Radiosulphate as a Measure of the Extracellular Fluid in Acute Hemorrhagic Shock

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For many years the major components of the body fluids have been recognized as existing in two primary areas. These two heterogeneous compartments are the fluids within the cell mass of the body and fluids in the supporting extracellular structure. The fluids in the extracellular structure are further recognized as existing in two compartments: The intravascular compartment of the extracellular fluid with a more or less well defined boundary in the vascular tree; the extravascular, or interstitial, component is the most ill-defined and, therefore, the most difficult segment of the body fluids to measure.

In the healthy adult, in a resting, steady state, dilution technics utilizing primarily dyes or isotopes, have been the primary method for measurement of body fluid compartments. These dilution technics produce consistent and reproducible values in the steady state in several compartments. Commonly used technics for well-delineated compartments include: (1) ^{51}Cr-tagged red blood cells, to measure the total body red blood cell mass [2]; (2) ^{125}I-tagged serum albumin, to measure total body plasma volume [3]; and (3) Tritium-tagged water,[12] to measure total body water.

Many attempts have been made to measure the extracellular fluid. The extravascular portion of the extracellular fluid has been the most controversial area for measurement. This is not surprising, in view of the fact that any material selected for measurement of this ill-defined space must have a volume of distribution equal to the extracellular fluid, but it must first be capable of passing through the capillary wall membranes and secondly, be effectively excluded by the cell mass of the body, or the cell walls of the body. Consequently, many large molecules which are excluded from body cells are restricted by slow rate of capillary diffusion. This is a particularly severe limitation when an attempt is made to categorize acute changes in extracellular fluid in response to a change in the steady state of the body, such as occurs following injury. Consequently, small molecules such as radioactive bromide and radioactive Sulfur-35-labeled sodium sulphate, have been used largely in an attempt to measure acute changes in extracellular fluid.

The extravascular extracellular fluid is further complicated by having, normally, a rapidly equilibrating or functional, component, as well as several slower equilibrating, or relatively nonfunctioning, components. Some of the non-functional components of the total extracellular fluid include connective tissue water, as well as water which has been termed transcellular [6] and is in places such as cerebrospinal fluid. This non-functional group, including the trans-

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cellular water, have totally inadequate boundaries for measurement.

Previous reports from this laboratory have discussed the measurements of acute functional, or early equilibratable extracellular fluid using small molecules, primarily Sulfur-35-labeled sodium sulphate. Extensive studies have been reported measuring reduction in early sulphate equilibratable extracellular fluid in response to severe hypovolemic shock.[11] Subsequent reports have documented the clinical value of replacement of extracellular fluid with an extracellular "mimic" in the form of balanced salt solution in hemorrhagic shock.[10] The value of such replacement when given in addition to replacement of whole blood, has been documented in experimental animals, as well as in man.[10, 13]

The reduction in acute equilibratable, or functional, extracellular fluid in hemorrhagic shock has been differentiated from the reduction in functional extracellular fluid which occurs in response to localized trauma, such as burns or surgical trauma.[5] Surgical trauma and burns appear to produce a parasitic interstitial edema which is largely non-functional but still in the extracellular compartment.

The present report is an attempt to further define the nature of the change in extravascular extracellular fluid in hemorrhagic shock.

Materials and Methods

In the present study, a total of 36 dogs have been studied in detail. All animals were mongrel dogs weighing between 14 and 28 kilograms. Splenectomy was performed on all animals 10 to 21 days prior to the experiment. All animals were anesthetized with intravenous pentobarbital sodium, 5 milligrams/kilogram. In one-half of the animals, both femoral veins and one femoral artery were then cannulated. The arterial cannula was a nonocclusive Cournand needle. In some, a polyethylene catheter was used. In others, a carotid artery and external jugular veins were utilized instead of femoral vessels. The arterial catheter was attached to a physiologic recording device for constant monitoring of blood pressures.

Samples were counted in duplicate or triplicate as described previously.[14] Each volume distribution curve of ^{35}S-tagged sodium sulphate was obtained by injecting 200 microcuries of the tagged material into a venous catheter. Three cc. blood samples were withdrawn from a venous catheter in a different vein at 5 and 10-minute intervals for one and one-half hours; and at 20 to 30-minute intervals thereafter. When multiple injections of ^{35}S-tagged sodium sulphate were used, a background sample or samples were withdrawn immediately prior to each reinjection.

The shock preparation used in the present experiments included arterial bleeding into a heparinized graduated cylinder at a rate of 50 cc. per minute. This was permitted until the mean blood pressure fell to below 50 mm. Hg. This usually required approximately 25–30% of the animals' estimated or previously measured blood volume. Occasionally, additional 25 to 50 cc. aliquots of blood were removed during the first 30 minutes of shock to maintain a blood pressure below 50 mm. Hg. Thereafter, the mean blood pressure during shock ranged between 50 and 65 mm. Hg. The treated animals received their shed whole blood with or without balanced salt solution over a 15-minute period and at least 15 to 30 minutes additional time was allowed before background samples were drawn prior to reinjection of isotopes.

