middle of the dye curve as the auricular catheter was inserted. During the subsequent 40 minutes the serum protein concentration and hematocrit values gradually returned to their initial levels, and hence the corrected and uncorrected curves give the same plasma volumes and disappearance rates. Temporary dilution clearly accounts for the irregularities in the original dye curve.

Curve 3 (A. de J.) shows the effect of gradual dilution. The catheter was inserted before the dye was injected and during the succeeding one and one-half hours while the plasma volume was being determined, approximately 300 ml. of normal saline were given in order to keep the catheter open. This caused a steady decline in hematocrit and serum protein values. Adjustment of the dye curve on the basis of the dilution of the proteins does not alter the value obtained for the plasma volume. It does, however, make clear that the slope of the original curve does not represent the true rate of dye disappearance. A similar situation obtains in curve 4, but here in addition the true time of mixing is obscured. From the original curve one might assume either that mixing was complete at 7 minutes (when the first dye sample was taken) or at 20 minutes; whereas the corrected curve permits only one interpretation, that mixing was complete at 18 minutes.

The original form of curve 5 is apparently quite satisfactory, but it will be seen that the corrections for fluid shifts shorten the time of mixing from 32 to 15 minutes. The infusion of saline, started at 15 minutes, caused an apparent increase in the mixing time.

In curve 6 the dye values display such irregularities that it would be difficult to calculate the plasma volume with any degree of certainty. Saline infusion was started before the injection of dye but later, during the determination, the flow was greatly reduced. The effects of these changes may be seen in the initial fall and subsequent rise in both hematocrit and serum protein concentration. In this rather complicated situation the correction for fluid shifts straightens the disappearance portion of the curve and reveals that the mixing time was about 20 minutes and the disappearance rate 6 per cent in the first hour.

It is clear from these illustrations that correction for fluid shifts should be made a routine part of any critical measurement of plasma volume. Indeed, it is an essential preliminary in the estimation of either the mixing time or the disappearance rate. The evidence from the present investigation indicates that fluctuations in the serum pro-

