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THE EFFECT OF SHOCK ON BODY FLUID DISTRIBUTION

EXPERIMENTAL SURGERY

MASTER OF SCIENCE

Recent reports have stressed the importance of maintaining the extracellular water (ECW) volume in the patient in shock by administering large volumes of balanced salt solution. The present study was undertaken to determine whether a significant decrease in the extracellular water volume occurs in experimental hemorrhagic shock or in a series of patients with septic shock.

Multiple isotope dilution techniques were employed for simultaneous measurement of the total body water (TBW), ECW and plasma volume. In addition, red cell mass was measured in many of the human studies.

In hemorrhagic shock multiple tracers of varying function and molecular size were used. Deficits observed were not statistically significant and could be accounted for by vascular compartment loss due to the withdrawal of blood samples.

In 11 patients in septic shock the observation of an increased ECW and increased total exchangeable Sodium not associated with an increase in TBW has been recorded. In addition, no correlation was found between this observation and the more usually performed tests of hemodynamic and metabolic functions in the shock patient.

These studies do not support the concept of selective extracellular fluid deficits in hemorrhagic shock

or septic shock. Unwarranted infusion of large amounts of salt-containing solutions should be avoided unless further scientific support for their use can be obtained.

# THE EFFECT OF SHOCK ON BODY FLUID DISTRIBUTION

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# CHAPTER I

## INTRODUCTION

In spite of intensive research, many aspects of the physiopathology of shock remain obscure. Shires has recently suggested that a decrease in extracellular water could be significant in some patients in shock and that maintenance of a normal extracellular water volume may be an important factor in survival.(1)

There are also many technical problems related to the measurement of body fluid distribution. These measurements are complex but recent technical developments have permitted improved measurements of body water composition. A continuing difficulty has been the lack of an acceptable technique for measuring the extracellular water.

In the present study, body water composition in experimental hemorrhagic shock and clinical septic shock has been measured. Particular attention was paid to the measurement of extracellular water volume. A modified inulin space technique was developed and the volume measured by using this large molecule of known distribution compared to values derived from the use of tracer amounts of several radioisotopes. Through the use of tracer substances of varying molecular size it was hoped to better identify intracellular water shifts if such occurred.

Using these tracer substances, we have employed the commonly accepted techniques for the calculations of extracellular water, total body water, plasma volume and red cell mass. The final concentration of a known quantity of a tracer after thorough mixing in an unknown volume can be used to calculate the latter volume.

## PROBLEMS OF INVESTIGATING BODY FLUID COMPARTMENTS

## HISTORICAL BACKGROUND WITH A SELECTIVE REVIEW OF THE LITERATURE

### Plasma Volume Tracers:

The dye method, using vital red duck dye, was introduced by Keith et al. in 1915.[2] This and many other vital dyes used in the past were criticized because hemolysis interfered with the colorimetric dye determination.

Dawson worked extensibly with up to 60 blue dyes. In 1920 he reported the use of T-1824,[3] later called Evans blue. No hemolysis was associated with the use of this material. The validity of this method was confirmed by Gregersen et al. in 1935[4] and Gibson and Evans in 1937.[5] Since only a limited number of repeated measurements could be done with the blue dyes, due to slow and incomplete elimination of these materials from the body, new methods were developed.

A most valuable contribution was made by Fine and Seligman in 1943[6] showing that the distribution space of serum albumin labeled with $I^{131}$ was the same as that of Evans blue dye. Since the introduction of semiautomatic isotope counting machines, $I^{131}$ has become the most frequently used tracer for blood volume studies. Recently, $I^{125}$ labeled serum albumin has been employed to permit a differential counting technique when $Chromium^{51}$ is used simultaneously.

Trivalent $Chromium^{51}$ - labeled albumin was produced by Gray and Sterling in 1950[7] and utilized for plasma volume determination by Frank and Gray in 1953.[8,9] However, this method has not become popular.

Problems in Measuring Plasma Volume:

Plasma protein-bound tracers begin to leak from the circulation into the interstitial space within a few minutes from the time of injection, as shown by Dellenback and Gregersen.[10,11] It is possible to plot this disappearance rate with time by sequential sampling. When values obtained are plotted with a logarithmic scale on the vertical axis, all but the very earliest sample points are found to fall on a straight line which can be extrapolated back to zero time for an estimation of dilution concentration as if mixing had been complete instantaneously, as shown by Gregersen and Huggins.[11,12] This method is generally accepted at the present time though its theoretical and empirical justifications have been questioned by Peters,[13] Remington and Baker.[14]

Another possible source of error in calculating plasma volume using Radioactive Iodinated serum albumin was pointed out by Albert.[15] Radioactive Iodinated serum albumin normally contains under 0.5 per cent free iodine. With storage and when kept at room temperature, the iodine tends to break away from the albumin and free iodine may increase to 1.5 per cent. This free iodine is lost rapidly from the blood stream and will result in larger volumes of plasma.

Red Cell Volume Tracers:

The first tracer used was Carbon Monoxide, reported by Grehant and Quinquand in 1882.[16] A modification of this method was first used in humans by Haldane and Smith.[17] Total hemoglobin of the body was calculated using the oxygen capacity of the blood to reflect Co capacity, and the

dilution of a measured amount of inhaled Carbon Monoxide was quantitated by the resultant concentration of the blood.

A modification of this technique was introduced by Sjöstrand in 1948[18] measuring concentration of Co in the alveolar air. A serious disadvantage of this method, presumably due to the equilibration of Co with hemoglobin in extravascular locations (bone marrow), was a red cell volume consistently larger than that obtained by modern methodology.

The work of Hahn in 1938[19] made it possible to use donor's red cells labeled with radioactive iron for measurement of blood volume.

In vivo tagging of red cells with $P^{32}$ for determining the blood volume was demonstrated in 1940 By Hahn and Hevesy[21] and in vitro $P^{32}$ labeling was performed for the same purpose in 1942 by Hevesy and Zarahn.[20] However, this tag is labile and has a loss rate of about 6 per cent per hour.[22] Gray and Sterling[7] demonstrated in 1950 that hexavalent Chromium$^{51}$ provided a rapid method of in vitro labeling combined with a slow disappearance rate of activity, eliminating the need for serial sampling and extrapolation back to zero time, as required by $P^{32}$ and $I^{131}$. This is the most widely used method of cell labelling at the present time. $K^{42}$,$^{23}$,$^{102}$ and Thorium $B^{103}$ have been used for cell labelling, but are technically more difficult to use than Chromium.$^{51}$

Problems in Measuring Red Cell Volume:

Technical difficulties in tagging the recipient(s), or donor red cells represent the main problem. Albert et al.[15] have found red cells in rouleaux formation and cell aggregates along with small clots,[23] responsible for

variations in duplication of results of ± 3.5 to 4 per cent. Also poorly prepared washed labeled cells, excessive washing and incomplete removal of supernatant fluid will result in excessively large volumes, due to the rapid loss of free chromate from the intravascular bed.

Blood Volume:

One of the oldest procedures for measurement of blood volume is that of exsanguination and flushing out of the remaining blood in the circulatory system, probably first recorded by Lehman and Weber in 1850,[24] who collected blood from two executed criminals and washed out the circulation. Welcker in 1854[25] used this technique in experimental animals known since as Welcker's method.

Dilution procedures were probably first used by Valentin in 1838.[26] Its principle is the measured fall in concentration of blood solids, red cells, or hemoglobin produced by the infusion of known quantities of fluid as the basis for calculation of blood volume. This method was subsequently used by Phillips et al.,[27] Strumia et al.,[28] and Lawson et al.[29] This technique was subjected to the errors introduced by the disappearance of fluid from the circulation during infusion.

A further step on this technique resulted in the injection of a known amount of an exogenous tracer substance and measurement of the dilution space. This method established the basis for all modern techniques of body fluid compartment measurement.

Blood volume is generally calculated for clinical use employing one of the following formulas:

$$BV = \frac{\text{Red cell volume}}{\text{Hctv} \cdot \text{Fcell}} \qquad BV = \frac{\text{Plasma volume}}{1 - (\text{Hctv Fcell})}$$

Hctv = Hematocrit of sample analysed, corrected for trapped plasma

Fcell = Relation between the whole body hematocrit and the hematocrit of the blood sample drawn from an accessible vessel, often called the large vessel hematocrit. $\frac{(LVH)}{(WBH)}$

Trapped plasma varies according to different authors,[30,31,32,33] from 2 to 8 per cent. In our laboratories, using the Micro-hematocrit tube, Adams Readecrit Micro-hematocrit centrifuge and International Micro-hematocrit Reader, Model CR we have been unable to demonstrate any significant change in trapping of plasma, with hematocrit varying from 20 to 50.

Fcell average value approximates 0.91 as described by Davies and Topley,[34,35] but Albert et al.[15] have shown that it can vary in the same individual from 0.98 to 0.83, and also varies in certain diseases states. The above mentioned variables can influence results of blood volume as calculated from formulas based in a single tracer technique. To measure accurately the blood volume, plasma and red-cell volume should be measured separately, as has been carried out in most of these studies.

Extracellular Water Volume Tracers:

Fenn made the first attempt to measure extracellular water space histologically by visual examination of frozen preparations of muscle.[36] The interstitial space thus

measured represented 15 to 17 per cent of the total muscle mass.

The fact that Chloride concentration in muscle and plasma was found identical led Fenn et al. to the conclusion that all Chloride was extracellular.[37] On the same presumption, Sodium was also considered, by Harrison et al. to be extracellular in distribution.[38] Based on the assumption that the distribution of Chloride and Sodium was identical throughout the body, estimates of extracellular water were made by Hastings et al. by measuring the total body Chloride or Sodium and dividing this value by the serum concentration.[39] This procedure demanded sacrifice of the animal. In order to perform this measurement in vivo, complicated Sodium and Chloride balance studies were performed by Harrison et al.[40] These methods have been criticized by Harrison and Hastings on the basis that both elements penetrate the cell [38,39,40,41] and that the ratio Sodium/Chloride is lower in various tissues than in ultrafiltrate of plasma.[38,41,42] The same criticism applies to the use of Bromide.[43,44,45] Gamble and Ross in 1923[46] attempted to measure the extracellular water as calculated from the exchange of Sodium. Lavietes et al. in 1935[47] employed a similar technique using Chloride with appropriate corrections being made of changes of concentrations of these ions in the fluids. Besides being technically difficult, these studies yield no absolute values for volume of extracellular water.

Sodium Thiocynate was proposed as a good tracer for extracellular water determination by Grandall, Hill, Elkinton and Gregersen[48,49,50,51] as it was simple to analyse, having rapid equilibration and slow renal excretion. However, it

was shown by Grandall et al.[48] that it enters the different tissue cells and this author suggested its use as an index of changes in extracellular water. This was denied by Oveman and Feldman [52] when they showed that permeability to Thiocynate ion is markedly increased in pathological states. It was also shown by Levitt and Gaudino[53] that reproducibility of results is poor.

The use of sulfate was based on the observations of Grennwald in 1918[54] who showed that it distributes itself through only about 20 per cent of the body weight. This method was criticized by several authors[55,56,57] on the basis of disadvantages of very rapid renal excretion and necessity for corrections due to the endogenous serum sulfate as well as endogenous sulfate excretion.

Attempts were then made to find a difussible, non-metabolized substance not normally present in the body, but which is restricted to the extracellular water. A method for determination of sugar in plasma introduced by Somogyi et al.[58,59] made it possible the use of sucrose for determination of extracellular water by Larietes et al.[55,56] Later, mannitol, Newman et al.[60,61,62] and inulin, Wilde, Kruhöffer[63,64] were studied. They offered smaller spaces of distribution and presumably more accurate indices of the extracellular water volume. The use of mannitol and sucrose was criticized by several authors[61,65,66] because, besides presenting the problem of more difficult analytic methods and rapid renal excretion, incomplete urinary recovery indicates some degree of utilization for which no simple correction could be made.

Inulin is a lipoid-insoluble with a large molecular weight (about 5000). These properties reduce the probability of its permeating the cell membrane. Smith[67] demonstrated that inulin does not penetrate the erythrocyte. Smith, Westfall and Hober [67,68,69] showed that it does not diffuse through the renal tubule. The work of Haywood and Hober [70] showed that it does not undergo concentration by liver cells. Its rapid and quantitative recovery in the urine, Smith et al. and Gandino et al. [66,69,71] argues against its being metabolized to any appreciable degree or stored in any tissue. It probably does not draw water from the cells as Smith et al. [69,72] showed that it is physiologically inert and exerts negligible osmotic pressure. However, uniform distribution is prevented after a single injection by the rapid glomerular excretion, which limited its use to the nephrectomized animal. This disadvantage was overcome by a method of constant infusion advocated by Gandino et al.[71] and confirmed by Schwartz et al.[74] which compensates for the renal excretion.