The animals were divided into seven groups of experiments: (1) The first group of animals received radiosulphate injection and the equilibration curve of sulphate was followed from 3 to 6 hours. Following a shock period of one hour, the isotope was reinjected and again samples were continued for 6 hours. In this group of animals, no therapy was given. Urinary losses were

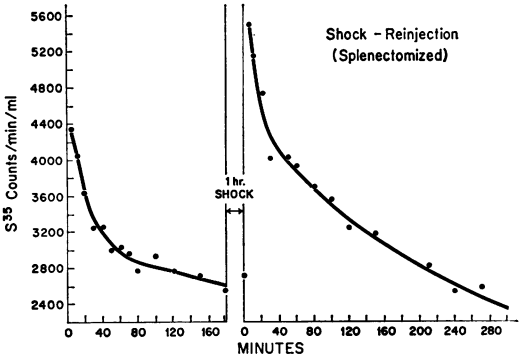

FIG. 1. Radiosulphate equilibration.

measured. Sequential urinary loss was measured via ureteral catheters and careful isotope monitoring of urinary loss of isotope was performed.

Detailed analyses of volume distribution curves in this group of animals reveals that there was a changing rate of primary loss of isotopes between the control and the shock state. In order to eliminate urinary loss from the volume distribution curve, all subsequent animals were prepared by the placement of polyethylene loops around the renal pedicles bilaterally, including the renal artery, vein, and ureter. These loops were put in position at the time of splenectomy 10 to 21 days prior to the experiment. The renal pedicle loops were located beneath the skin in the flank at the time of the original operation. At the time of the experiment, a 1 to 2 cm. skin incision was made, and the renal pedicle loops were tightened. All animals were catheterized and in none was there urinary output after tightening the renal pedicle loops. Furthermore, autopsy was done at the completion of experiments to determine the adequacy of the renal pedicle occlusion.

(2) Animals in Group 2 had previous splenectomies and renal pedicle loops were tightened prior to the beginning of the experiment. Radiosulphate was injected and 3 to 4-hour equilibration curves were determined. Following a one hour period of light anesthesia but without any blood loss or shock, radiosulphate was reinjected. Similarly, 3 to 5-hour equilibration curves were then obtained. These animals served as controls.

(3) In Group 3, animals were injected with radiosulphate and volume distribution curves were determined over a 3 to 5-hour period. Subsequently, reinjection with sodium sulphate was performed after shock had been established for one hour. These animals were then untreated and remained with a blood pressure from 40 to 80 mm. Hg throughout the reinjection period. Again, 3 to 5-hour equilibration curves were determined while the animals were in shock.

(4) Group 4: This group of animals had 3 to 5-hour equilibration curves after ^{35}S-sodium sulphate injection. Shock was then induced for one hour. The shed blood was then reinfused and 15–30 minutes later, a reinjection volume distribution curve for sodium sulphate was measured. In one-half of this group of animals, the reinfusion of shed blood was performed after a 2-hour period of shock. Reinjection technic was again used and volume distribution determined after reinfusion of blood.

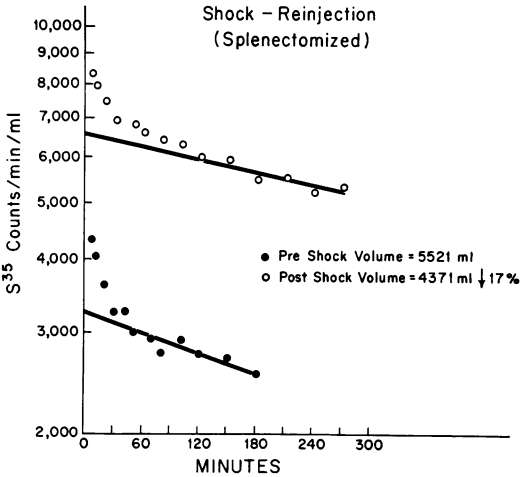

FIG. 2. Radiosulphate equilibration—semilogarithmic plot.

An additional three animals were studied, receiving the same technic as animals in Group 4, but without the renal pedicle looping.

(5) Group 5: A study was made in this group of animals by performing a radiosulphate volume distribution curve over a 6-hour period, followed by production of hemorrhagic shock for one hour. Subsequently, as in Group 4, the shed blood was reinfused, but in Group 5, an additional infusion of 5% of body weight as Ringer's lactate solution was administered. Subsequently, reinjection of sodium sulphate was made with measurement of volume distribution curves.

(6) Group 6: This group of animals had radiosulphate volume distribution curves measured, followed by 4-hour periods of hemorrhagic shock with reinjection. Subsequently, the shed blood was reinfused and in addition, 5% of body weight as Ringer's solution was administered. Following reinfusion of blood and infusion of balanced salt solution, reinjection of radiosulphate was made and 3–6-hour volume distribution curves were obtained.