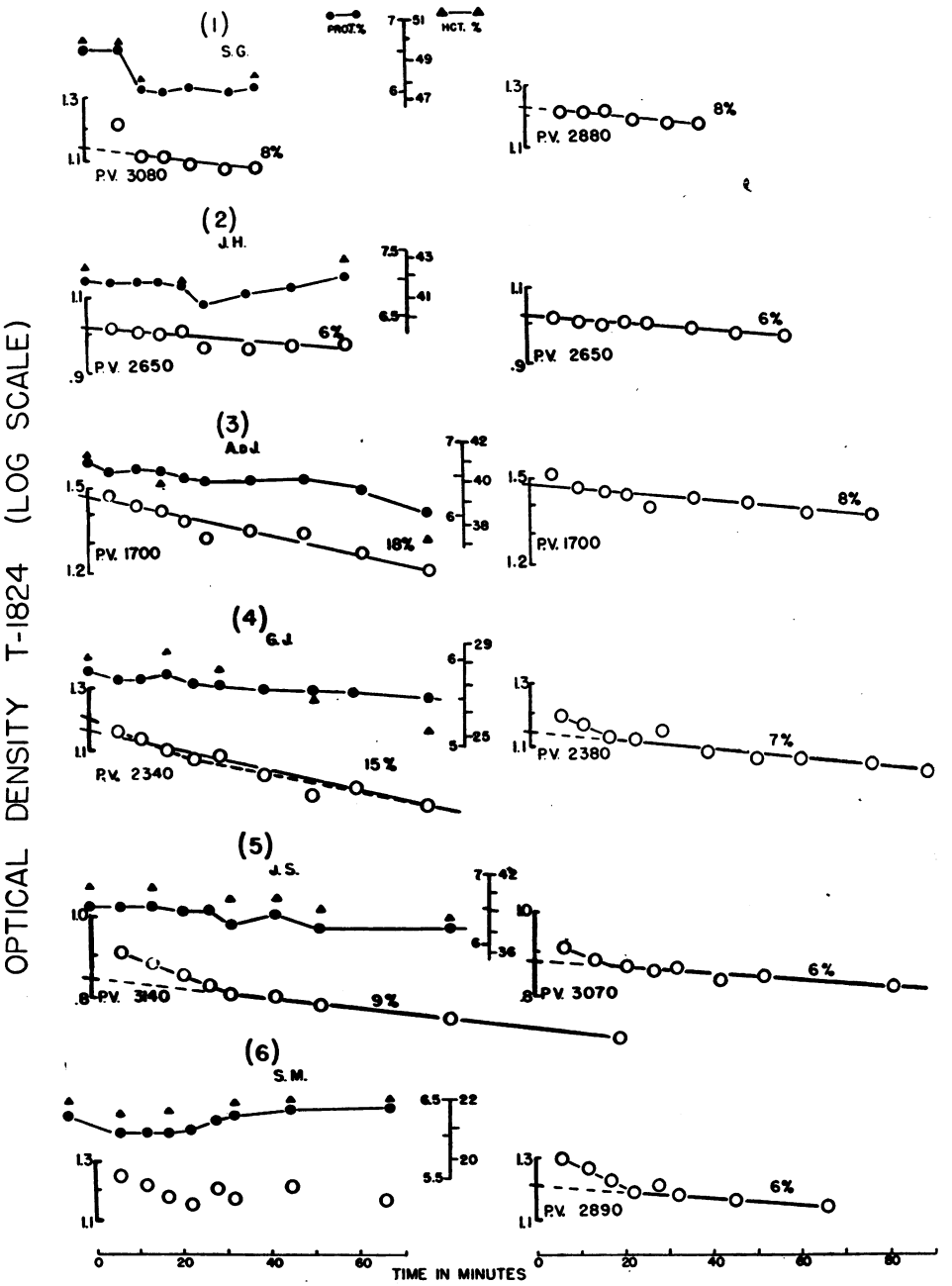

FIG. 1. TIME-CONCENTRATION CURVES OF T–1824 (OPEN CIRCLES), BEFORE (ON THE LEFT) AND AFTER (ON THE RIGHT) BEING CORRECTED FOR FLUID SHIFTS (SEE TEXT)

The magnitude and direction of the fluid shifts are shown by the change in serum protein and hematocrit levels which are indicated by solid circles and solid triangles respectively. The value of the disappearance rate (percentage) is shown above each dye curve, the plasma volume (P.V.) below the curve.

Fluid shifts occurring during a determination may give a falsely rapid disappearance rate (curves 3, 4, and 5). The mixing time may be erroneously estimated (curves 1 and 3) or obscured entirely (curve 4). In some instances, when fluid shifts take place both in and out of the plasma compartment, the original uncorrected data may defy analysis. It is apparent, therefore, that the correction for fluid shifts must be employed in any critical study of the mixing time and disappearance rate.

tein level form a satisfactory basis for evaluating the effect of changes in plasma volume on the dye curve.[7]

Dye curves in cases of skeletal trauma, hemorrhage, head injuries, and burns. Several characteristic time-concentration curves obtained with

[7] It may be mentioned here that in some of the cases of severe shock requiring emergency treatment the determination was interrupted by the transfusion of stored blood. Because of the degree of hemolysis occasionally present in bank blood, subsequent portions of these curves could not be used. It is not practicable to attempt to correct the dyed serum densities for hemolysis. Spectrophotometric analysis of hemolyzed samples by Dr. J. L. Nickerson (personal communication) have shown that several forms of hemoglobin may be present, and in variable proportions, giving an unpredictable spectral absorption curve.