Inulin space was found to be 19.4 per cent of the body weight in the dog and 16 per cent in the human by the same group of investigators. [71,73,74]

The theoretical arguement against an extracellular water space smaller than that measured by isotopes is that extracellular volume may have compartments unaccessible to inulin. Wilde and Kruhoffer [63,75] showed identical distribution of sucrose and inulin, substances of markedly different molecular weights, in the nephrectomized rabbit

and rat, which makes that theoretical arguement unlikely. At the present time, inulin represents probably the best measure of extracellular water available.

Just recently, a new technique has been developed in our laboratories[110] for the measurement of the extracellular water volume using a single injection of inulin. This technique will be described in detail in Chapter, II, Methodology.

Simplified techniques for measurement of extracellular water were facilitated by the introduction of radioisotope tracers. These included Sodium, ($Na^{22}$), Sulfate labelled with Sulphur, ($S^{35}$) and Bromine$^{82}$ among the most widely accepted.[100, 107, 114]

One considerable advantage of using radioisotope tracers for extracellular water determinations is that with the development of new counting techniques, it is possible to select several isotopes which give maximum counts per minute at specific windows for each isotope. These techniques have made possible the simultaneous measurement of body fluid spaces, namely, total body water, extracellular fluid volume, plasma volume and red cell mass. Details of the development and present utilization of these newer methods will be found at the end of the present chapter.

Total Body Water Tracers:

The earliest method available for the measurement of total body water developed by Mitchell et al. depended on total or partial dessiccation of the organism.[76] Newburgh[77] attempted to study total body water exchange by accurate intake and all avenues of output. This method was criticized by Lavietes[78] on the basis that errors involved made it quite

impossible.

From observation that total base and water excreted are in the same proportions as they appear in plasma, Gamble[79] suggested that the water exchange could be estimated from cation balance studies. Tedius balance studies are subjected to frequent errors. Furthermore, Elkinton et al.[80] contested some of its basic assumptions.

Rathburn et al[81,82,83] proposed specific gravity of the whole body to calculate the Total Body Water from an empirical equation derived from measurement of body specific gravity, total body water and body fat in sacrificed animals. This formula is lacking evidence that its use may be applicable during pathological variations and besides, gases in respiratory and gastrointestinal tract interfere with accurate measurement in the living organism, as shown by Marshell et al.[84]

Marshell, Painter and Danowski[84,85,86,87] proposed the use of urea, thiourea and sulfanilamide as tracers for Total Body Water determination. These methods were criticized by several investigators[88,89,90,91,92] as they found an equal distribution of these substances in body tissues, with urea presenting also the disadvantage of significant variation in endogenous formation.

The use of Deuterium Oxide ($D_2O$) gained rapid popularity due to its biological and chemical similarity to $H_2O$. It can be quantitatively detected,[93] is nontoxic,[94] and has rapid diffusion through the cell membrane[95,96,97,98] and equilibrium after a single injection was found to oscilate from nine minutes in the guinea pig[99] to one hour in man.[100] Numerous publications[96,97,98,101] supported its rapid equilibration in vitro and in vivo between plasma, red blood

cells and other tissues.

Rate of $D_2O$ elimination is extremely show: one half remains in the body after nine days and after equilibrium is reached the plasma concentration remains constant for nineteen hours, as shown by Elkinton et al.[80]

Ussing and Krogh[102,103] observed that a certain proportion of deuterium atoms may interchange with the hydrogen atoms in the organic compounds other than water, but Moore[100] calculated that no more than five per cent will enter metabolic processes and he also demonstrated by total desiccation of the animal that $D_2O$ measures the total body water within five per cent. Using this method, Total Body Water has been found to be 65 per cent of body weight in the guinea pig,[99] 63 per cent in dog,[73] and 53 per cent in man.[95]

Antipyrine has been recommended by Soberman et al.[105] for measuring Total Body Water with a simple and inexpensive method. Its use has been questioned, however, by Marshell et. al.[84] because a small amount is bound to plasma protein for which no correlation can be made. It is also metabolized and tissues (lung, kidney and liver) do not attain concentrations identical with plasma. There is also a discrepancy between antipyrine and heavy water spaces which is greater in edematous than in normal patients, questioning the value of its use in pathological states.

The in vivo measurement of Total Body Water with Tritium in the form of THO was introduced by Pace et al.[104] and observed in man a space of distribution of 64.8 per cent of body weight. This is the currently favored method since a rapid equilibration is achieved and no extrapolation is

necessary since no sensible loss occurs.

Simultaneous measurement of Body Fluid Spaces:

A most valuable contribution in recent years has been the development of new counting techniques in order to determine simultaneously various body fluid compartments.

The multiple isotope-dilution technique was introduced by Moore et al.[100,107,108] The basis of this method consists of selecting several isotopes which give maximum counts per minute at specific windows for each isotope. A series of simultaneous equations can be set up to differentiate the activities due to the individual isotopes.

The first multiple isotope technique used by Moore et al. consisted of Deuterium for measurement of total body water, Chromium$^{51}$ for measurement of red cell mass and Evans Blue for determination of plasma volume. Simultaneous measurements of extracellular water was later introduced by incorporating Bromine - 82 as a tracer for extracellular water volume. Another important contribution was the use of $Na^{24}$ for total exchangeable sodium studies and $K^{42}$ to determine total exchangeable potassium. These studies were performed in a 24 hour period.

Using the same principle, Shires et al. reported in 1960 a method of simultaneous measurement of extracellular water volume, plasma volume and red blood cell mass,[109] using radioactive sulfate labeled with Sulfur$^{35}$ for determination of extracellular water volume, as shown by Walser and co-workers.[110] $I^{131}$ - tagged human serum albumin for plasma volume and chromate$^{51}$ as a tracer for red blood cell mass. All measurements were made from a venous blood

blood sample, approximately 20 minutes after injection, in the assumption that this was the time required for each isotope to obtain a mixing equilibrium. The importance of this new technique is that it would make possible a rapid determination of the most important body fluid compartments. In order to make the necessary calculations, they were able to separate the different isotopes, $S^{35}$, beta emitter was separated from gamma emitters by using a well type scintillation chamber. $I^{131}$ and $Cr^{51}$ were separated by using two electrical window openings on the Packard instrument, as the activity of each isotope in each window is determined by counting standards solutions.

Shires et al. reported in 1961 (111) that no change occurs in extracellular water volume following minor surgical procedures, however, in 12 patients undergoing elective major surgical procedures, extracellular water loss varied from 0 to 28 per cent in one patient, with an average loss of 13.1 per cent, considered as a marked reduction in functional extracellular water volume. In 1964 (112) they reported a reduction of extracellular water in dogs following hemorrhagic shock, and alleviation of this reduction by the use of salt solution. They suggested that isotonic swelling of skeletal muscle and perhaps other cells was responsible for this phenomenon.

Using the same technique, Virtue et al.(113) studied 25 patients undergoing cholecystectomy and were unable to demonstrate an extracellular water volume loss.

# CHAPTER II

## METHODS

The tracer-dilution methods are all based on the concept that the amount of a tracer injected into an unknown volume is the same both before and after mixing in that volume, consequently:

$$\text{unknown volume} = \frac{\text{amount of tracer injected}}{\text{final concentration after mixing}}$$

This concept can be applied to a color or chemical tracer as well as to any radioactive isotope.

In these studies the radioactive isotopes used have been tritiated water, Sodium ($Na^{22}$), Sulfate labelled with Sulphur ($S^{35}$), Cobalt (Cobalt$^{57}$ labelled Vitamin $B_{12}$), Chromium ($Cr^{51}$) and Iodine ($I^{125}$ and $I^{131}$ labelled Serum Albumin). We have also used Inulin as the only color tracer.

There are specific problems related to the clinical use of each of the above tracers.

Chromium$^{51}$ and tritiated water are lost from the body at a very slow rate, consequently they reach a stable equilibrium lasting for at least a few hours after a mixing period which varies from less than 20 minutes in the case of Chromium to an average of two hours for tritiated water. (Fig. 1) Thus, the mean concentration of these isotopes after mixing are used for calculations.

The $Co^{57}$, $Na^{22}$, $I^{125}$, $I^{131}$ and $S^{35}$ do not obtain a constant equilibrium after the initial rapid mixing in body fluids. They "leak" significantly out of the initial

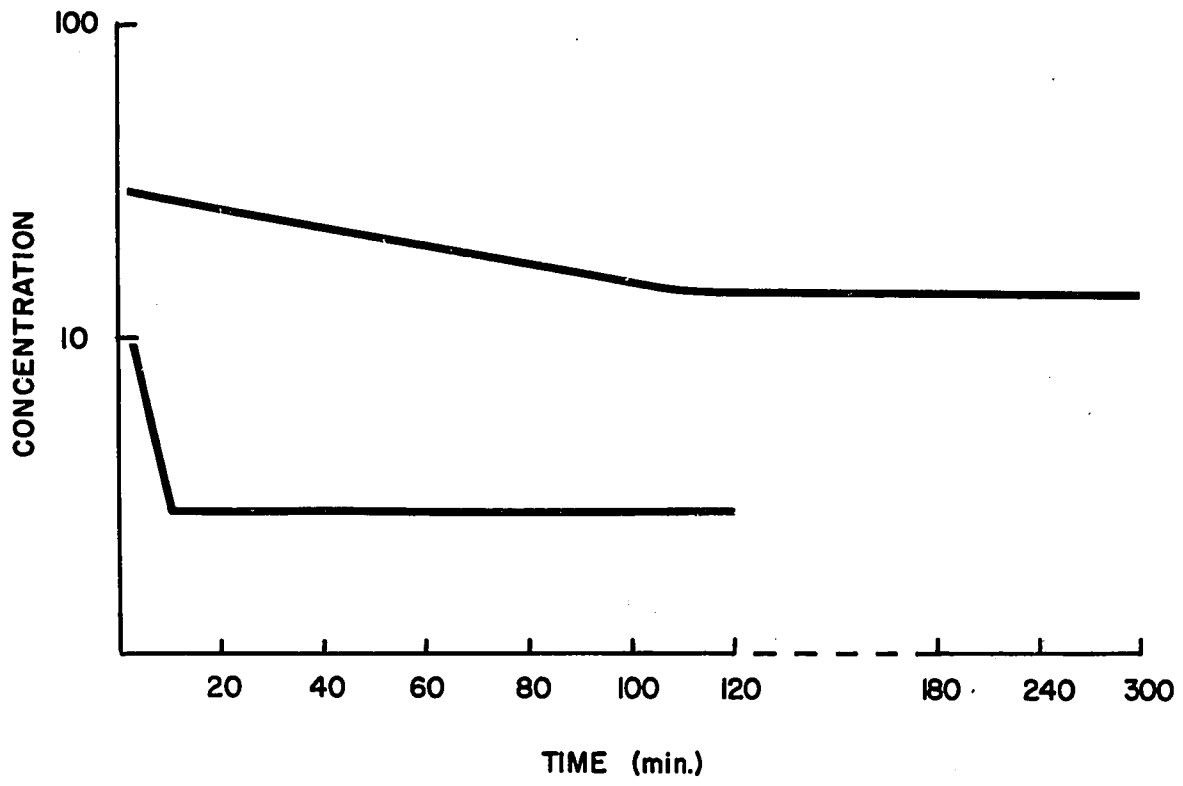


FIGURE 1

Stable equilibrium reached by Chromium$^{51}$ and tritiated water after mixing period.

spaces where rapid mixing occurs. Iodine is lost from vascular space into the interstitial space and into the kidneys. $Co^{57}$, $Na^{22}$, and $S^{35}$ gradually leak out of the extracellular water into the cells and kidneys. Kidney excretion is particularly high for $Co^{57}$.

The best available method for correcting gradual loss of these isotopes after the initial rapid mixing period consists of collecting several blood samples over a two hour period and plotting the log of concentration against time (Fig. 2), the resultant straight line is extrapolated to the Y axis to obtain the plasma concentration at the time of injection. The volume of distribution is then calculated by dividing the latter concentration into the total counts per minute injected.