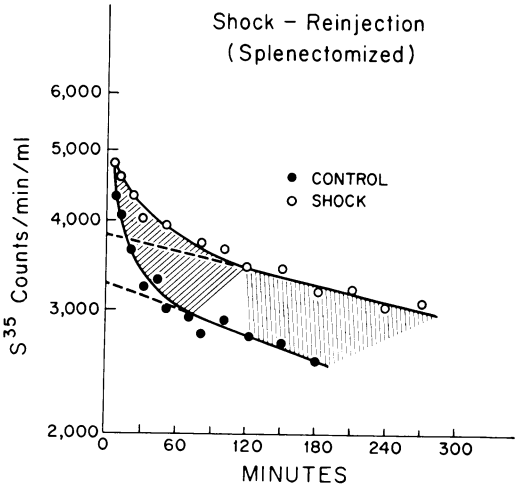

FIG. 3. Radiosulphate equilibration—semilogarithmic plot, normalized.

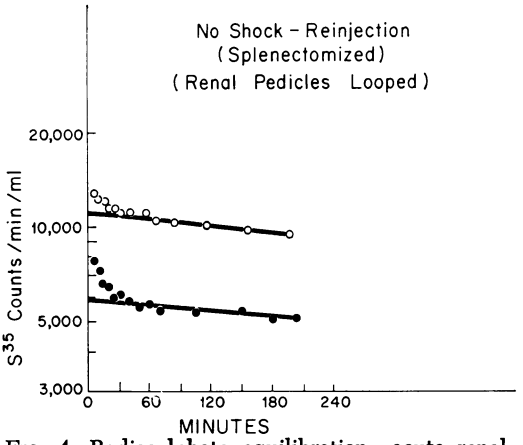

FIG. 4. Radiosulphate equilibration—acute renal pedicle looping.

Results

Group 1. The results of the equilibration curves of radiosulphate before and after untreated hemorrhagic shock can be seen in Figure 1. The normal sulphate volume distribution curve is seen in the pre-shock state. As obtained by Walsesr, Ryan, Murphy and others, the normal early volume of distribution of sulphate demonstrates a 20–30-minute mixing period, followed by a slower second phase disappearance slope. This figure represents a linear plot of the volume distribution curve. The initial early mixing stage which lasts from 20–40 minutes, is generally presumed to be a combination of intravascular, as well as extravascular, mixing of the injected isotope. The second, or slow, linear disappearance curve is presumed to be a combination of urinary loss of sulphate, as well as sulphate penetration into either a non-functional extracellular fluid, such as connective tissue, or into an organic binding in various organs of the body.[16] After one hour of hemorrhagic shock, untreated, the reinjection of radiosulphate shows a similar early mixing dilution curve which then assumes a sharp slope linear disappearance curve. Urinary losses were measured during the equilibra-

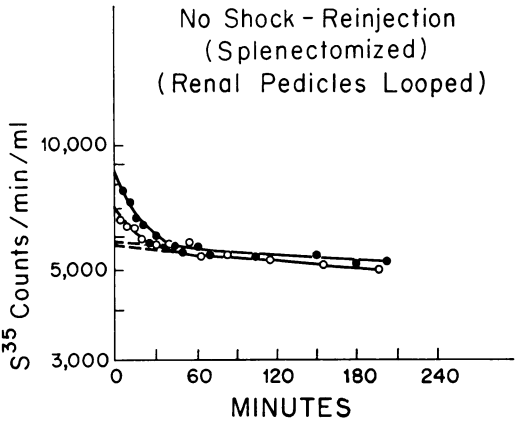

FIG. 5. Radiosulphate equilibration—acute renal pedicle looping, normalized.

tion period and were subtracted from the injected isotope in the control state. During the shock study, urinary output was reduced to virtually zero and, consequently, urinary isotope loss is greatly reduced.

Most of our early studies on the extracellular fluid in hemorrhagic shock showed similar curves. Volume distribution can be calculated at any point from 40 minutes to 5 hours on a linear plot using the formula $VD = D - E/P$

where: VD = Volume of distribution in ml.
D = Administered dose
E = Amount excreted, and
P = Plasma concentation in units per ml.

Consistent reduction in volume of equilibrated extracellular fluid is obtained. Using this linear plot, it is obvious that the calculated volume of distribution during hemorrhagic shock is consistently reduced, in that the early equilibrating extracellular fluid is reduced.

However, for materials such as those used to measure acute changes in the extracellular fluid, which do not come to final stable equilibration at any time, the formula used above is inadequate. To compensate for this, an equation as outlined by Ryan [8] is used to calculate for zero time: that theoretical moment when all the injected material is uniformly distributed in its assumed volume of distribution, thereby compensating for the material being subject to excretion, metabolism, or slower diffusion into relatively less functional fluid compartments. This equation would then become: $VD_0\ D/P_0$

where: VD_0 = Volume of distribution in ml. at "zero time"
D = Administered dose, and
P_0 = Extrapolated "zero time" plasma concentration in units per ml.