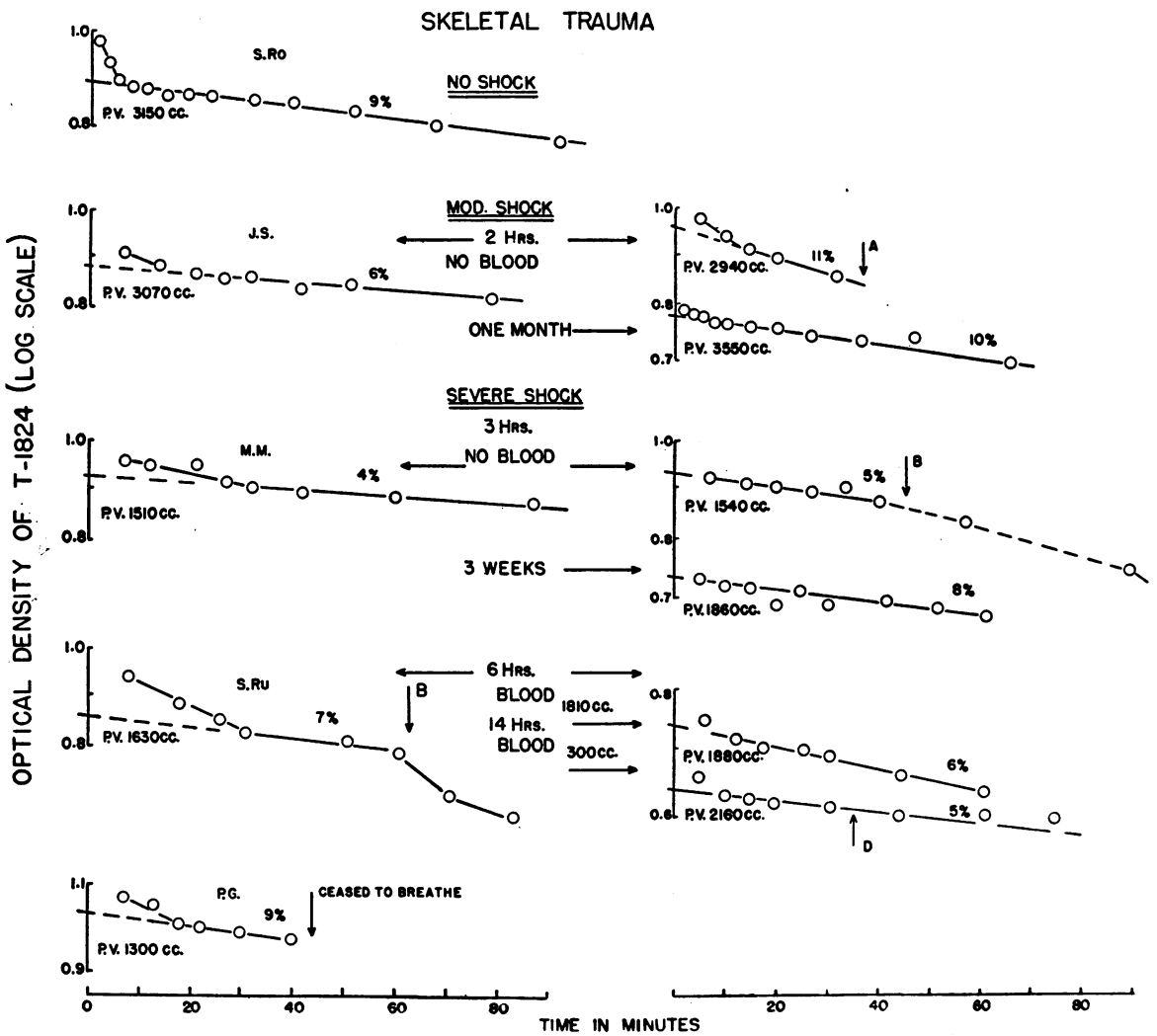

FIG. 2. T–1824 TIME-CONCENTRATION CURVES ON PATIENTS WITH SKELETAL TRAUMA. SEE TEXT FOR A BRIEF DESCRIPTION OF EACH CASE

The time interval and the amount of blood transfused between the determinations are shown between the horizontal arrows. The vertical arrow "A" denotes the time at which the plasma volume measurement was terminated by transfer of the patient to the operating room. The vertical arrow "B" shows the time at which blood transfusion was begun and "D" gives the time at which an infusion was started for the study of renal clearance. All the dye curves have been corrected for the fluid shifts as described in the text, and shown in Figure 1. Except for some prolongation of the mixing time in the shock cases, the dye curves obtained during shock appear similar to the curves after recovery or during convalescence. It may be seen that shock does not alter the fundamental pattern of the time-concentration curve.