This method of determining distribution volume is valid only if the mixing phase of the dilution is relatively rapid as is the case for Sodium and Sulphate, Iodine and Cobalt. Since the mixing phase, in the case of inulin lasts up to 60 minutes, a correction factor designed by Shizgal is required. (115)

The inulin dilution curve is composed of two simple exponentional decay curves (Fig. 3) as a result of the amount of inulin secreted by the kidney during the equilibration time. This is corrected by the use of the following formula:

$$V = \frac{Ai}{C_o - (b/a)\ C_o' \ (e^{-60a} - 1)}$$

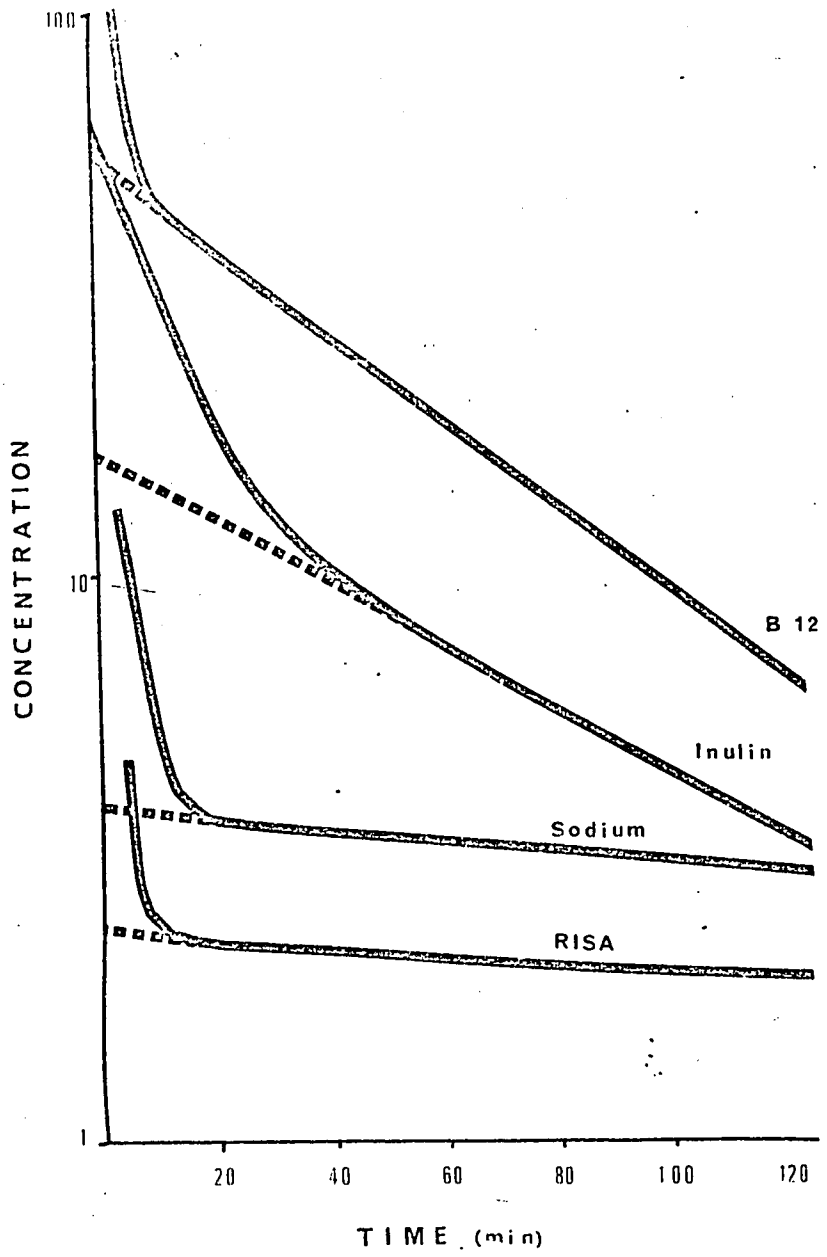


FIGURE 2.

Semi-log plot of concentration against time to demonstrate the time course of the dilution curves. The mixing phase is prolonged only in the case of inulin.

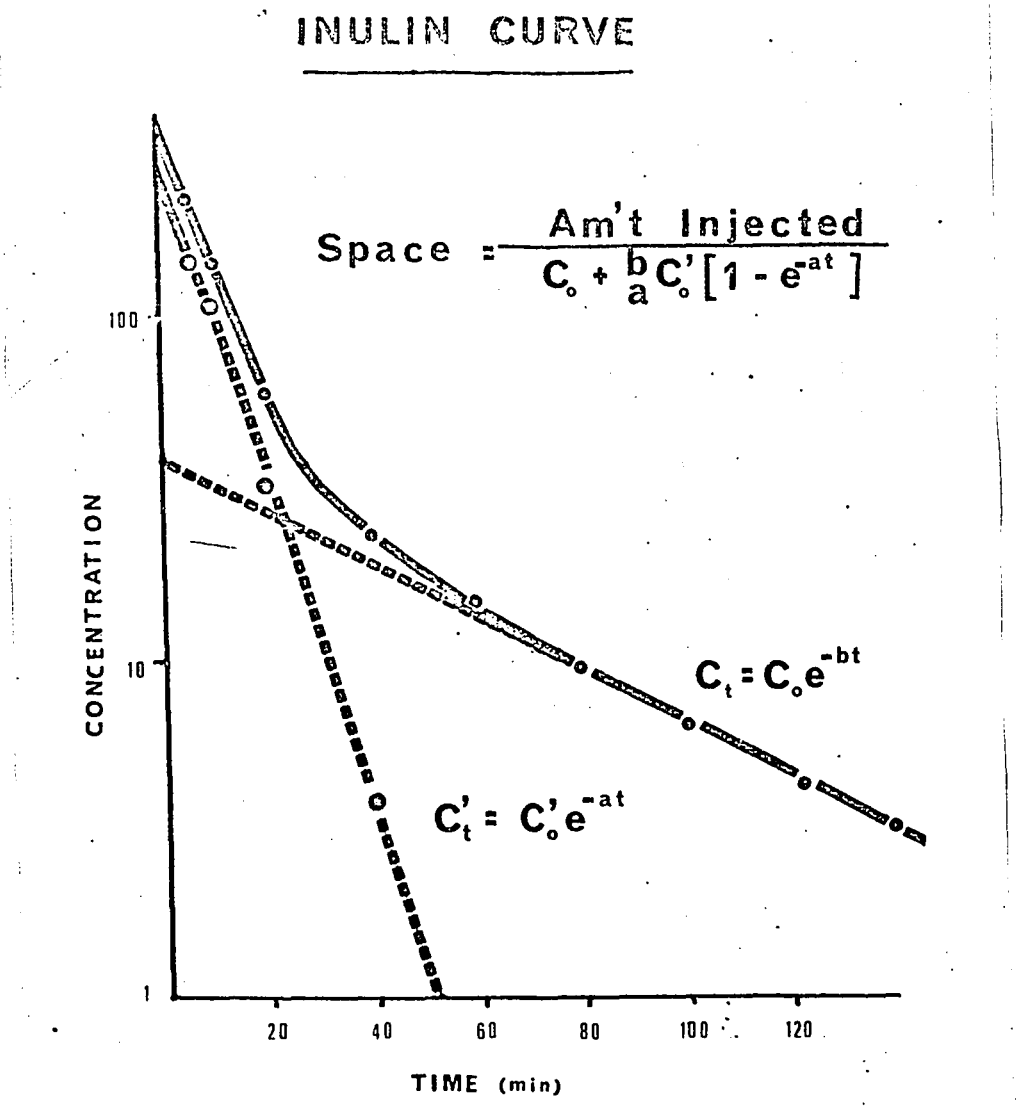


FIGURE 3.

The dilution curve of inulin (solid line) is the sum of two simple experimental decay curves (broken lines). The first represents the mixing phase and the second represents the renal excretion.

where: Ai = amount of inulin injected

$C_o$ = plasma concentration at time = 0 of the equilibration curve

$C_o'$ = plasma concentration at time = 0 of the mixing curve

b = slope of the equilibration curve

a = slope of the mixing curve

In this study, two types of trauma have been investigated. Hemorrhagic shock was studied in ten experimental animals. The changes associated with septic shock are available in eleven patients and ten normal volunteers were also studied as a control group.

Hemorrhagic Shock:

A modified Wiggers shock preparation was studied in 10 splenectomized mongrel dogs. Control measurements were carried out just prior to bleeding. The animals were bled to a pressure of 50 mm. of mercury which was maintained for 90 minutes. This was followed by a 45 minute period with the blood pressure at 35 mm. mercury. (Fig. 4) In some of the experiments, small amounts of the shed blood had to be retransfused occasionally in order to maintain the blood pressure at the desired level. The space measure-

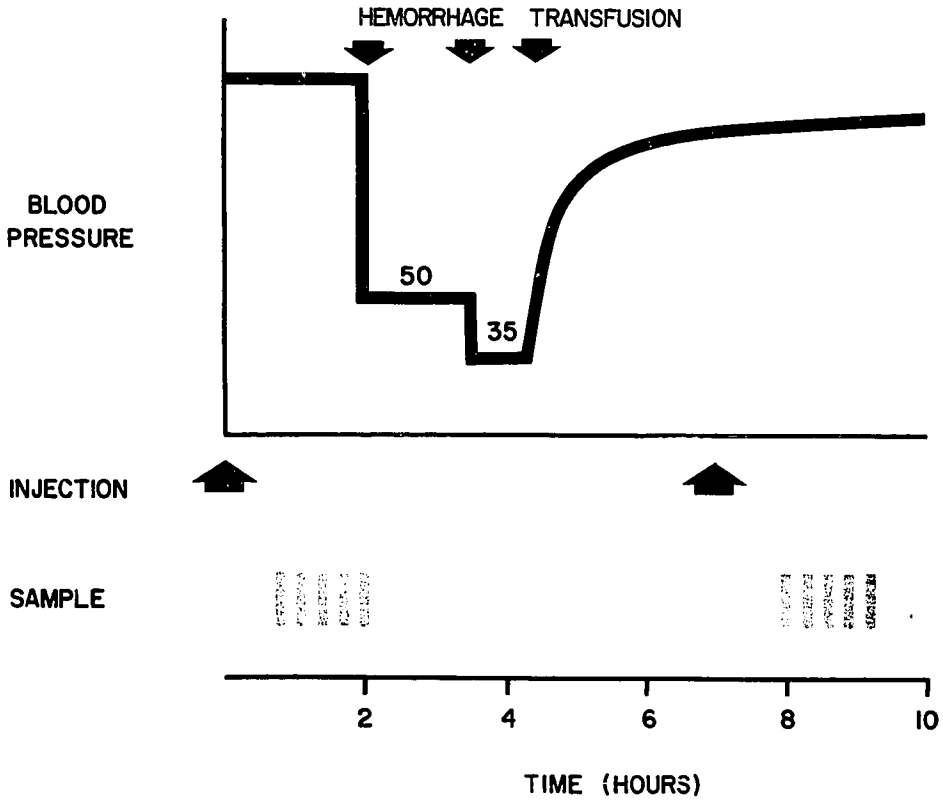


FIGURE 4

Modified Wigger's shock preparation utilized in our experimental studies.

ments were repeated two hours after the shed blood was returned to the animal. The amount of each isotope injected was four times that of the first injection in order to minimize the risk of error of the re-injection technique. In all experiments, the total amount of saline solution administered in order to maintain patency of the intravascular catheters was less than 200 cc. Shed blood was collected in a heparinized bottle under sterile conditions.

Space Measurement Techniques:

All radioactive materials were given as a single intravenous injection. The extracellular fluid was estimated by simultaneously determining the volume of distribution of $Na^{22}$, Sulphur$^{35}$ labelled $SO_4$, inulin and cobalt$^{57}$ labelled Vitamin $B_{12}$. The plasma volume was measured with iodine$^{131}$ labelled serum albumin (RISA). In order to saturate the animal with Vitamin $B_{12}$ and avoid later intracellular incorporation of the radioactive material, 5000 mgm. of Vitamin $B_{12}$ were administered intravenously. Half an hour later, a single intravenous injection of the above substances was given through a catheter placed in one of the femoral veins. Continuous monitoring of central venous pressure and blood pressure was carried out through catheters placed in a jugular vein and femoral artery. Five to six blod samples were drawn over a two hour period. Differential counting techniques were employed as described in the appendix. The plasma concentration of the various isotopes was obtained by solving a series of simultaneous equations. The inulin concentration of each plasma sample was determined in duplicate by the resorcinol method.

The logarithm of the concentration of each of the substances injected was plotted against time. The method of least squares was employed to fit the best straight line to the experimental data in order to obtain the extra-polated concentration at the time of injection. This concentration was corrected for plasma water and the Gibbs-Donnan effect and then divided into the amount injected to obtain the volume of distribution. The water concentration factor employed was 0.93 and the correction for $Na^{22}$ due to the Gibbs-Donnan effect was 0.95 while 1.05 was used for the correction of $S^{35}$.

In the case of inulin, previously described corrections were necessary because of its long mixing time. An IBM 7044 computer was used for the above calculations. The detailed steps for the procedure are outlined in the Appendix.

Clinical Studies in Normal Humans:

Methods and Materials:

Studies were carried out in 10 normal patients awaiting minor elective surgical procedures such as inguinal hernia repair, biopsy of the breast and varicose vein stripping. The age ranged from 24 to 76 years. Extra-cellular water volume was estimated by determining the volume of distribution of $Na^{22}$ in all the patients and $Cr^{51}$ as a tracer for red blood cell mass was added in the last four patients.

Plasma volume was measured with $Iodine^{131}$ labelled serum albumin, later converted to $I^{125}$ in order

to be able to inject $Cr^{51}$ simultaneously without compromising accuracy.