When the values in Figure 1 are transposed to a semilogarithmic plot in order to obtain extrapolatable "zero time," the resultant configuration is shown in Figure 2.

Two things can be seen using this semilogarithmic plot. First, it can be seen that the equilibration time in the normal state is between 20 and 40 minutes, whereas in the post-shock state, the time for early second phase equilibration is delayed to somewhere between 2 and 3 hours. Therefore, the linear disappearance slope during the shock phase appears at approximately 120–

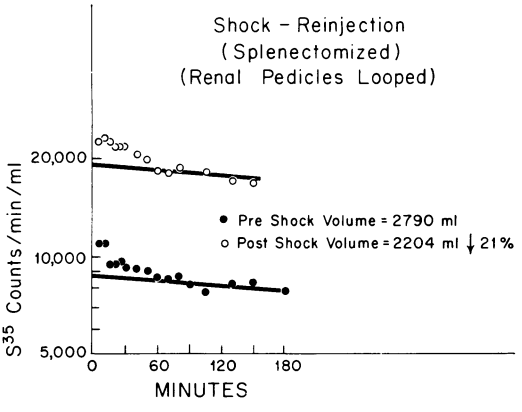

FIG. 6. Radiosulphate equilibration—acute renal pedicle looping, before and after hemorrhagic shock.

180 minutes. Consequently, it is obvious that the normal pre-shock, two-phase volume distribution curve has changed to one with three components. There is an early mixing component lasting 20–40 minutes, a second component lasting from 40 minutes to 120 minutes, and a third linear component beyond 120 minutes. Secondly, the disappearance slope of the final equilibrated curve can be seen to be different in the normal and in the post-shock state. The steeper final slope in the normal state undoubtedly represents additional loss via the urinary route which was not present in the shock state.

Using semilogarithmic plot, it is also possible to compare the early points in a volume distribution curve with the late equilibration points. This has been done as presented in Figure 3. In the pre-shock period, a final equilibrated "zero time" extrapolation point can be ascertained during shock. When these curves are "normalized" from Figure 2 by withdrawing the background from the "zero time" normal equilibrated volume, the two curves are then comparable, as illustrated in Figure 3. It is obvious from this comparison that there is a marked second component of the early equilibration curve during hemorrhagic shock. It is also apparent in this untreated shock preparation, that the final equilibrated volume represents a greatly reduced, slowly equilibrating, or total, extracellular fluid. Consequently, there is a reduction in the early equilibratable extracellular fluid and, in this preparation, also a reduction in the total or slowly equilibrating extravascular extracellular fluid. Once again, the difference in final disappearance slope is doubtless due to the difference in urinary formation during the normal state distribution curve.

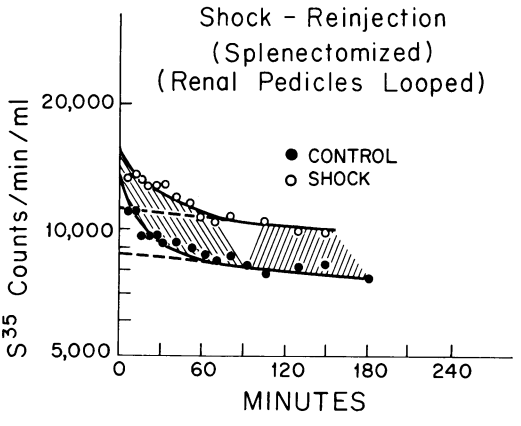

FIG. 7. Radiosulphate equilibration—acute renal pedicle looping, normalized, before and after hemorrhagic shock.

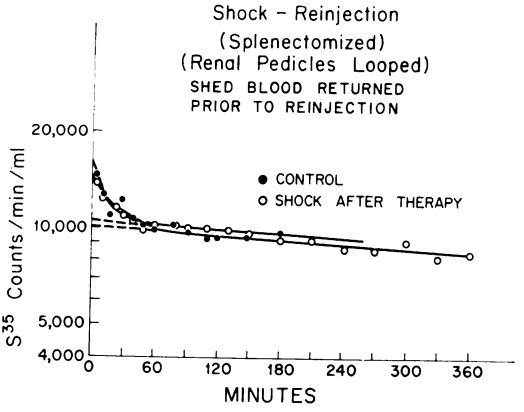

FIG. 8. Radiosulphate equilibration curve—renal pedicle looping, normalized before and after shock treated with whole blood.