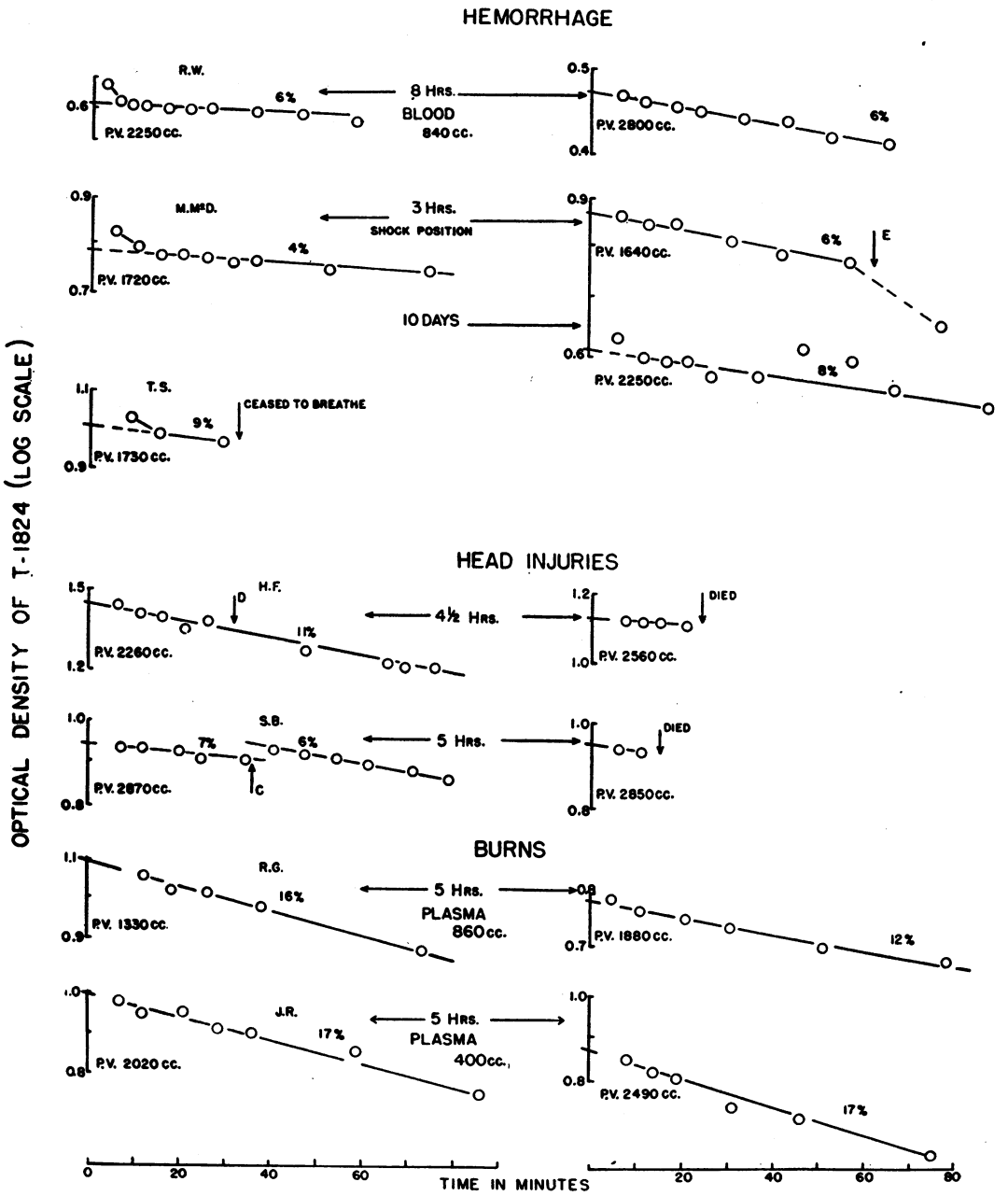

FIG. 3. T–1824 TIME-CONCENTRATION CURVES ON PATIENTS WITH HEMORRHAGE, HEAD INJURY, OR BURNS

The time interval and the amount of blood or plasma given between the determinations are shown between the horizontal arrows. The vertical arrow "C" denotes the time at which a second dye injection was given for another plasma volume measurement. The vertical arrow "D" denotes the start of infusion for the study of renal clearance. The vertical arrow "E" shows the time at which a rapid (1500 ml.) saline infusion was begun.

Note the disappearance rate of the dye in the burn cases. In shock from burns, the mixing may also be retarded, but it returns to normal at once after resuscitation. In the two cases of head injuries, note the short mixing time, even in the terminal stage.

T–1824 in cases of skeletal trauma, hemorrhage, head injuries, and burns, are shown in Figures 2 and 3. The data have been corrected for fluid shifts and plotted in the manner described. The curves on the left were obtained on patients at the time of admission and before treatment was begun unless otherwise noted (see legend). Subsequent dye curves on the same patients appear on the right. The time interval between the determinations and the treatment given are also noted.

The data on S. Ro. were obtained 3 hours after the patient had suffered a compound fracture of the left leg. The patient displayed no clinical or physiological evidence of shock at any time. The mixing time was 6 minutes and the disappearance rate 9 per cent per hour.

J. S. represents a case with more severe injury (compounded fracture of left ankle associated with extensive muscle damage) who was clinically considered to be in moderate shock. The dye curve on this patient on admission indicates a mixing time of 15 minutes and a disappearance rate of 6 per cent per hour. However, during the succeeding 2 hours the swelling around the fracture increased visibly and the patient abruptly went into severe shock. At this time the mixing time was still 15 minutes and the disappearance rate was 11 per cent per hour. One month later during convalescence the mixing time was 8 minutes and the disappearance rate 10 per cent per hour. The prolonged mixing time on the first determinations was taken in retrospect as evidence of the retarded blood flow which preceded the appearance of clinical shock.

M. M. had multiple fractures of the extremities and a fractured pelvis. She was in severe shock during the time the first two determinations were done. The first dye-curve, obtained immediately after admission, indicating a mixing time of 20 to 30 minutes, but in the second curve taken 3 hours later mixing was complete in 5 minutes. During the interim the patient had received 1300 ml. of crystalloid solutions intravenously. It will be seen that the dye disappearance rate (4 per cent and 5 per cent per hour), when the patient was in shock, was less than that (8 per cent) observed three weeks later during convalescence.

Patient S. Ru. was a case of profound shock with multiple comminuted or compounded fractures of all extremities. The dye curve obtained soon after admission indicates that complete mixing of the dye with the circulating plasma did not occur until almost 30 minutes after the dye injection. The disappearance rate (7 per cent per hour) was essentially the same as that found after the patient had been given blood and had emerged from shock.

The determination on patient P. G. is included to show that a dye curve obtained in the terminal stages of shock is comparable to those obtained on patients who recovered from shock. The dye was injected only 40 minutes before the patient died; the mixing time was 20 minutes, and as far as one can judge from the data, the disappearance rate was 9 per cent per hour.

T–1824 time-concentration curves from three patients in severe shock from hemorrhage are included in Figure 3. Although the reduction in blood volume in each case was at least 40 per cent, there was no indication of an increase in the disappearance rate of the dye. The time of mixing shows a tendency to increase with the severity of shock, as was found in cases of traumatic shock. The curve on T. S. illustrates a case in which the determination was terminated by death thirty minutes after the injection of dye.