Total body water determinations were carried out by injecting a known amount of Tritiated Water and measuring the volume of distribution in plasma after three or four hours when equilibrium of concentration had been obtained. All radioactive materials were given a single intravenous injection. For the determination of RISA, $Na^{22}$ and $Co^{57}$ concentration, six to eight blood samples were drawn over a two hour period from one of the antecubital veins of the opposite arm to the one in which injections had been made. Differential counting techniques were employed as described in the Appendix. The plasma concentration of the various isotopes was obtained by solving a series of simultaneous equations. The logarithm of the concentration of each of the substances injected, with the exception of Tritiated Water and $Cr^{51}$, was plotted against time and the straight line resulting was extrapolated to time zero. Concentration was corrected for plasma water and the Gibbs-Donnan effect and then divided into the amount injected. Correction factor for water concentration was 0.93 and for $Na^{22}$ due to the Gibbs-Donnan effect was 0.95.

The red blood cell volume was obtained by dividing the product of the mean $Cr^{51}$ concentration and the hematocrit into the total amount of $Cr^{51}$ injected.

24 hour urine was collected in seven patients and total amount of radioactive $Na^{22}$ excreted was determined as well as the $Na^{22}$ concentration in plasma in order to measure

the total exchangeable sodium ($Na_e$) using the formula:

$$Na_e = \frac{A - B}{1000} \times \frac{C}{D}$$

A = total counts injected

B = total counts excreted (in urine)

C = sodium concentration in plasma

D = counts of radioactive sodium in 24 hours plasma.

Septic Shock in Man:

Two women and nine men with the clinical picture of septic shock were studied. Infection complications of surgical procedures precipitated septic shock in all but three patients who suffered one, a pulminating pseudomonas urinary infection and two, severely burned patients with profound sepsis. All patients had the clinical picture of septic shock and positive blood cultures were obtained in all but two patients. The majority of the patients died within a few hours following measurement; one long survival and one survived 17 days. Space measurements were performed according to the technique described in 'clinical studies in normal humans.' In addition, cardiac index, serum lactate levels, blood pressure, central venous pressure and serum phosphatase were measured in all these patients.

# CHAPTER III

# RESULTS

## A. EXPERIMENTAL STUDIES IN DOGS

### Hemorrhagic Shock:

Pre-shock measurements obtained in 18 normal dogs were within acceptable limits for all the substances with the exception of Vitamin $B_{12}$. (Table I) The $Na^{22}$ space was 24.7%. Standard deviation (S.D.) = 4.2 of body weight. The $SO^4$ and inulin spaces were 21.3% (S.D. = 3.6) and 21.4% (S.D. = 5.9) respectively. The RISA volume was 4.4% (S.D. = 0.9). The Vitamin $B_{12}$ space at 29% (S.D. = 7.7) of body weight is greater than generally accepted for extracellular water. Furthermore, since the latter varied from 20.9% to 54%, it probably does not accurately reflect the extracellular water.

Of the 10 dogs in which a modified Wiggers shock preparation was studied, in spite of an 80% mortality rate, only a few animals had a significant decrease in the extracellular water following hemorrhagic shock. (Fig. 5) There was a mean decrease of 5.5% in the $Na^{22}$ space, 4.7% in the $SO^4$ space, 8.4% in the inulin space, and 12.4% in the RISA space. Because of the difficulty in accurately measuring a small change in extracellular water, a difference in excess of 10% was considered significant. A decrease in excess of 10% in the $Na^{22}$ space occurred in only three animals. In these dogs, the mean decrease was 16%. In the three animals with a significant decrease in the $SO^4$ space the mean was 20%. An average decrease of 24% was

| | | RISA | | CO-57 | | NA-22 | | S-35 | | INULIN | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dog | Weight | c c | %B.Wt. | c c | %B.Wt. | c c | %B.Wt. | c c | %B.Wt. | c c | %B.Wt. |
| 1 | 20 | 853 | 4.3 | 4386 | 21.9 | 5576 | 27.9 | 4771 | 23.9 | 6384 | 31.9 |
| 3 | 23.6 | 1038 | 4.4 | 5944 | 25.2 | 5966 | 25.3 | 4728 | 20.3 | 5222 | 22.3 |
| 4 | 20 | 1054 | 5.3 | 6516 | 32.6 | 5211 | 26.0 | 4685 | 23.4 | 3025 | 15.1 |
| 5 | 24.7 | 1270 | 5.1 | 9094 | 36.8 | 8037 | 32.5 | 5751 | 23.3 | -- | -- |
| 6 | 25.6 | 1318 | 5.1 | 8799 | 34.4 | 7037 | 27.5 | 6042 | 23.6 | 5608 | 21.9 |
| 7 | 21.4 | 1131 | 5.3 | 11630 | 54.3 | 7031 | 32.9 | 6430 | 30.0 | 5880 | 27.5 |
| 9 | 17 | 714 | 4.2 | 5430 | 31.9 | 3531 | 20.8 | 2893 | 17.0 | 3371 | 19.8 |
| 10 | 25.7 | 996 | 3.9 | 6479 | 25.2 | 5493 | 21.4 | 4450 | 17.3 | 4078 | 15.9 |
| 11 | 37.3 | 1467 | 3.9 | 7784 | 20.9 | 7439 | 19.9 | 5272 | 14.1 | 5063 | 13.6 |
| 12 | 19.3 | 817 | 4.2 | 6793 | 35.2 | 4459 | 23.1 | 4356 | 22.6 | 3855 | 20.0 |
| 13 | 24.1 | 1335 | 5.5 | 7066 | 29.3 | 5017 | 20.8 | 5136 | 21.3 | 5155 | 21.4 |
| 14 | 22.7 | 993 | 4.4 | 5455 | 24.0 | 4100 | 18.1 | 5581 | 24.6 | -- | -- |
| 16 | 17.5 | 577 | 3.3 | 4838 | 27.6 | 4027 | 23.0 | 3375 | 19.3 | 2936 | 16.8 |
| 19 | 14.8 | 389 | 2.6 | 4047 | 27.3 | 3164 | 21.3 | 2672 | 18.0 | 1887 | 12.8 |
| 22 | 32.7 | 1109 | 3.4 | 9188 | 28.1 | 7673 | 23.5 | 6795 | 20.8 | 6528 | 20.0 |
| I | 21.1 | 1209 | 5.7 | 4545 | 21.5 | 5467 | 25.9 | 4084 | 19.4 | 6356 | 30.1 |
| II | 22.7 | 1093 | 4.8 | 4939 | 21.8 | 5606 | 24.7 | 4759 | 21.0 | 6464 | 28.5 |
| III | 21.8 | 769 | 3.5 | 5092 | 23.4 | 6560 | 30.1 | 5157 | 23.7 | 5458 | 25.0 |
| Mean | | | 4.4 | | 29.0 | | 24.7 | | 21.3 | | 21.4 |

TABLE I

PRESHOCK MEASUREMENTS IN 18 NORMAL DOGS

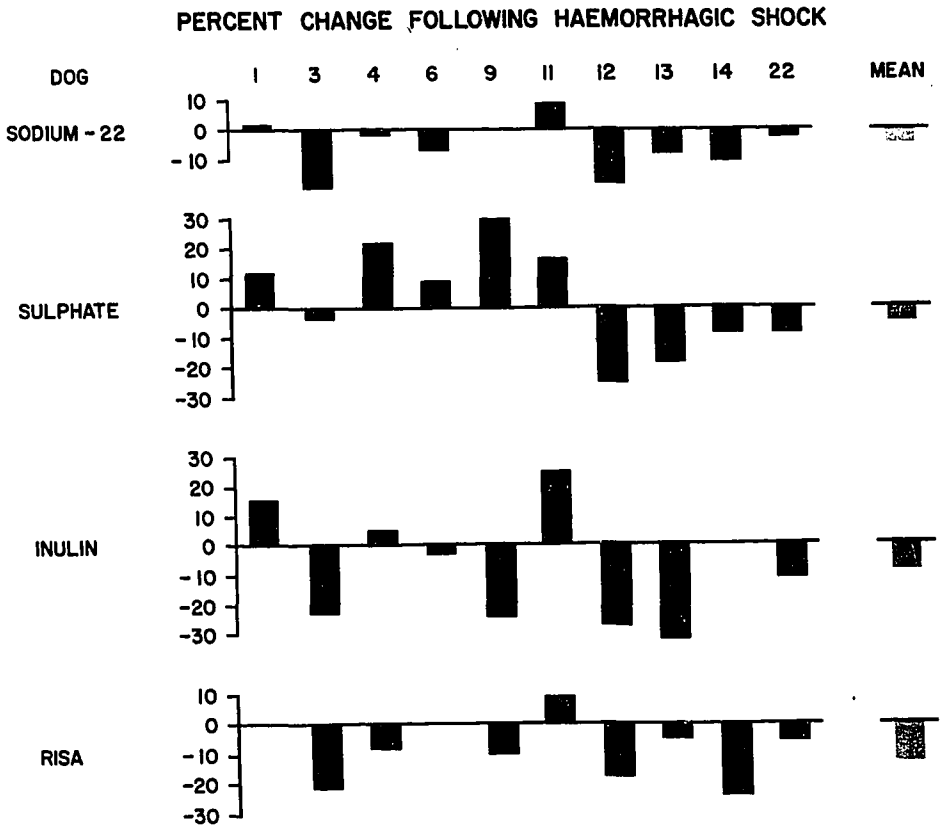


FIGURE 5

Modified Wigger's shock preparation: 10 dogs. Only a few animals showed a significant decrease in the extracellular water following hemorrhagic shock.

observed in five animals with significant decrease in the inulin space. (Table II) The mean for each type of extracellular water measurement was tested for significance against all the other extracellular water measurements, both pre and post shock, using a student's "T" test, as shown in Tables III and IV. 'A' represents pre shock measurements and 'B' represents post shock. Except for the pre shock Vitamin $B_{12}$ space, the differences were not statistically significant. The small differences noted are most probably related to the losses due to blood sampling. The control $Co^{57}$ Vitamin $B_{12}$ space was invalidated since an inadequate period of time was allowed following loading with non radioactive Vitamin B12. In the animals studied there was no evidence for a consistent major shift of extracellular water following hemorrhagic shock. However, the possibility exists that a significant decrease may occur in only some animals. The factors accounting for this variation are not yet understood.

B. CLINICAL STUDIES:

Control Studies in Normal Humans:

Measurements obtained in 10 normal humans were within acceptable limits for all the spaces studied. (Table V)

The mean $Na^{22}$ space was 20.5% of the body weight (S.D. = ±2.2) The RISA volume was 3.91% (S.D. = ±1.08) of body weight. Total body water was 52.6% (S.D. = ±7.9) and red cell mass in three patients 2.4% (S.D. = ±0.3). In order to minimize variance of percentage of body weight of the above spaces due to the unknown variable of body fat contained, we exposed our results for plasma and extracellular water in

| | | | RISA | | CO-57 | | NA-22 | | S-35 | | INULIN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | 20 | A | 853 | 4.3 | 4386 | 21.9 | 5576 | 27.9 | 4771 | 23.9 | 6384 | 31.9 |
| | | B | 867 | 4.3 | 4858 | 24.3 | 5664 | 28.3 | 5355 | 26.8 | 7399 | 37.0 |
| | | Diff | +14 | | +472 | | +88 | | +584 | | +1015 | |
| | | % Diff | 0 | | +10.8 | | +1.6 | | +12.2 | | +15.9 | |
| 3. | 23.6 | A | 1038 | 4.4 | 5944 | 25.2 | 5966 | 25.3 | 4728 | 20.3 | 5222 | 22.1 |
| | | B | 809 | 3.4 | 5408 | 22.9 | 4848 | 20.5 | 4541 | 19.2 | 4034 | 17.1 |
| | | Diff | -229 | | -536 | | -1118 | | -187 | | -1188 | |
| | | % Diff | -22.1 | | -9.0 | | -18.7 | | -4.0 | | -22.7 | |
| 4. | 20 | A | 1054 | 5.3 | 6516 | 32.6 | 5211 | 26.0 | 4685 | 23.4 | 3025 | 15.1 |
| | | B | 965 | 4.8 | 5050 | 25.3 | 5103 | 25.5 | 5706 | 28.5 | 3155 | 15.8 |
| | | Diff | -89 | | -466 | | -103 | | +1021 | | +130 | |
| | | % Diff | -8.4 | | -7.1 | | -2.0 | | +21.7 | | +4.3 | |
| 6. | 25.6 | A | 1318 | 5.1 | 8799 | 34.4 | 7037 | 27.5 | 6042 | 23.6 | 5608 | 21.9 |
| | | B | 1309 | 5.1 | 8606 | 33.6 | 6594 | 25.8 | 6625 | 25.9 | 5482 | 21.4 |
| | | Diff | -9 | | -193 | | -443 | | +583 | | -126 | |
| | | % Diff | 0 | | -2.2 | | -6.3 | | +9.6 | | -2.2 | |
| 9. | 17 | A | 714 | 4.2 | 5430 | 31.9 | 3531 | 20.8 | 2893 | 17.0 | 3371 | 19.8 |
| | | B | 642 | 3.8 | 3407 | 20.0 | 3536 | 20.8 | 2013 | 11.8 | 2538 | 14.9 |
| | | Diff | -72 | | -2023 | | +5 | | -880 | | -833 | |
| | | % Diff | -10 | | -37.3 | | 0 | | -30.0 | | -24 | |
| 11. | 37.3 | A | 1467 | 3.9 | 7784 | 20.9 | 7439 | 19.9 | 5272 | 14.1 | 5063 | 13.6 |
| | | B | 1606 | 4.3 | 7445 | 20.0 | 8176 | 21.9 | 6129 | 16.4 | 6283 | 16.8 |
| | | Diff | +139 | | -339 | | +737 | | +857 | | +1220 | |
| | | % Diff | +9.5 | | -4.4 | | +9.9 | | +16.3 | | +24.1 | |
| 12. | 19.3 | A | 817 | 4.2 | 6793 | 35.2 | 4459 | 23.1 | 4356 | 22.6 | 3855 | 20.0 |
| | | B | 668 | 3.5 | 4099 | 21.2 | 3618 | 18.7 | 3265 | 16.9 | 2798 | 14.5 |
| | | Diff | -149 | | -2694 | | -841 | | -1091 | | -1057 | |
| | | % Diff | -18.2 | | -39.7 | | -18.9 | | -25.0 | | -27.4 | |