In order to obviate the change in the extrapolatable equilibration slope due to urinary loss, all subsequent animals in the present study received a polyethylene renal pedicle loop which was applied to both renal pedicles at the time of splenectomy, as described previously. This was done not only to avoid the urinary loss of isotopes during equilibration, but also to avoid the addition of operative surgery at the time of the shock experiment. Furthermore, the renal pedicle loop was brought about following a series of experiments in which the ureters were looped in order to achieve a closed extracellular fluid. When this was done, it was found that there was concentration of isotope, or dye, within the kidney

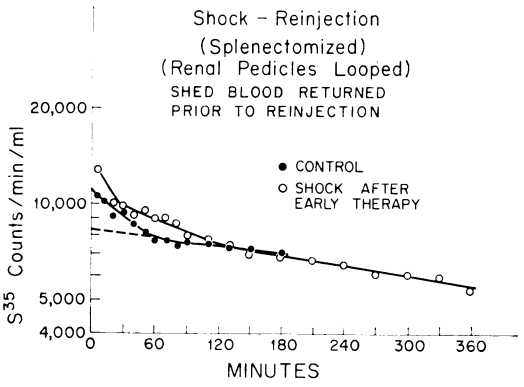

FIG. 9. Radiosulphate equilibration curve—renal pedicle looping, normalized before and after shock treated with whole blood.

which, when the animal was shocked, would reappear in the plasma. As a consequence, it was learned that ureteral ligation will not produce a closed extracellular fluid. No such problem existed when the entire renal pedicle was looped acutely just prior to the beginning of all subsequent experiments.

Group 2. In this control group of animals previously splenectomized, the renal pedicle loops were drawn tight to occlude the renal pedicle immediately prior to the experiment. At this time, radiosulphate was injected and the volume distribution curve was followed for 3–5 hours as shown in Figure 4. In these control animals, a reinjection of radiosulphate was made after 5 hours and extracellular fluid volume measured again.

When these results are plotted on semilogarithmic plot, it can be seen that in the control state the reinjection technic adequately measures the extracellular fluid. Furthermore, as demonstrated in Figure 5, when these volume distribution curves are normalized by subtracting the "zero time" equilibrated background remaining after the first injection, the volume distribution curves are then comparable. In these control animals the early equilibratable fluid, or early functional extracellular fluid, is identical in the same animal on two different occasions. Similarly, it can be seen that the total extracellular fluid or final equilibrated extrapolatable fluid volume is identical using the reinjection technic in control animals. Arterial sampling was done via both the femoral and the carotid artery in this group. Ligation of the femoral artery for catheterization did not change the functional or total extracellular fluid.

Group 3. The Group 3 animals had long equilibration curves with radiosulphate followed by hemorrhagic shock which was untreated and then followed by reinjection of radiosulphate with volume distribution curves being remeasured. It can be seen from Figure 6 that the pre- and post-shock volumes of distribution follow the same pattern as was demonstrated in Figure 3, with one exception. In Figure 6, there had been renal pedicle looping and, consequently, the decay slope of the final equilibrated curve was the same in the pre- and post-shock periods. It can also be seen that there is a delay in the early equilibration curve in the post-shock period.

When these curves are normalized, as described previously (Figure 7), it can be seen that there is a delay in the early equilibration curves and that there is also a remarkable change in the final equilibrated volume of distribution measured from the extrapolatable "zero time" volume distribution. Consequently, this group of animals confirms the findings of the early work which was depicted in Figure 3, in that there is a reduction in early equilibratable or functional extracellular fluid induced by severe hemorrhagic shock. Furthermore, there is a reduction in the total or final equilibrated extracellular fluid as measured by radiosulphate during untreated shock.

Consequently, it can be seen that in prolonged untreated shock there is a reduction in both the early equilibratable or functional extracellular fluid and the late equili-

bratable or total extracellular fluid. When the renal pedicles are looped, the final equilibrated radiosulphate volume presents a linear slope which is not quite "zero." This slope probably represents loss of sulphate into either a non-functional area or, as Walser has demonstrated, represents loss of sulphate into organic binding sites in organs such as muscle and liver. In any event, the continued slow loss of sulphate following equilibration does not influence the final equilibrated value when extrapolated to zero, since the rate of fall of the final equilibrated slope is identical in the pre-shock and shock experiments.

Group 4. In this series of experiments, animals had previously been splenectomized, renal pedicle loops were drawn tight at the beginning of the experiment and normal 3 to 5 hour volume distribution curves for radiosulphate were determined. Following this, the animals were put into hemorrhagic shock for *one* hour by the technic described, following which the shed blood was returned. Following the return of shed blood, volume distribution curves for radiosulphate were remeasured. A similar group of animals was also studied in which the shock period was held for *two* hours prior to reinfusion of shed blood and reinjection determination of the volume distribution of radiosulphate.