In patients with head injuries (H. F. and S. B., Figure 3) the time of mixing was invariably short, not exceeding five minutes. This is to be expected since in these cases the blood volume was essentially normal and the cardiac output and blood pressure elevated. The mixing time continued to be short up to the time of the respiratory failure and death (see second determination on H. F. and S. B.).

Figure 3 includes also two typical dye curves observed in burns. It may be seen that these differ from all the preceeding curves in one particular, namely, the abnormally high disappearance rate.

MIXING TIME AND DISAPPEARANCE RATE OF T–1824 IN NORMAL SUBJECTS AND IN PATIENTS IN SHOCK

Determinations of mixing time and disappearance rate were made on 88 patients with various types of injuries. The results are summarized in Table II and Figure 4.[8] Included for comparison

[8] It should be noted that a considerable number of dye curves on patients had to be excluded from this analysis

TABLE II

Groups		Disappearance rates				Mixing time*			
		No. pts.	No. suitable cases†	No. of determinations	Average dis. rate 1st. hr. (S.D.)	No. pts.	No. suitable cases‡	No. of determinations	Average mixing time
					per cent				*minutes*
Arranged with respect to shock	Normal males	51	51	71	5.2 (1.65)	51	27	40	8.9
	Patients not in shock	47	43	43	8.62 (3.14)	43	40	40	7.4
	Patients in shock	41	33	33	8.56 (3.34)	28	27	29	14.0
	Recovery		22	22	7.2	21	18	25	7.0
Arranged with respect to type of injury	Skeletal trauma	38	30	30	8.9 (3.22)				
	Hemorrhage	24	17	17	7.7 (2.97)				
	Head injury	8	8	8	9.3				
	Abdominal injury	8	8	8	9.6				
	Burns	21	18	18	13.4 (5.36)				
	Miscellaneous	10	8	8	7.5				
Convalescent cases			17	17	7.7	29	17	17	7.6
All cases—all determinations†		88	88	147	7.83 (3.41)	70‡	66	121	8.8

* Includes data only to March, 1943, after which time the routine determination of mixing time was discontinued.
† Except burns. ‡ Including burns.

is an analysis of similar data obtained on 51 normal male subjects.

The first column in Table II gives the number of patients in each group; the second column lists the cases in which the disappearance rate and mixing time could be reliably estimated, while the third column shows the number of such determinations. It should be noted that the disappearance rates in burns are listed separately and are not included in the graphic presentation in Figure 4.

In normal subjects the average disappearance rate in 71 determinations after the first injection of dye was 5.24 per cent (S.D. 1.63) and in 95 determinations including re-injections it was 5.26 per cent. The range and frequency are shown in Figure 4A (open circles). The average disappearance rate in patients after injury was calculated on 43 patients not in shock and on 33 patients in shock at the time of admission. Both groups consist of patients who had suffered skeletal trauma, hemorrhage, or abdominal injuries. The average disappearance rate for patients *in shock* (8.6 per cent) is exactly the same as for those *not in shock* (8.6 per cent). In Figure 4A are found the range and frequency of these values. The cross-hatched columns represent the disappearance rates on patients in shock and the single striped columns show the values on patients not in shock. A comparison of the disappearance rates on 22 patients in shock at the time of admission and on the same patients immediately after emergence from shock following treatment (Table II and Figure 4B) show little significant change. The cross-hatched columns (Figure 4B) give the frequency and distribution of the data on the 22 patients when in shock, and the open columns give the disappearance rates after recovery. It may be concluded from these results that the presence of shock has little if any effect on the disappearance rate.

In Table II (second section) the results are grouped to bring out the relation of the disappearance rate to the type of injury. In the cases of skeletal trauma and abdominal injuries the disappearance rate is somewhat higher than in the cases of hemorrhage. This difference, if significant, may perhaps be attributed to the fact that skeletal trauma and abdominal injury are associated with dye and protein leakage from capillaries damaged locally, a factor which is absent in pure hemorrhage. The effect of local tissue damage on the disappearance rate is well illustrated by the burns in which the loss of dye during the first hour after injection ranged from 6.5 to 26.5 per cent with a mean value of 13.4 ± 5.4 per cent. These observations indicate that the change in the

because urgency of treatment prevented the dye curves from being completed. Also in a number of the normal subjects the first dye samples had not been taken until 15 or 20 minutes after the injection of dye, and consequently the length of the mixing period could not be calculated.

rate of dye disappearance is mainly related to the nature and the extent of the injury.

The frequency and distribution of the mixing time found in 40 determinations on 27 normal subjects are shown in Figure 4C (open circles). The mean values are summarized in Table II. In normal subjects the average time of mixing was 9 minutes, but from the distribution curve it will be seen that in 3 instances the mixing time exceeded 15 minutes. In 40 injured patients not in shock the dye was completely mixed in 7 minutes (single striped columns in Figure 4C).