table continued on next page please

TABLE II

| | | | RISA | | CO-57 | | NA-22 | | S-35 | | INULIN | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 13. | 24.1 | A | 1335 | 5.5 | 7066 | 29.3 | 5017 | 20.8 | 5136 | 21.3 | 5155 | 21.4 |
| | | B | 745 | 3.1 | 4819 | 20.0 | 4624 | 19.2 | 4115 | 17.1 | 3503 | 14.5 |
| | | Diff | -590 | | -2247 | | -393 | | -1021 | | -1652 | |
| | | % Diff | -44 | | -31.8 | | -7.8 | | -19.9 | | -32 | |
| 14. | 22.7 | A | 993 | 4.4 | 5455 | 24.0 | 4100 | 18.1 | 5581 | 24.6 | | |
| | | B | 752 | 3.3 | 4473 | 19.7 | 3680 | 16.2 | 5077 | 22.4 | 3862 | 17.0 |
| | | Diff | -241 | | -982 | | -420 | | -504 | | | |
| | | % Diff | -24.3 | | -18 | | -10.2 | | -9.0 | | | |
| 22. | 32.7 | A | 1109 | 3.4 | 9188 | 28.1 | 7673 | 23.5 | 6795 | 20.8 | 6528 | 20.0 |
| | | B | 1043 | 3.2 | 6018 | 18.4 | 7459 | 22.8 | 6167 | 18.9 | 5769 | 17.6 |
| | | Diff | -66 | | -3170 | | -214 | | -628 | | -759 | |
| | | % Diff | -6.0 | | -34.5 | | -2.8 | | -9.2 | | -11.6 | |

TABLE II.

HAEMORRHAGIC SHOCK IN DOGS:

VOLUMES OF DISTRIBUTION BEFORE AND AFTER SHOCK

## TABLE III

## HEMORRHAGIC SHOCK (10 DOGS)

| SPACE MEASURED | PER CENT OF BODY WEIGHT | |
|---|---|---|
| | CONTROL | POST-SHOCK |
| RISA | 4.5 ± 0.65 | 3.9 ± 0.7 |
| $CO^{57}$ | 28.4 ± 5.2 | 22.5 ± 4.5 |
| $NA^{22}$ | 23.3 ± 3.4 | 22.0 ± 3.7 |
| $S^{35}$ | 21.2 ± 3.3 | 20.4 ± 5.4 |
| INULIN | 20.6 ± 5.2 | 18.7 ± 6.8 |

## TABLE IV

## SIGNIFICANCE OF CHANGE IN PRE AND POST SHOCK MEASUREMENT

| | RISA A | RISA B | CO-57 A | CO-57 B | NA-22 A | NA-22 B | S-35 A | S-35 B | INULIN A | INULIN B |
|---|---|---|---|---|---|---|---|---|---|---|
| RISA A | - | | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| RISA B | | - | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ |
| CO-57 A | +++ | +++ | - | ++ | ++ | ++ | +++ | ++ | ++ | ++ |
| CO-57 B | +++ | +++ | ++ | - | | | | | | |
| NA-22 A | +++ | +++ | ++ | | - | | | | | + |
| NA-22 B | +++ | +++ | ++ | | | - | | | | |
| S-35 A | +++ | +++ | +++ | | | | - | | | |
| S-35 B | +++ | +++ | ++ | | | | | - | | |
| INULIN A | +++ | +++ | ++ | | | | | | - | |
| INULIN B | +++ | +++ | ++ | | | | | | | - |

+ $p \lhd 0.02$ ++ $p \lhd 0.01$ +++ $p \lhd 0.001$

| Pt. | Age | Sex | Wt.Kg. | Risa | %BW | Na-22 | %BW | Tot.BW | %BW | RBC | %BW | %TBW Risa | %TBW Na-22 | Nae M.Eq. | Nae.M.Eq. /Kg. | Nae. /Liter |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 5 | 28 | M | 48.3 | 1413 | 2.9 | 10147 | 21.0 | 30266 | 62.7 | --- | --- | 4.7 | 33.5 | 1995 | 41.3 | 65.9 |
| 6 | 53 | M | 65.9 | 3574 | 5.4 | 12835 | 19.5 | 35354 | 53.6 | --- | --- | 10.1 | 36.3 | 3067 | 46.5 | 86.7 |
| 9 | 35 | M | 74.6 | 4495 | 6.0 | 13710 | 18.4 | 39966 | 53.6 | --- | --- | 11.2 | 34.3 | 2773 | 37.2 | 69.4 |
| 10 | 50 | M | 67.8 | 2702 | 4.0 | 16505 | 24.3 | 35010 | 51.3 | --- | --- | 7.7 | 47.1 | 3086 | 45.5 | 88.1 |
| 12 | 25 | M | 68.2 | 2088 | 3.1 | 13374 | 19.6 | 34347 | 50.3 | --- | --- | 6.1 | 38.9 | | | |
| 13 | 30 | F | 60.7 | 1719 | 2.8 | 11937 | 19.7 | 36238 | 59.7 | --- | --- | 4.7 | 32.9 | | | |
| 52 | 54 | F | 63.64 | 2436 | 3.8 | 13006 | 20.4 | 27760 | 43.6 | 1609 | 2.5 | 8.7 | 46.8 | | | |
| 55 | 76 | F | 36.8 | 1557 | 4.2 | 8360 | 22.7 | 20916 | 56.8 | 994 | 2.7 | 7.4 | 40.0 | 1745 | 47.4 | 83.4 |
| 56 | 24 | F | 74.95 | 2179 | 2.9 | 12305 | 16.4 | 29135 | 38.9 | --- | --- | 7.5 | 42.2 | 2152 | 28.7 | 73.9 |
| 57 | 56 | F | 39.8 | 1575 | 4.0 | 9078 | 22.8 | 21880 | 55.0 | 842 | 2.1 | 7.2 | 41.5 | 1730 | 43.5 | 79.1 |
| Mean | | | | | 3.91 | | 20.5 | | 52.6 | | 2.4 | 7.5 | 39.4 | | 41.4 | 78.1 |
| | | | | | ± 1.08 | | ± 2.2 | | ± 7.9 | | ± 0.3 | ± 2.1 | ± 5.4 | | ± 6.6 | ± 8.6 |

TABLE V

Control measurements in 10 normal humans of plasma volume (RISA), extracellular water ($Na^{22}$), total body water (tot. BW) and red blood cell mass (RBC) expressed in per cent of body weight (% BW) and per cent of total body water. (% TBW) Total exchangeable Sodium ($Na_e$) is represented as M.Eq./Kg. of body weight and M.Eq./Liter of total body water.

per cent of total body water. The values so obtained were 7.5% of total body water (S.D. = ±2.1) for plasma and 39.4 (S.D. = ±5.4) for extracellular water. Mean $Na_e$ was found 41.4 (S.D. = ±6.6) per kilogram of body weight or 78.1 (S.D. = ±8.6) per liter of measured total body water.

Clinical Studies in Septic Shock:

Table VI shows the group of patients in septic shock. Mean plasma volume was found 3.9% of body weight or 7.3% of total body water; red blood cell mass 2.4% of body weight; $Na^{22}$ space 26.9% of body weight or 51.2% of total body water and finally, total body water was measured 53.0% of body weight. Considering the presence of two obese patients, (ages 17 and 38) the reason of a smaller $Na^{22}$ space and total body water when related to body weight in comparison to the overall group becomes obvious.

Figure 6 shows the Na-22 or extracellular water volume expressed as % of total body water in 17 normal patients and the 11 patients in septic shock. The central line represents the regression curve for our normals, the area between lines above and below represents the 95% confidence limit of all the normal patients between these two lines, however, the septic shock patients represented by the open dots are located above the regression curve and only three within the 95% confidence limit. This difference was highly significant with "P" less than .001.

Having recorded an increase in extracellular water volume it became important to see if there was any correlation between this and the rest of the parameters measured.

| Pt. | Age | Sex | Wt.Kg. | Risa | %BW | RBC | %BW | Na-22 | %BW | TBW | %BW | %TBW Risa | %TBW Na22 | Nae.M.Eq. | Nae.M.Eq. /Kg. | Nae.M.Eq. /Liter |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 7 | 78 | M | 65.5 | 2237 | 3.4 | --- | --- | 19569 | 29.9 | 35036 | 53.5 | 6.4 | 55.9 | --- | --- | --- |
| 14 | 22 | M | 70.5 | 2759 | 3.9 | --- | --- | 17925 | 25.4 | 41085 | 58.2 | 6.7 | 43.6 | --- | --- | --- |
| 17 | 51 | M | 152.7 | 2349 | 1.5 | --- | --- | 32189 | 21.1 | 71027 | 46.5 | 3.3 | 45.3 | 5677 | 37.2 | 79.9 |
| 18 | 70 | M | 76.6 | 4019 | 5.3 | --- | --- | 24812 | 32.8 | 44544 | 58.9 | 9.0 | 55.7 | 6178 | 81.7 | 138.7 |
| 19 | 69 | F | 54.0 | 2064 | 3.8 | --- | --- | 17191 | 31.8 | 38213 | 70.7 | 5.4 | 45.0 | --- | --- | --- |
| 20 | 19 | M | 64.1 | 1872 | 2.9 | --- | --- | 19704 | 30.7 | 30500 | 47.6 | 6.1 | 64.6 | 4171 | 65.1 | 136.8 |
| 22 | 70 | M | 56.6 | 2341 | 4.1 | 954 | 1.7 | 16858 | 29.8 | 28521 | 50.4 | 8.2 | 59.1 | 4441 | 78.5 | 155.7 |
| 31 | 56 | M | 68.6 | 2525 | 3.7 | 1007 | 1.5 | 14985 | 21.8 | 34454 | 50.2 | 7.3 | 43.5 | --- | --- | --- |
| 34 | 63 | M | 56.4 | 2041 | 3.6 | 2144 | 3.8 | 16530 | 29.3 | 33295 | 59.0 | 6.1 | 49.6 | --- | --- | --- |
| 38 | 59 | F | 99.5 | 3900 | 3.9 | 2297 | 2.3 | 18603 | 18.7 | 38231 | 38.4 | 10.2 | 48.6 | 3599 | 36.2 | 94.1 |
| 41 | 43 | M | 71.0 | 4140 | 5.8 | 2254 | 2.8 | 18649 | 26.3 | 35431 | 50.0 | 11.7 | 52.6 | 3774 | 53.2 | 106.5 |
| Mean | | | | | 3.9 | | 2.4 | | 26.9 | | 53.0 | 7.3 | 51.2 | | 58.6 | 118.6 |
| | | | | | ± 1.1 | | ± 0.93 | | ± 4.5 | | ± 8.5 | ± 2.3 | ± 7.0 | | ± 19.8 | ± 29.5 |

TABLE VI

Measurements of 11 patients in septic shock: plasma volume (RISA), red cell mass (RBC), extracellular water ($Na^{22}$), total body water (TBW) and total exchangeable Sodium ($Na_e$) expressed as per cent of body weight and total body water.