It can be seen from Figure 8 that in those animals in which the shed blood was reinfused after *one* hour of hemorrhagic shock, there was no deficit in either early equilibratable or functional extracellular fluid nor in the total extracellular fluid. These animals' blood pressure recovered promptly following this relatively mild episode of hemorrhagic shock and, consequently, there was no alteration in the extracellular fluid remaining. In the subsequent group of animals in which shock was allowed to continue for *two* hours prior to return of shed blood, the results can be

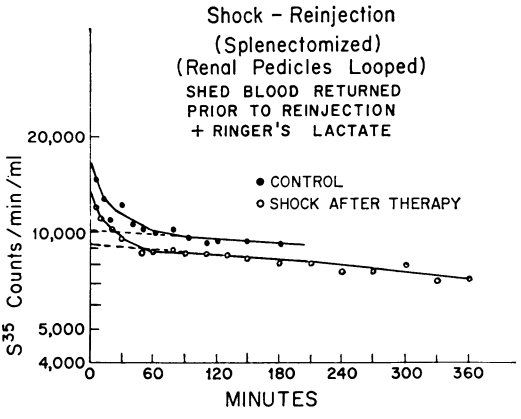

FIG. 10. Radiosulphate equilibration curve—renal pedicle looping normalized before and after shock treated with whole blood and lactated Ringer's solution.

seen in Figure 9. In this group of animals, a reduction in early equilibratable or functional extracellular fluid can be seen persisting for approximately two hours. Since these animals at this time had had blood pressures restored to normal levels, there appeared no defect in the total extracellular fluid in that the delayed or final equilibrated extracellular fluid volume, from extrapolated zero time, was identical pre- and post-shock, with treatment. Additional animals were studied in this group without renal pedicle looping in order to be certain that renal pedicle looping in itself was not influencing the change in functional extracellular fluid and identical results were obtained.

It is likely that the first 20 minutes of a radiosulphate volume distribution curve, in the normal state, represents intravascular mixing. The second phase lasts only a few minutes and may well represent transcapillary equilibration. This is followed by the third, and final diluted concentration which is final radiosulphate equilibratable extracellular fluid.

In the shock state, the first and second phases are prolonged and slowly equilibrating, demonstrating what may well be a "perfusion" defect, or reduction in early

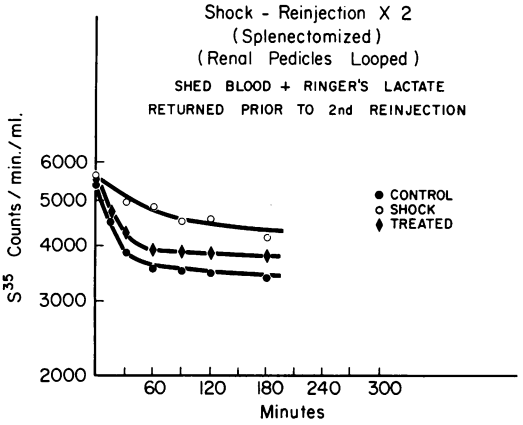

FIG. 11. Radiosulphate equilibration before and during shock and repeated after therapy.

functional ECF. If shock persists, then the total radiosulphate ECF is also reduced. On the other hand, if shock is of short duration only the perfusion defect, if any, may be seen.

This group of experiments resolved much of the conflict concerning the role of the functional or the total extracellular fluid in response to hemorrhagic shock. It is apparent from this group of experiments that if the shock is treated early by reinfusion of blood, there will be no deficit in either functional or total extracellular fluid. If the period of shock is prolonged prior to reinfusion, then one sees a functional deficit in extracellular fluid only. On the other hand, if shock goes untreated, as demonstrated in Group 3, then there is consistently a reduction in both functional or early equilibratable and total or late equilibratable extracellular fluid. If treatment is delayed for several hours, then there may be a reduction in functional and total extracellular fluid which is not correctible by the return of shed blood alone.

Group 5. This group was similar to the previous group, in that the animals had radiosulphate volume distribution curves determined, followed by one hour of shock, followed by reinfusion of shed blood. In this group, an additional infusion of 5% of body weight as balanced salt solution was given. Following this therapy, volume distribution curves for radiosulphate were again determined. It was found that in this group of experiments there was no reduction in either early functional or late total extracellular fluid. Furthermore, the final equilibrated extrapolatable volume distribution curve for the extracellular fluid reflected the expansion of the extracellular fluid by the amount of balanced salt solution administered. Since renal pedicles were looped, one would expect to see the extracellular "mimic" reflected, since the extracellular fluid was essentially a closed system. This can be seen in Figure 10.

Group 6. The Group 6 experiments were planned to demonstrate the production of functional and total extracellular fluid volume deficits by hemorrhagic shock and the effect of return of shed blood plus an extracellular "mimic" upon these volumes. To this end, volume distribution curves were determined, the animal was placed in shock for one hour, volume distribution curves were then again determined over an additional 3-hour period while the animal was still in shock. Following this total of 4 hours of shock, the shed blood was returned and in addition 5% of body weight as Ringer's solution was infused. Following therapy, volume distribution curves for radiosulphate were determined for a third time. The results indicate: (1) the second volume measurements in this group of experiments always showed a reduction in both functional and total extracellular fluid; (2) the third volume distribution determination following infusion of shed blood plus balanced salt solution showed an abolition of the functional and total extracellular fluid deficit or a lessening of these deficits. In some instances, as shown in Figure 11, there is near repair of the extracellular fluid defect.