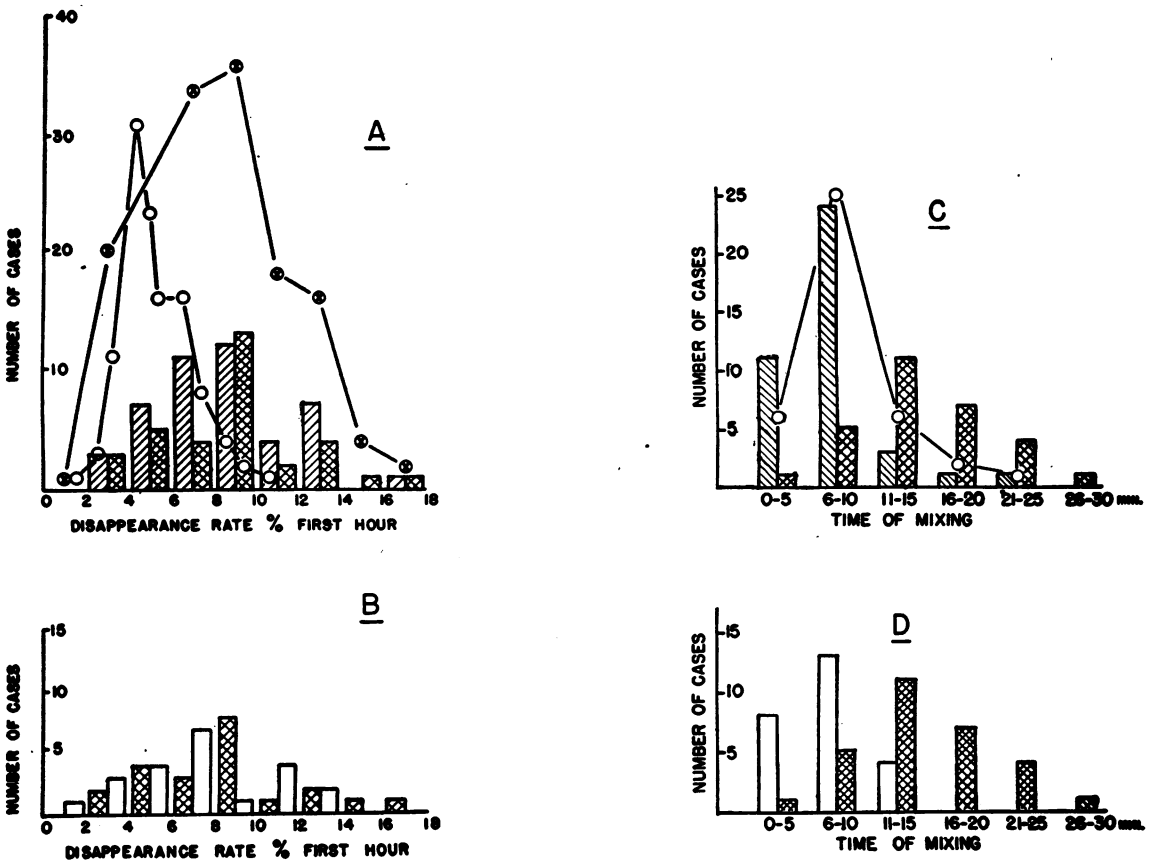

FIG. 4

CHART A SHOWS THE FREQUENCY AND DISTRIBUTION OF THE DISAPPEARANCE RATE OF T–1824 IN NORMAL SUBJECTS (OPEN CIRCLES) AND IN ALL INJURED PATIENTS EXCEPT THE BURN CASES (CROSSED CIRCLES)

The single striped columns represent the frequency and distribution of disappearance rates in injured patients *not in shock*. These are compared with the disappearance rates in patients *in shock*, shown by the crossed-hatched columns. It may be seen that the distribution of the disappearance rate is the same for both groups.

CHART B SHOWS THE FREQUENCY AND DISTRIBUTION OF DISAPPEARANCE RATES IN PATIENTS IN SHOCK (CROSS-HATCHED COLUMNS) FOR COMPARISON WITH THE DISAPPEARANCE RATES IN THE SAME PATIENTS IMMEDIATELY AFTER RESUSCITATION OR DURING CONVALESCENCE (OPEN COLUMNS)

It is apparent that the disappearance rate of T–1824 is not altered by shock (see text).

CHART C SHOWS THE FREQUENCY AND DISTRIBUTION OF THE MIXING TIME OF T–1824 IN NORMAL SUBJECTS (OPEN CIRCLES) AND IN INJURED PATIENTS

The single striped columns represent the mixing time in patients *not in shock* while the crossed-hatched columns show the mixing time in the patients *in shock*.

CHART D SHOWS THE MIXING TIME IN PATIENTS IN SHOCK (CROSSED-HATCHED COLUMNS) FOR COMPARISON WITH THE MIXING TIME IN THE SAME PATIENTS IMMEDIATELY AFTER RESUSCITATION OR DURING CONVALESCENCE (OPEN COLUMNS)

It is apparent that the mixing time of T–1824 is somewhat prolonged in shock. The averages for the various groups are to be found in Table I.