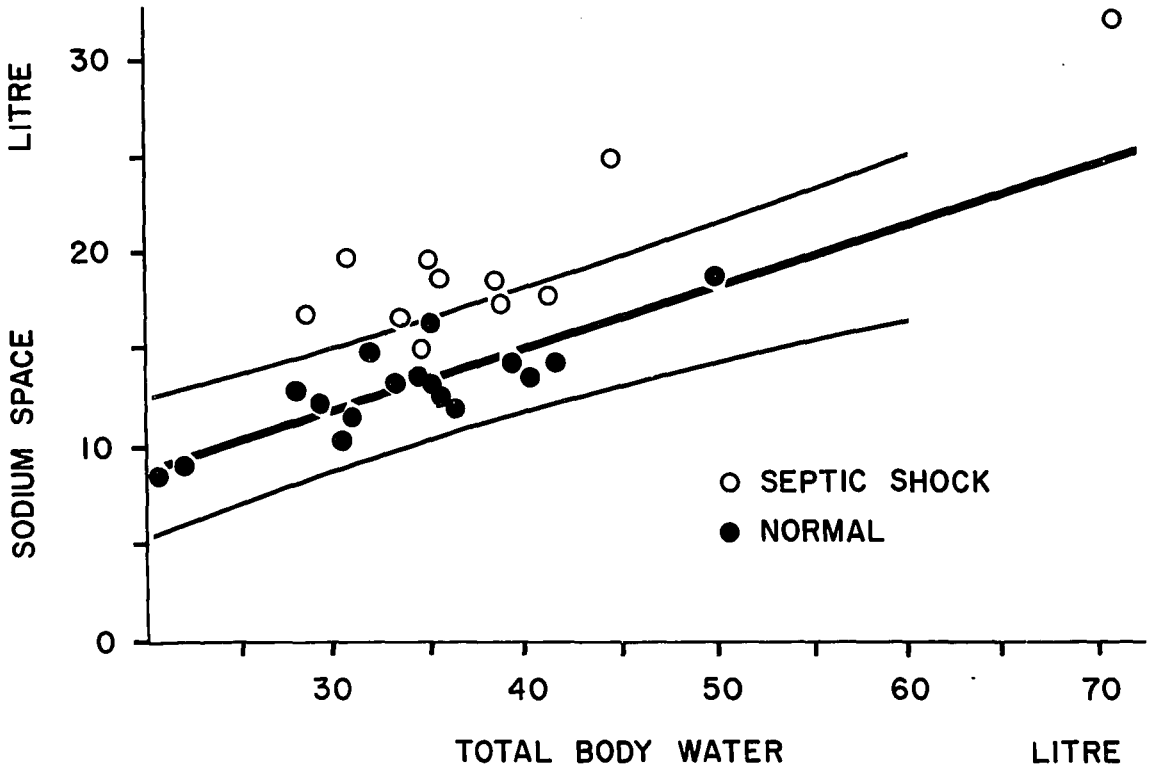


FIGURE 6

Extracellular water volume as % of total body water: regression curve (central line) with the area representing the 95% confidence limit.

This relationship is demonstrated in Figure 7 where we have represented extracellular water versus cardiac index. The shaded areas represent the normal values although the majority of the patients have a high cardiac index, there is no linear relationship between the extracellular water and the cardiac index.

In figure 8 we have plotted extracellular water versus central venous pressure. There is random distribution between low, normal and high central venous pressure in relationship to an extracellular water in general elevated or in the upper limits of normal and once again, there is no linear relationship between extracellular water and the observed central venous pressure.

The serum lactate levels were compared to extracellular water volume, as could be expected in severely ill patients, the majority of lactates were elevated. Again, there was no correlation between the degree of this elevation and the increase of extracellular water. (Fig. 9)

We have also plotted ph measurement versus extracellular water. (Fig. 10) Again the observation recorded reflects the serious nature of the patient's illness, rather than any correlation between the abnormal ph and the extracellular water volume.

There was no difference between the total body water as % of body weight of the 10 normal patients and the septic group, and our results are similar to those reported by others. There was no difference between normals and patients with septic shock in the values obtained

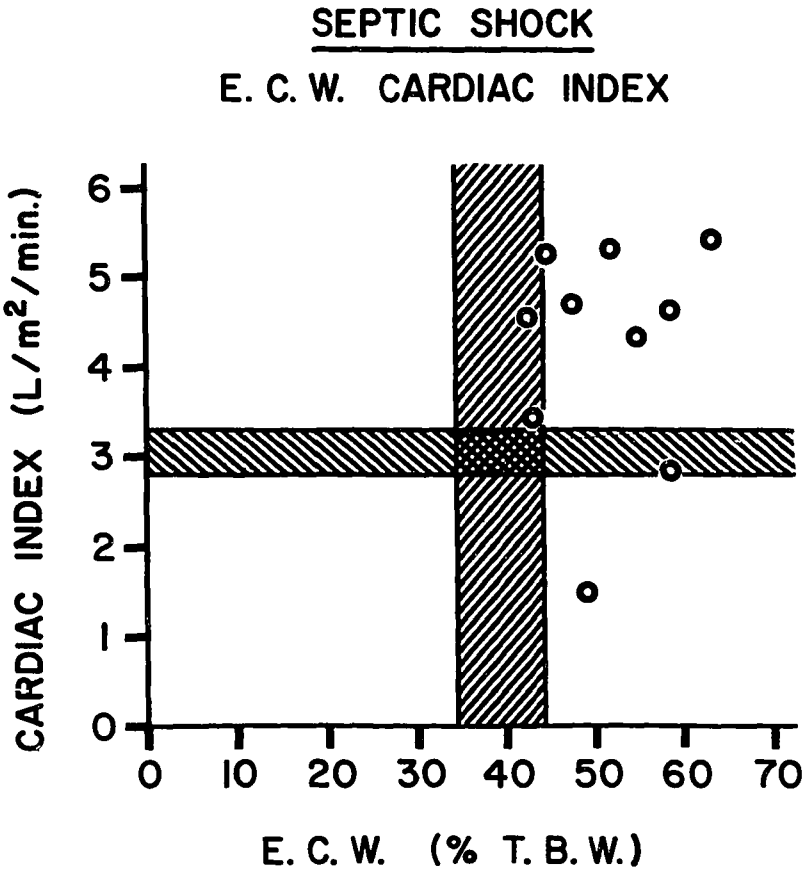


FIGURE 7

The shaded areas represent the expected normal limits.

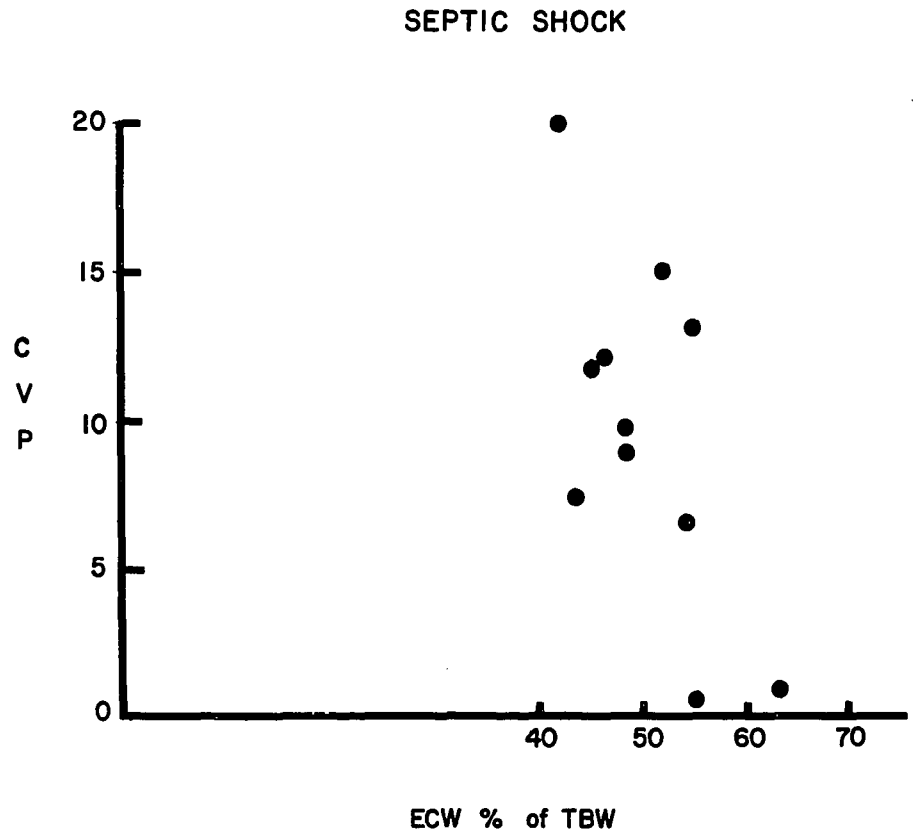


FIGURE 8

Extracellular water versus central venous pressure.

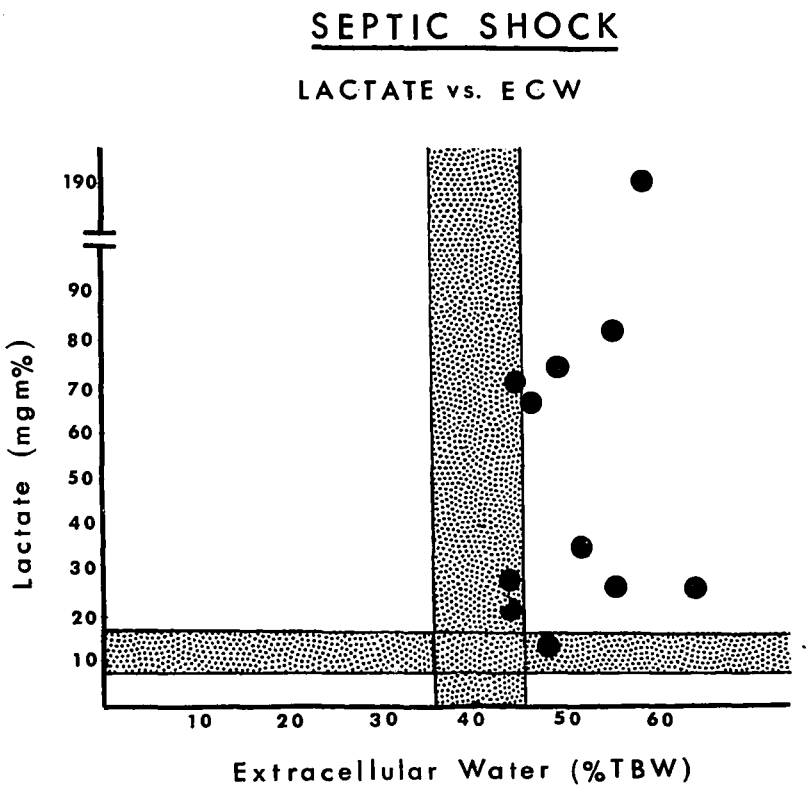


FIGURE 9

Shaded areas represent the expected normal limits.

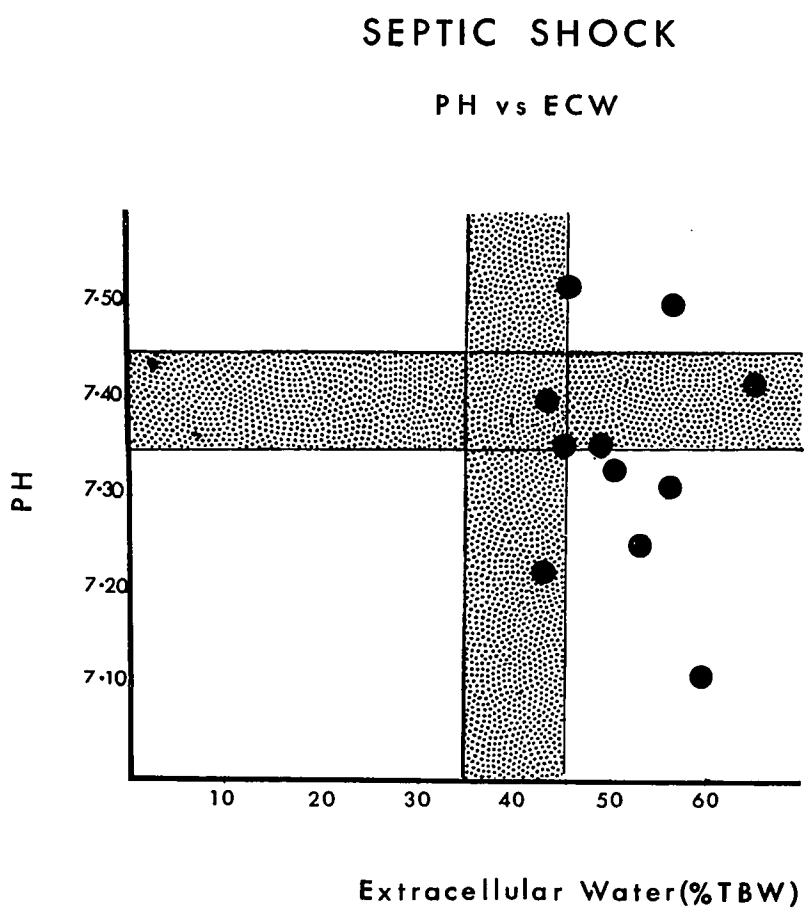


FIGURE 10

The shaded areas represent the expected normal limits.

for plasma volume and red blood cell volume. However, the extracellular water was greater in the septic group, this difference was highly significant with "p" less than .0001.

The reverse observation was appreciated in the intracellular water which would be expected since it is an indirect measurement resulting from the subtraction of extracellular water from total body water.