Consequently, the spectrum of change in the early and late equilibratable extracellular fluid can be seen in Figure 12. When

hemorrhagic shock is induced and no therapy is administered, there is a consistent reduction in functional extracellular fluid, as well as a consistent reduction in total extracellular fluid. When shock is of short duration and treated with either blood or blood plus balanced salt solution, the total extracellular fluid defect is abolished and a functional extracellular fluid defect may remain for 3 hours. If, on the other hand, the shock is of very short duration and is treated quite early, then subsequent 3 to 5-hour measurements would reveal no remaining deficit in functional or total extracellular fluid.

Discussion

The present group of experiments have served to clarify some of the earlier work on the extracellular fluid in response to hemorrhagic shock.[11] As illustrated in Figure 1, the earlier determinations of volume distribution of small molecules such as radioactive Sulfur-35-tagged sodium sulphate, demonstrated quite clearly that there was a change in the distribution of the small molecule. The linear plot of volume distribution sampling, as demonstrated in this figure, reveals a distinct slowing of the early equilibration of radiosulphate in response to hemorrhagic shock. It was also seen that this was true when the urinary loss of isotopes was measured and accounted for in the control and in the shock period. Further examination of the long-term equilibration curves for radiosulphate as demonstrated in Figures 2 and 3, illustrates a change in the characteristics of the distribution curve of radiosulphate in hemorrhagic shock. It can be seen that in the normal pre-shock state, radiosulphate volume distribution has a two-component curve. The first component lasting 20–40 minutes is assumed to represent intravascular and extravascular mixing. The second portion of the normal curve represents a final equilibrated concentration which can

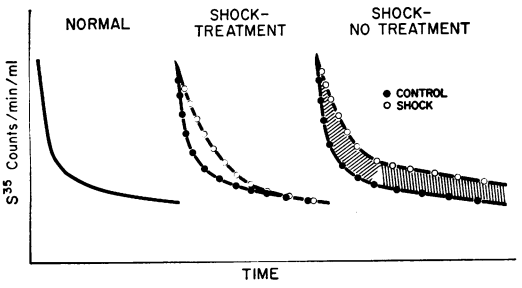

FIG. 12. Radiosulphate equilibration curve—semilogarithmic plot, summary model.

be plotted semilogarithmically as demonstrated in Figure 2 and extrapolated to zero time to get a theoretical instantaneous mixing volume. When this is done, there is apparently a continuation of the fall in the plasma activity of radiosulphate. This is presumed due to at least two components: (a) urinary loss, and (b) penetration of sulphate into a nonfunctional space or into an organified form. In the post-shock state the urinary loss component of the declining sulphate slope is obviated. As demonstrated in Figure 3, when the pre- and post-shock volume distribution curves are normalized, by subtracting the remaining background of radiosulphate at the time of reinjection, volume distribution curves can be compared before and after shock. It is demonstrated that there is a consistent defect in the early equilibratable or functional extravascular extracellular fluid. Further, in hemorrhagic shock which is untreated, it can also be seen that there is a defect in the late or final equilibrated or total extracellular fluid. Consequently, detailed examination of the volume distribution curves in untreated hemorrhagic shock in splenectomized animals demonstrates a reduction in both the functional and total extracellular fluid.

Since there is obviously a small but definite continuing penetration of sulphate into some space or some area other than the extracellular fluid, the experiments were designed to eliminate urinary loss of sulphate.

in order to make the extracellular loss of radiosulphate constant in the pre- and post-shock periods. Consequently, all subsequent experiments were performed with splenectomized animals, placing polyethylene loops about the renal pedicles at the time of splenectomy, 2 or 3 weeks prior to experimental procedure. Renal pedicle loops were drawn tight at the time of the experiment, the extracellular space was essentially converted to a closed space by eliminating urinary loss. In this fashion, using semilogarithmic plot, the final equilibrated slope of radiosulphate could be extrapolated to "zero time," or that theoretical moment when all injected radiosulphate is uniformly distributed in its volume of distribution. Since any subsequent loss of sulphate was not due to excretion, but rather to metabolism or diffusion into other areas, these components were converted to a constant that was then comparable in the pre- and post-shock period. As demonstrated in Figures 4 and 5, this allows comparison of the early, as well as the final equilibrated values of radiosulphate. By normalizing these curves, by removing background from previous radiosulphate injection, the accuracy of repeated measurement of the extracellular fluid under these circumstances can be seen.