In 29 cases of shock, however, the average time of mixing was 15 minutes (cross-hatched columns of Figure 4C). The effect of shock on the time of mixing is further shown by the shortening of the average mixing time to 7 minutes in 16 of these patients after emergence from shock. This change may be seen in Figure 4D where the cross-hatched columns show the frequency distribution curve for the mixing time on patients in shock and the open columns represent the mixing time after treatment on the *same* group. It is clear from these results that the mixing time is prolonged in shock.

DETERMINATION OF PLASMA VOLUME FROM 10-MINUTE SAMPLES

The preceding analysis of the dye curves obtained on normal subjects and on patients suffering from trauma and/or hemorrhage provide, for the first time, the data necessary for estimating the probable error introduced into the measurement of plasma volume by utilizing only one dye-tinged blood sample drawn at some fixed time after the injection. Inspection of the dye curves in Figures 2 and 3 and of the frequency distribution curves of mixing time shown in Figure 4C reveals that mixing is usually complete within 10 minutes except in cases of profound shock. Even among the latter there are but few instances in which the 10-minute point is markedly higher than the initial density (C_0). This means that the fraction of the plasma remaining unmixed with the dye at 10 minutes is rarely large. It is also apparent from the data presented above that the loss of dye during the same period is relatively small. Even with a disappearance rate as high as 12 per cent per hour the loss in 10 minutes would be only 2 per cent of the total amount injected. It may be noted further that the error arising from incomplete mixing is opposite in sign to that produced by disregarding dye loss; hence the plasma volume calculated from the 10 minute point is not likely to be far different from that determined by extrapolation.

The plasma volume calculated from the 10-minute sample has been compared with the values obtained from extrapolation of the dye curve in 42 normal subjects and in 99 patients. The results are shown in Table III and in Figure 5 where open circles denote normal subjects and solid circles the patients with injuries. The results plotted in the upper graph were obtained from the dye curves after they had been corrected for fluid shifts. Here the plasma volume derived from the 10-minute sample is on the average 1.2 per cent greater than the volume obtained by extrapolation. The standard deviation is ± 1.7 per cent. The results shown in the lower graph were calculated from the uncorrected dye density at 10 minutes. As one would expect from the fluid shifts that may occur, the uncorrected 10-minute point yields a somewhat greater spread of values; the average plasma volume is 3.2 per cent greater than the value determined by extrapolation.

The order of magnitude of the additional error introduced by extending the time for sampling beyond 10 minutes is indicated in Table III by the calculations made from the 20-minute sample.

The measurement of plasma volume from the dye concentration in the 10-minute sample thus appears to be adequate for practical purposes. The error in the method is slight compared with the changes in plasma volume that are clinically significant. However, in utilizing the simplified method it is obviously important to observe every precaution in obtaining fair samples. In cases of shock, for example, the blood flow in peripheral veins is characteristically deficient, and the samples must be drawn from deeper veins or preferably from an artery. It should also be emphasized that, although the 10-minute sample is sufficiently accurate for practical purposes in normal subjects and apparently also in patients with the various types of traumatic injuries considered here, the technique may not apply in other clinical conditions. In congestive heart failure, for example, the mixing time may be extremely variable and often greatly prolonged. Whenever such variation is suspected, the plasma volume should be calculated from extrapolation of a time-concentration curve.

SUMMARY AND CONCLUSIONS

The evidence presented above indicates that shock does not cause any profound change in T–1824 dye curves. The only significant effect is the prolongation of the mixing time which is to be expected in view of the marked slowing that is characteristic of the circulation in shock. Some increase in the disappearance rate was observed

in burns and to a lesser degree in cases with peritonitis, but it should be emphasized again that by utilizing the extrapolation method the determination of plasma volume is made independent of these variations in mixing time and disappearance rate. This applies to injured patients with or without shock as well as to normal subjects. In either condition the blood samples must, of course, be representative of the circulating blood. It seems likely that inadequate attention to this detail gave rise to the inconsistent results reported by some investigators (8), who attempted to use the dye method in shock-like states.

Another source of irregularities in the dye curves is rapid shift of fluid in and out of the plasma compartment. The changes in serum protein associated with such shifts, especially if accompanied by parallel changes in the hematocrit values, appear to constitute a reliable basis for correcting the dye curves over a short period. One must not lose sight of the possibility that, under some circumstances, considerable amounts of proteins may accompany the fluid and will therefore invalidate the correction.