Finally, the total exchangeable sodium was found significantly higher in the six patients in which this measurement was performed. (Table VI)

# CHAPTER IV

# DISCUSSION

Our observations fail to support the concept of a major extracellular fluid volume deficit in hemorrhagic shock or septic shock. In order to confirm the validity of these observations, an additional study was carried out to verify the reproducibility of our results.

Following the techniques described in Chapter II, the space of distribution of I-131, Co-57, Na-22, S-35 and Inulin was measured twice within a four hour period in three normal dogs under light pentobarbital anesthesia which was the type of anesthesia used for all animals in our studies. (Table VII)

The $SO_4$ and Na-22 spaces were reproducible within 5% while the inulin space was reproducible within 10%. Co-57 space was reproducible within 7%. The RISA space showed a uniform decrease of 50 to 86 ml. corresponding to the amount of blood removed for sampling.

In 1960 Shires et al.[114] reported the simultaneous measurement of plasma volume, red cell mass and extracellular volume in 9 normal adults. Extracellular water varied from 18.8 to 24.7 per cent of body weight, the mean figure was 21.64 per cent of body weight. They concluded that their results agree closely with those of plasma volume, red blood cell mass and extracellular fluid as measured individually by various methods in other normal adults.

A paper on changes in extracellular fluid associated with major surgical procedures was published by

| Dog | Weight Kg | | RISA c c | %B.W. | CO-57 c c | %B.W. | NA-22 c c | %B.W. | S-35 c c | %B.W. | INULIN c c | %B.W. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | 21.1 | A | 1209 | 5.7 | 4545 | 21.5 | 5467 | 25.9 | 4084 | 19.4 | 6356 | 30.1 |
| | | B | 1123 | | 4726 | | 5503 | | 4289 | | 5731 | |
| | | Diff | -86 | | +181 | | +36 | | +205 | | -625 | |
| | | % Diff | -7.1 | | +4.0 | | +0.7 | | +5.0 | | -9.8 | |
| II | 22.7 | A | 1093 | 4.8 | 4939 | 21.8 | 5606 | 24.7 | 4759 | 21.0 | 6464 | 28.5 |
| | | B | 1036 | | 4630 | | 5432 | | 4946 | | 6845 | |
| | | Diff | -57 | | -309 | | -174 | | +187 | | +381 | |
| | | % Diff | -5.2 | | -6.3 | | -3.1 | | -3.9 | | +5.9 | |
| III | 21.8 | A | 769 | 3.5 | 5092 | 23.4 | 6560 | 30.1 | 5157 | 23.7 | 5458 | 25.0 |
| | | B | 718 | | 4699 | | 6316 | | 5177 | | -- | |
| | | Diff | -51 | | -393 | | -244 | | +20 | | | |
| | | % Diff | -6.6 | | -7.7 | | -3.7 | | +0.4 | | | |

TABLE VII

REPRODUCIBILITY OF RESULTS IN THREE NORMAL DOGS

the same group in 1961.[111] They studied two groups of patients, a control group consisting of five patients having general anesthesia and minor surgical procedures involving minimal tissue trauma and minimal blood loss. These patients received no fluids during the surgical procedure. Simultaneous measurement of plasma volume, red blood cell mass and extracellular fluid volume were made at the beginning of the operation and were then re-measured after two hours of the surgical procedure, using the method previously described.[114] The total blood loss measured was consistently 200 cc. or less, while there was no significant change in the extracellular water volume.

The experimental group consisted of 12 patients undergoing elective major surgical procedures. The measured total blood loss in this group varied from 215 cc. to 968 cc. which agreed with blood loss predicted from laparotomy, pack and sponge weights.

The extracellular fluid volume loss varied from 0 to 28 per cent in one patient of the original extracellular fluid, with an average loss of 13.17. This generally followed a pattern of increasing loss of functional extracellular fluid with increasing trauma, but was independent of whole blood loss. Serum sodium and potassium concentration stay within normal limits. The authors concluded that there was a loss of isotonic fluid due to internal redistribution in 1) the tissue adjacent to the surgical wound, 2) translocated into an area where salt tends to be sequestrated such as in the splachnic bed or 3) intracellular.

The same group presented a paper at the Surgical Forum in 1963 on Distributional Changes in Extracellular Fluid During Acute Hemorrhagic Shock. They used 16 splenectomized dogs. In three dogs, measured spaces at two hour intervals varied in less than 3 per cent. In group II, five dogs were bled 10 per cent of measured blood volume. Repeated measurements after two hours showed no change in extracellular fluid and a decrease in red blood cells and PV equal to the amount of blood removed. Group III (8 dogs) spaces were measured before and two hours after sublethal hemorrhage of 20 per cent of blood volume showing decreases in extracellular fluid of 18 to 26 per cent of the original values. By drawing blood samples every two minutes, they also found that equilibration time was 8 minutes for S-35 in the shock group as opposed to 22 minutes in the control group. The determination of red blood cells and plasma volume showed a loss proportional to the amount of blood removed. They concluded that hemorrhagic shock produces a shift of extracellular fluid within the body resulting in a decreased extracellular fluid volume.

In April 1964 Shires et al.[112] presented the follow-up of the preliminary report made at the Surgical Forum under the title "Fluid therapy in Hemorrhagic Shock". 45 mongrel dogs were submitted to the Wiggers preparation which details were presented in Chapter II, Method. Both ureters were cannulated operatively for collection of urine during isotope equilibration. Blood samples were collected

at 30 and 35 minutes following injection. They followed the previously described method for counting of the isotopes. The three spaces, red blood cells mass, PV and extracellular fluid volume were measured previously to hemorrhage and during severe shock. For treatment, the dogs were separated in three groups of ten each for a total of 30 dogs in the survival study. Ten animals were treated with only shed blood, 10 were given an additional 10 cc./kg. of donor plasma, 10 animals received Ringer's solution equal to five per cent of their body weight, followed by the shed blood. All fluids and blood were returned after the two and a half hour period of shock. The survival was only two dogs of the group treated with shed blood alone, three out of the group of shed blood plus donor plasma and seven out of the 10 treated by shed blood plus Ringer's lactate. In a parallel study, 15 dogs were subjected to the Wiggers technique and the three spaces measured before and during shock, then were treated with Ringer's lactate five per cent of their body weight, shed blood and 10 cc./kg. of plasma, respectively to each group of five dogs. One hour after treatment spaces were measured for a third time. In the dogs treated with shed blood there was a remaining deficit of extracellular fluid of 28 per cent. This deficit was 30 per cent in the group treated with shed blood and plasma. In the dogs treated with salt solution, the extracellular fluid returned to the control levels, with slightly increased plasma volume, which return to normal levels in the other two groups previously described.

They claimed to have demonstrated a disparate

reduction of functional extracellular fluid induced by hemorrhagic shock and indicated the alleviation of this reduction in extracellular fluid by the use of salt solution added to the shed blood. The 80 per cent mortality of standard Wiggers preparation had been reduced to 30 per cent by restoration of functional extracellular fluid which they claimed was not due to expansion of intravascular volume alone, since the addition of plasma to shed blood still resulted in a 70 per cent mortality, they recommended the administration of salt solutions as well as replacement of blood loss for treatment of hemorrhagic shock.

Crenshaw presented a paper at the Surgical Forum on "Changes in extracellular fluid during acute hemorrhagic shock". (Vol. XIII, 1962). The method dexcribed by Shires was applied to 18 pts. who had sustained acute hemorrhagic shock. Prior to administration of blood, simultaneous PV, red blood cell mass and extracellular fluid volume determinations were performed and these studies were repeated a week later as controls for each patient. The data obtained indicated an average reduction of 18.7 per cent, representing a 1131 cc. loss of blood volume. The functional extracellular fluid was reduced an average of 31 per cent which represents a functional loss of 5200 cc. or 4414 cc. loss of functional extravascular extracellular fluid.

Shires suggested that the decrease in extracellular fluid during acute hemorrhagic shock is due to isotonic

swelling of skeletal muscle cells and perhaps other cells in the body. They claimed to have demonstrated a consistent and sustained fall in intracellular membrane potential in acute hemorrhagic shock in dogs, considered consistent with increased intracellular volume.

Following the work of Shires, Virtue et al.[113] decided to investigate extracellular fluid changes following elective surgery and studied 25 patients undergoing cholecystectomy. All patients were placed on balanced diets and plasma volume and extracellular fluid volume were determined before surgery and immediately post-operatively using $I^{131}$ and $S^{35}$ as tracers for plasma and extracellular fluid volume. All patients had the same anesthesia; six control patients received less than 100 cc. of Ringer's Lactate. The other 19 patients received either Ringer's Lactate or G/W 5% or N/S, at the rate of 15 ml./kg. body weight during the first hour and 7.5 ml./kg. body weight during each succeeding hour.

Their results were summarized as follows:

1) extracellular fluid loss did not vary regularly with the degree of trauma
2) extracellular fluid loss was not consistently greater than blood loss in the control group
3) extracellular fluid loss can be offset by administering Ringer's Lactate solution and equally effective by administering 5% G/W or physiologic saline.

They suggested that as Shire's post-operative determinations were done about two hours after operation, fluid may have shifted considerably during these two hours.

In our study, the volumes recorded by the use of four different methods of extracellular water volume measurement do not show a significant change after hemorrhagic shock. The validity of our extrapolation technique is supported by the similarity of control values for each substance. Walser, Seldin and Grollman[116] studied the technique of single sample radiosulfate space measurement used by Shires and found no difference between the inulin and radiosulfate volumes. This suggests that the time required for equilibrium of the tracer material may not be the same before and after shock. This possibility is supported by the observation of Schloerb[117] et al. and Roth,[118] in which the equilibration time was studied and found to be extended in animals after hemorrhagic shock. Schloerb[117] has also pointed out that under these circumstances sulfate distribution may be abnormal since its distribution in various body organs is uneven. Vineyard[119] has reported studies which suggest that sulfate may be bound to plasma for a time after injection which again invalidates its use as a single sample method of measuring extracellular water.

It is important to recognize that many studies of body composition were originally based on single samples taken at the time of equilibrium. More recently the back extrapolation technique has been developed and would seem a more accurate method particularly in the traumatized patient where equilibrium is delayed. The twenty minute sample utilized by Shires and the resultant measurement of

a deficit may merely be the result of failure to equilibrate at this time as described above. Anderson et al. in using radiosulfate in Viet Nam injuries and the present experimental studies have clearly shown that the sulfate space determined by back extrapolation of a straight line based on multiple samples is not decreased under these circumstances. Our studies and those of Vineyard suggest that, using the multiple sample technique, no difference exists between spaces measured by a variety of substances before or after this type of hemorrhagic shock.

Nevertheless, it may be that internal redistribution of body water does occur after hemorrhagic shock. It is possible that isotope dilution techniques, especially with small radioactive ions, fail to differentiate between a functioning and a sequestered extracellular compartment. This possibility is less likely since the larger molecule inulin, which should not penetrate the transcellular fluid described by Edelman,[120] did not demonstrate a different extracellular fluid volume in our experimental studies. It may be that the improvement in survival using balanced salt solution results from increased flow rather than fluid deficit replacement as has been suggested by Baue.[121]

Significant increase in the extracellular water volume was identified after septic shock. It is quite possible that the distribution of radioactive ions includes the transcellular fluid. In future studies it will therefore be important to combine the radioactive ion studies with measurements using Inulin or Sucrose[121, 122, 123] which do not normally penetrate these possible areas of

sequestration. The use of Inulin for such studies is simplified by the method of Shizgal.[115]

Parenteral fluid therapy is a common denominator in the management of most surgical patients. The recent concept of a decrease in functional extracellular fluid volume has been examined in hemorrhagic shock and septic shock. Our findings do not support the occurrence of a deficit in this space. Nevertheless, a functional deficit may exist during major abdominal surgery in particular since the use of radioactive ions may, through their distribution into the gastrointestinal tract, measure both functional and sequestered fluid. Certainly, balanced salt solutions in the treatment of body injury should be used in moderate amounts until the basis for their use has been documented.

Since we can find no evidence that an increased total exchangeable sodium has been caused by high administration of sodium at a time prior to measurement of body water composition, the most likely explanation is that the process of septic shock has led to a mobilization of non exchangeable sodium. It may be that the high mortality rate associated with septic shock might be improved by measures desiring to increase sodium losses.

## SUMMARY

Body water composition has been studied in hemorrhagic shock and septic shock. In hemorrhagic shock multiple tracers of varying function and molecular size were used. Deficits observed were not statistically significant and could be accounted for by vascular compartment loss due to the withdrawal of blood samples.

In eleven patients in septic shock, the observations of an increased extracellular water and increased total exchangeable sodium not associated with an increase in total body water have been recorded. In addition, no correlation was found between this observation and the more usually performed tests of hemodynamics and metabolic functions in the shock patient.

These studies do not support the concept of selective extracellular fluid deficits in hemorrhagic shock or septic shock. Unwarranted infusion of large amounts of salt-containing solutions should be avoided unless further scientific support for their use can be obtained.