Using this reinjection technic in splenectomized and acutely renal pedicle looped dogs, the volume of distribution curves in various types and degrees of hemorrhagic shock can be measured. It can be seen from Figures 6 and 7 that the volume distribution of radiosulphate in hemorrhagic shock which is untreated demonstrates two distinct changes during the shock period: (a) there is a delay in the functional or early equilibratable extracellular fluid which lasts from 1 to 2 hours; (b) the subsequent final equilibrated value for radiosulphate during hemorrhagic shock reveals a volume of distribution which is far less than the volume of distribution of the same tagged anion in the normal state in the same animal. Consequently, a profound defect in the early sulphate equilibratable or functional extracellular fluid exists in untreated hemorrhagic shock. Similarly, a profound defect in the total, or final radiosulphate equilibrated, extracellular fluid exists in the untreated hemorrhagic shock.

Several workers have demonstrated difficulty in determining functional extracellular fluid in hemorrhagic shock in both animals and man. Maloney recently reported a "delay in equilibration" in the radiosulphate volume distribution curves during hemorrhagic shock.[7] Anderson recently reported a "delay in equilibration" in radiosulphate in shock casualties when measurements were made after therapy had been started.[1] Shizgal [4] reported varying changes in results of 2-hour measurements of extracellular fluid volume following hemorrhagic shock. Vineyard [15] reported on the use of radiosulphate and radiobromide in measuring extracellular fluid volume in animals in hemorrhagic shock that had been shocked one hour and treated prior to measurement. Schloerb [9] reported delay in equilibration in the measurement of extracellular fluid with Sulfur-35-tagged sodium sulphate in animals that had been acutely nephrectomized. It occurred to us that the variation in these experimental results might well depend upon several critical factors: (1) the degree of severity and duration of hemorrhagic shock; (2) the length of time used for the equilibration curves; (3) the effect of varying forms of therapy upon the measurement of the extracellular fluid with small anions; and (4) the effect of acute surgical trauma, such as nephrectomy. Consequently, as reported in the present experiments, the severity and duration of the shock preparation was varied as was the therapy, including early or late return of shed blood prior to measurement of the extracellular fluid. In these experiments, each animal served as its own control and the normal volume distribution curves over a 3 to 6-hour period were measured prior

to shock or shock and therapy, then remeasurement was made of extracellular fluid volume. When this was done, as demonstrated in Figures 8, 10 and 11, it can be seen that the differences in measurement of extracellular fluid can be easily explained.

It is apparent from the results reported here that, if long-term equilibration curves are measured prior to and during hemorrhagic shock without therapy, there is a consistent reduction in functional, as well as in total extracellular fluid. It is equally apparent that if hemorrhagic shock is of short duration or is treated very quickly after its onset with the return of shed blood, the reduction in total extracellular fluid upon reinjection is no longer measurable, and there may only be measurable a functional extracellular fluid deficit up to 2 hours by the reinjection technic. Similarly, if therapy is instituted quite early and extracellular fluid volume measurements are made prior to and immediately following early therapy, such as return of shed blood, no defect in extracellular fluid may be seen. In these experiments, the blood pressure was returned to normal in every instance very quickly. All animals survived and there was apparently no measurable remaining deficit in functional or total extracellular fluid.

It appears at the present time there is no ideal substance to measure the extracellular fluid during rapid and acute changes in the body fluid composition. The difficulties with even small molecule measurement of the extracellular fluid, as outlined, include:

1. The use of a pure, untreated shock preparation without concomitant surgical trauma;

2. Loss of small molecule into urine and non-functional sites;

3. Change in slope and equilibration time induced by the abnormal state.

Consequently, there appears to be a real need for a more direct measurement of changes in body fluids. Studies at the present time are therefore being directed toward direct measurents of intracellular and extracellular composition.

Summary

1. Six groups of experiments have been designed to study in detail the use of radioactive Sulfur-35-labeled sodium sulphate, for measurement of the extracellular fluid in normal state and in severe hemorrhagic shock.

2. It has been determined that there is a constant defect in the early equilibratable, or functional, extracellular fluid induced by untreated, sustained hemorrhagic shock. There has also been determined a defect in the late, final equilibrated, or total, extracellular fluid as measured by radiosulphate in untreated hemorrhagic shock.

3. The extracellular space has been converted to an essentially closed system by the use of acute renal pedicle occlusion with polyethylene loops. Surgical trauma at the time of the shock preparation is avoided by placing polyethylene loops at the time of splenectomy 10–21 days prior to experiments. This technic eliminates urinary loss of radiosulphate and reduces the disappearance slope of equilibration to a small constant.

4. It is apparent that the variation in experimental results obtained by different investigators would depend upon several critical factors:

a. The degree of severity and duration of hemorrhagic shock.
b. The length of time used for equilibration curves.
c. The effect of varying forms of therapy.
d. The avoidance of surgical trauma at the time of shock studies. In states of acute change, meticulous and rigid control of these variables is necessary for determination of extracellular fluid.

5. It is apparent that no ideal tracer substance is currently available which will

quickly and adequately measure the extracellular fluid during acute change. Direct technics for measurement of intracellular and extracellular composition are now being carried out.

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