The evidence from the dye curves is quite clear in demonstrating that increased capillary leakage is not an essential and invariable feature of shock. This is borne out by the fact that the rate of dye loss in cases of hemorrhagic and traumatic shock was essentially the same whether or not the patients were in shock and by the relatively slight increase in the disappearance rate in those cases above the normal average value. A marked increase in the rate of dye loss was observed only in cases of burns and abdominal injuries with peritonitis. In these conditions it will be noted that there is direct damage to extensive areas of the capillary bed and evidence of continued "weeping" from the injured surfaces. Thus it appears that increased capillary leakage as measured by the dye T–1824 is related to the character and extent of the injury, and that it is not an inherent feature of shock. This is in accord with recent reports that the hematocrit and plasma protein values are below normal before treatment in shock cases among civilian and war casualties (4, 25). From our experience also it appears that in cases of skeletal trauma and hemorrhage, uncomplicated by exposure or dehydration, the initial determinations of hematocrit and plasma protein after the injury fall below the average normal values (3, 10). Evidence of hemoconcentration has been observed only in the cases of burns and abdominal injuries, which, as noted above, provide the only clear instances of increased disappearance of dye.

In reviewing the experimental evidence of hemoconcentration in shock one is impressed by the coincidence that this evidence was obtained in experiments in which the type of injury was such as to cause direct damage to a considerable area of the capillary bed and predispose toward a large local loss of fluid unaccompanied by red cells (26).

One of the important gains from the systematic analysis of a large series of dye curves has been to provide a much-needed objective basis for any practical method of measuring plasma volume in which only one dye-tinged sample is drawn at some fixed time after the injection (7, 27). The

TABLE III

Average percentage of error in plasma volume calculated from single sample

	From 10 min. sample			From 20 min. sample		
	Number determinations	Corr.* Average (S.D.)	Uncorr.* Average (S.D.)	Number determinations	Corr.* Average (S.D.)	Uncorr.* Average (S.D.)
Normal subjects	42	1.15 (1.7)	3.10 (2.6)	50	2.81 (1.9)	2.8 (3.2)
Injured patients All cases	99	1.33 (1.9)	3.30 (3.1)	116	4.08 (2.8)	5.8 (4.8)
Injured patients In shock	24	0.5 (2.5)	3.01 (4.5)	24	3.89 (2.4)	7.46 (3.2)

* Volumes as calculated from sample corrected or uncorrected for intervening fluid shifts.

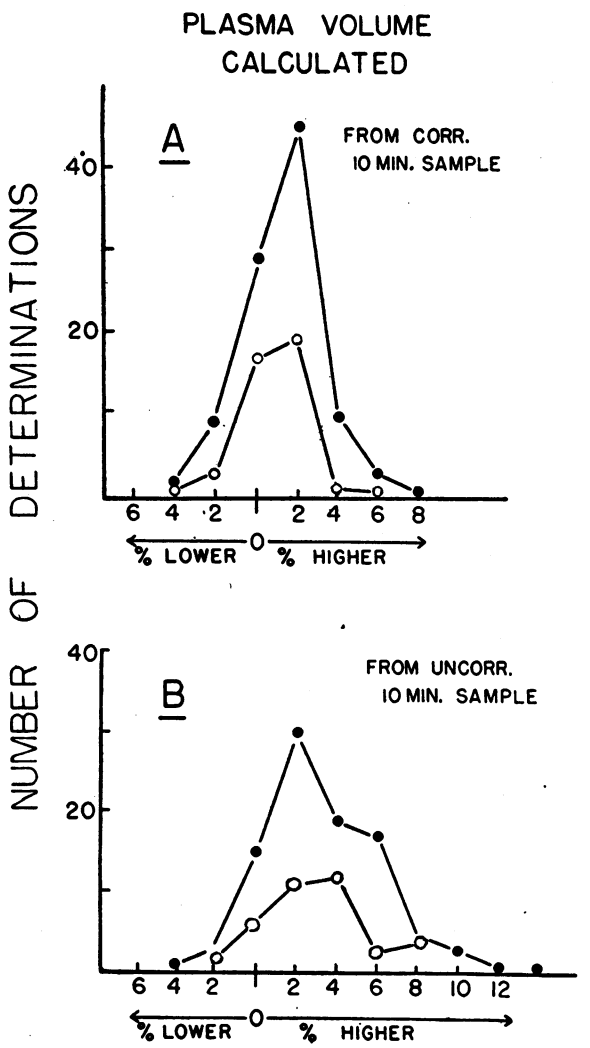

FIG. 5. THE FREQUENCY DISTRIBUTION CURVE OF THE DIFFERENCES BETWEEN THE PLASMA VOLUMES CALCULATED FROM THE DYE DENSITIES OF THE 10-MINUTE SAMPLES AND THE PLASMA VOLUMES CALCULATED BY EXTRAPOLATION OF THE TIME-CONCENTRATION CURVES

The open circles give the results on normal subjects, the solid circles the results on all injured patients.

Chart A shows the results after correction has been made for fluid shifts occurring between the time of the dye injection and the time of sampling. In chart B this correction has not been made.

evidence (see Table III and Figure 5) shows that the probable error in the plasma volume calculated from the dye-concentration in the 10-minute sample (provided this sample is collected with due precautions) is negligible (1 to 3 per cent) in comparison with changes in plasma volume that are clinically significant.

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