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# APPENDIX

Detailed laboratory procedures using multiple isotope techniques were used for measurement of body fluid spaces.

Extracellular water volume measurements:

The extracellular water volume was estimated in the experimental animals by measuring the volume of distribution of:

1. Sodium - 22
2. Sulfate labelled with sulphur - 35
3. Cobalt - 57 labelled Vitamin $B_{12}$
4. Inulin

Following a single intravenous injection of the above several blood samples were taken over a two hour period. The concentration of Na-22 and Cobalt -57 was determined by counting in a deep well scintillation counter. S-35 counting was carried out in a Packard Tri-carb liquid scintillation counter using the scintillation solution described by Bruno and Christian.[122] Internal standards were used to correct for quenching variations. Inulin concentration was determined in duplicate using the resorcinol method.

The Cobalt, Sulfate and Sodium spaces were obtained by plotting the log of concentration against time. (Fig. 2) The resultant straight line is extrapolated to the Y axis to obtain the plasma concentration at the time

of injection. The volume of distribution was calculated by dividing the latter concentration into the total counts/min. injected, which was obtained by accurately weighing the solution injected and counting a standard solution. The latter is prepared by diluting a weighed aliquot of the injection solution in a known volume.

In clinical studies, $Na^{22}$ was the tracer chosen for measurement of extracellular water volume, following the technique described above.

Injection of Isotopes

The amounts of isotopes usually injected were as follows:

a) I-125 labelled RISA 10-15 uc

b) Cr-51 50-100 uc

c) Sodium-22 10-20 uc

d) Tritiated water 500-1000 uc

e) S-35 labelled Sodium Sulphate 5-10 uc

It should be noted that it is not necessary to accurately know the amount of uc injected for volume determinations. It is important that the volume of the solution injected be accurately known. This is accomplished by accurately weighing the syringe with the needle and needle guard before and after the solution has been injected into the subject. This should be carried out on a scale which is correct to 1/10 of a gram. The volumes injected should usually be an excess of 5 cc. to maintain proper accurracy. The amount of isotope injected is usually adjusted so that the samples will give 40,000 counts in 20 minutes. The isotope solution was usually made up in 100 cc. saline vials.

Sterile precautions were undertaken at all times. The solutions were kept in a special refrigertor at all times. The concentration of the injection solutions were as follows:

a) I-125 labelled RISA - 1 uc per cc.

b) Cr-51 50 uc per cc.

CR-51 is usually supplied at this concentration and therefore it is not necessary to further dilute it. It is not important to accurately record the amount of isotope used to tag the red cells, however, it is necessary to know within 1% accuracy the volume of tagged cells injected and the concentration of isotope in the solution injected.

c) Sodium -22 luc per cc.

d) Tritiated water 50 uc per cc.

e) S-35 labelled Sulphate 10 uc per cc.

Red Blood Cell Measurements in Clinical Studies

Red blood cell volume was determined by tagging red cells from the patient with chromium$^{51}$. Approximately 30 ml. of blood were mixed with 50 microcuries of Chromium$^{51}$ and incubated for 45 minutes at 37$^{o}$C. with frequent mixing to permit Cr$^{51}$ tagging of the red cells. The cells were washed three times with normal saline and weighed amount was reinjected. When studying patients in shock only one washing with saline was carried out because of obvious time saving purposes. In all these cases plasma and 24 hour urine was examined to determine Chromium$^{51}$ activity.

In all experiments no appreciable amount of radioactivity due to free Chromium$^{51}$ could be detected. A standard was prepared by diluting a weighed aliquot of the injection solution in a known volume of distilled water.

Several venous blood samples were obtained over a period of time hemolyzed with 0.2 ml. of Saponin and counted in a deep well scintillation counter. The red blood cell volume was obtained by dividing the product of the mean $Cr^{51}$ concentration and the hematocrit into the total amount of $Cr^{51}$ injected.

Plasma Volume:

The plasma volume was obtained using $Iodine_{131}$ labelled serum albumin. As with sodium and sulphate spaces determinations, several blood samples were taken to determine the extrapolated concentration at the time of injection. The method of least squares was used to fit the best straight line to the experimental data.

In some patients, Iodine-125 was used instead of Iodine-131 in order that Cr-51 could be simultaneously injected without compromising accuracy. Furthermore, because of the greater counting efficiency of I-125, increased counting accuracy can be achieved with less radiation ezposure to the patient.

Total Body Water:

Total body water determinations were carried out by injecting a weighed amount of tritiated water. Blood samples were taken at 3 and 4 hours. An equal volume of 10% TCA is added to the plasma to precipitate the proteins. The resultant supernatant is added to the scintillation solution. All the standards and samples are prepared in triplicate and counted in a tri-carb liquid scintillation counter. Quenching variation is corrected for by using

internal standards. The volume is obtained by dividing the plasma concentration at equilibrium into the total amount of tritiated water injected.

Differential Counting Techniques:

Whenever more than one isotope is injected, it is necessary to employ differential counting techniques in order to determine the activities due to the individual isotopes. For each isotope injected it is necessary to count each sample once at a specific window setting on the pulse height analyser. The window was chosen so that there is a maximum counts per minute of one isotope and a minimum counts per minute due to the other isotopes. (Fig. 11) It is also necessary to count each standard solution in each of the windows. This is usually done in duplicate for each series of tubes counted. In this manner, a series of simultaneous equations can be set up for each sample, the solution of these equations will give the activities due to the individual isotopes. When I-125, Cr-51, Na-22 and H-3 are injected at the same time it is necessary to solve the following equations:

$$E_1 = a_1 I + b_1 Cr + c_1 Na + d_1 H$$
$$E_2 = a_2 I + b_2 Cr + c_2 Na + d_2 H$$
$$E_3 = a_3 I + b_3 Cr + c_3 Na + d_3 H$$
$$E_4 = a_4 I + b_4 Cr + c_4 Na + d_4 H$$

Where: $E_1$, $E_2$, $E_3$, $E_4$ = counts/min. of the sample in window 1, 2, 3, and 4

I = counts/min. due to I-125 in window 1

Cr = counts/min. due to Cr-51 in window 2

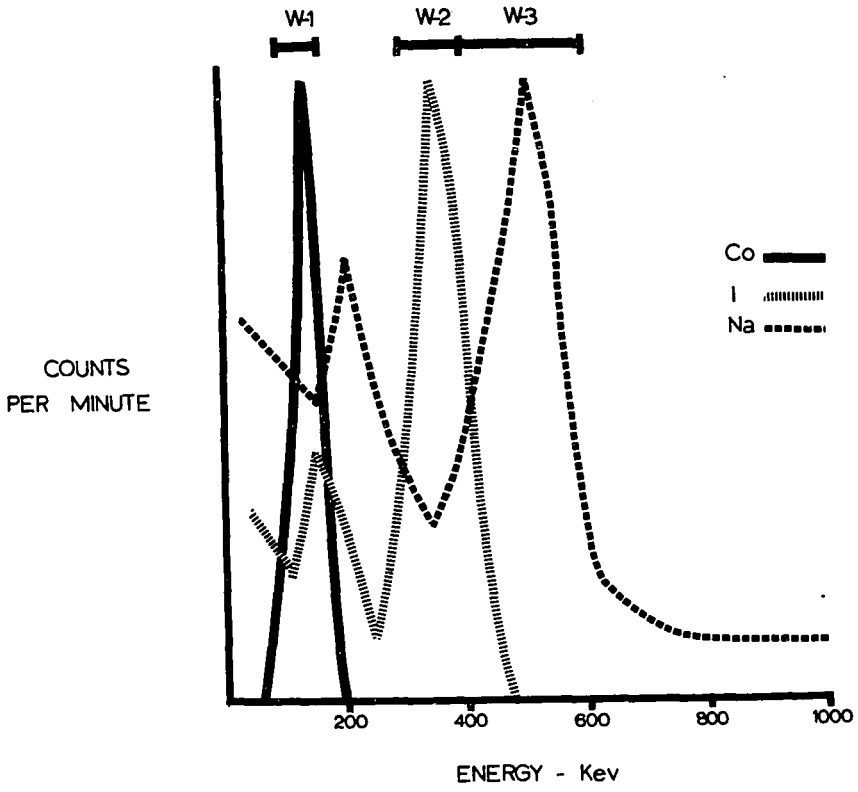



FIGURE 11

Differential counting techniques: the window was chosen so that there is a maximum counts per minute of one isotope and a minimum counts per minute due to the other isotopes.

where: Na = counts/min. due to Na-22 in window 3

H = counts/min. due to H-3 in window 4

$a_1$, $a_2$, $a_3$, $a_4$ = cts/min. of I-125 std. in windows 1,2,3 and 4 divided by the cts/min. of I-125 std. in window 1

$b_1$, $b_2$, $b_3$, $b_4$ = counts/min. of the Cr-51 std. in windows 1,2,3 and 4 divided by the cts/min of the Cr-51 std. in window 2

$c_1$, $c_2$, $c_3$, $c_4$ = cts/min. of the Na-22 std in windows 1,2,3 and 4 divided by the cts/min of the Na-22 std in window 3

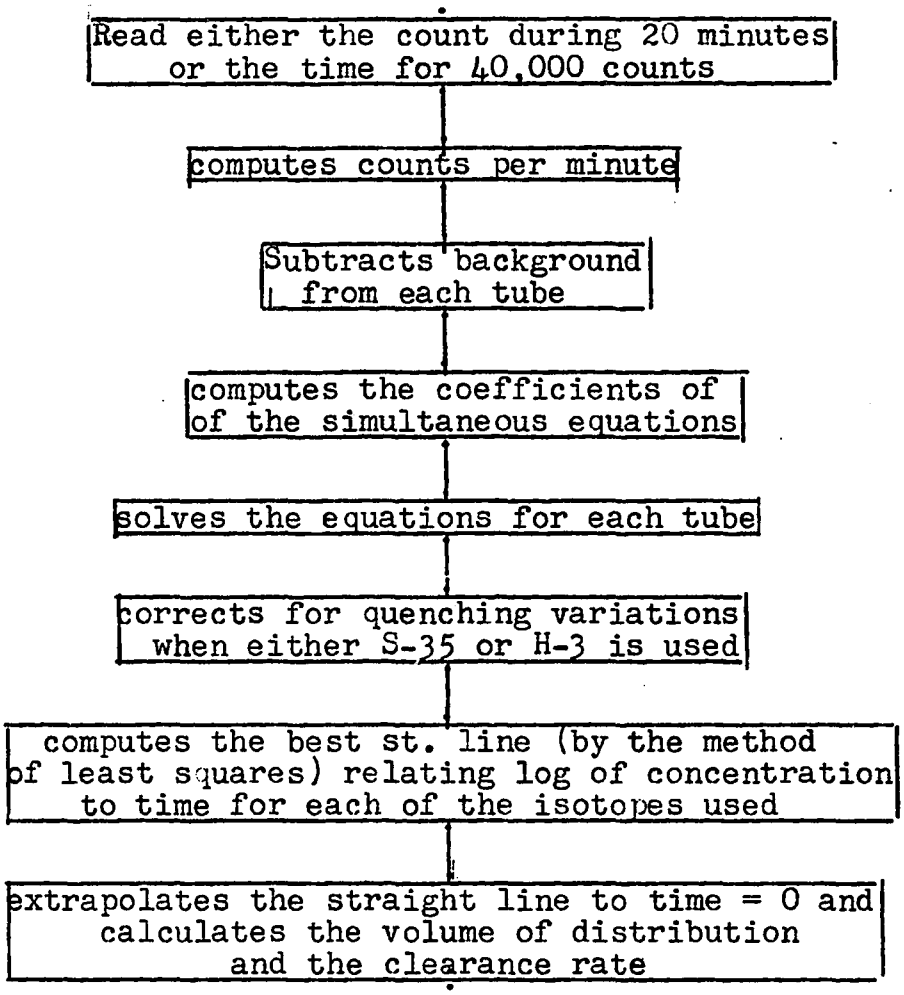
$d_1$, $d_2$, $d_3$, $d_4$ = cts/min. of the H-3 std in windows 1,2,3 and 4 divided by the cts/min. of the H-3 in window 4

A solution of the above exists because there are 4 equations and 4 unknowns (I, Cr., Na., H)

One of the difficulties with differential counting techniques is that in order to obtain the solution it may become necessary to subtract one large number from another. The resultant small number would tend to be less accurate. This is not a problem with the isotopes we used because most of the coefficients were zero and the remaining ones were less than 0.3

Computor program

A fortran IV program has been developed for use on the IBM 7044 at the McGill computing centre to carry out all the calculations outlined above. A block diagram of the program is listed on the following page.




All the input information is also included in the print-out so that errors in keypunching can be easily picked up. Furthermore, all the data is plotted on semi-log graph paper so that we are not forcing a straight line to data which do not lie as a straight line.

The greatest value of the computor lies in the fact that a typical experiment requires approximately 30 minutes of keypunching. Fifteen to twenty man hours would be required to carry out the same calculations on a desk calculator.