THE USE OF RADIOSULFATE IN THE MEASUREMENT OF DIURNAL FLUCTUATIONS IN EXTRACELLULAR FLUID VOLUME

by

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A. INTRODUCTION:

Clinically, relatively large changes in the body water of animals are made apparent by the signs characteristic of dehydration and edema. However the measurement of the distribution of the water among the various body fluid compartments *in vivo* is a largely unsolved problem. It is the purpose of this thesis to study the sulfate space as a measure of extracellular fluid volume, to apply this method to the study of diurnal changes in this body compartment under various conditions, and to attempt to correlate any changes which might occur with the electrolyte balance.

B. HISTORICAL REVIEW

1. BODY FLUID COMPARTMENTS:

a) Definition of various compartments:

The living cell is an aggregate of fluid protoplasm separated from the external environment by a cell membrane. Any sudden changes in the external environment lead to death of the cell. In the evolution of complex multicellular organisms, organ systems have developed such that the environment surrounding the cells is maintained relatively constant allowing the organism as a whole to adapt itself to many different environments. However, although the composition of the body fluids does remain remarkably constant, this state is a dynamic one since the actual constituents are continuously changing with ingestion, excretion, and cellular metabolism. The significance of the constancy of this internal environment was first recognized in 1859 by Claude Bernard when he described the "milieu interieur".

Body fluids may thus be classified as intracellular (ICF), the protoplasmic fluid of individual cells, and extracellular (ECF), the fluid surrounding the cells. The two together make up about 70% of body weight. These two fluids differ markedly in their ionic composition, the principal electrolytes of the ICF being K, Mg, PO_4, and protein, those of the ECF Na, Cl, HCO_3, and SO_4.

The ECF may be further subdivided into the plasma and the interstitial fluid which differ only in the high protein content of the plasma. If account is taken of the effect of the non-diffusible protein according to the Gibbs-Donnan equilibrium, their ionic concentrations are the same (1).

b) Concept of extracellular fluid volume:

Theoretically the term extracellular fluid includes the plasma, interstitial fluid, lymph, eye humors, cerebrospinal fluid, synovial, pleural and pericardial fluids, glandular secretions and urine; but since these anatomic entities do not behave as a single unit physiologically, a more practical concept of extracellular fluid, according to Ryan et al. (2), and a more physiologic one, is "the plasma and those fluid compartments with which it freely exchanges ions and small molecules". By this definition are excluded the cerebrospinal fluid, urine, synovial fluid and glandular secretions which have been termed by Edelman, Olney, James, Brooks, and Moore (3) the "transcellular water" because it has had to pass through and be processed by cells other than capillaries in order to reach its present site.

It has been suggested by Cotlove (4) that the concept of the extracellular fluid has been oversimplified and that it should really by considered as consisting of two subphases, the interstitial fluid which is an ultrafiltrate of plasma, and connective tissue.

Different tissues vary with respect to their relative proportions of water and electrolytes (5,6,7); for example, skin contains a higher proportion of extracellular fluid than does muscle. However the proportions of solids, ECF and ICF are similar enough to justify consideration of the fluids of the body as a single system; as pointed out by Elkinton and Danowski (1), this simplification becomes mandatory when dealing with abnormalities created by disease states.

c) Expression of data for body fluid volumes:

A problem arises as to what reference point should be used to relate body fluid volumes. Generally they are quoted as % body weight, but since

excess fat, which has a low water content (8), varies greatly in the human - from one to forty per cent or more - this is by no means an ideal referent. Estimation of fat content from skin thicknesses is not sufficiently accurate to be useful (9).

Lean body mass has been cited by Behnke (10) as being much more meaningful since its water content is comparatively constant at about 72%. Muldowney (11) showed that red cell mass correlated well with lean body mass, a result differing from that of Huff and Feller (12). A study by Muldowny, Crooks, and Bluhm (13) showed that there is a close correlation of exchangeable chloride and of exchangeable potassium with lean body mass while correlation with body weight is poor.

Lean body mass can be calculated from the specific gravity (14), but this method is not readily applicable clinically. It can also be estimated from the relation: Lean body mass = total body water x 100/73 (15), but this relationship does not hold true necessarily in disease states. Because of the difficulties of determining lean body mass, body fluid volumes expressed in the literature as per cent body weight usually have a wide normal range unless subjects of the same body build are compared.

d) Variation with sex and age:

It has been demonstrated that in health the total body water and ECF are significantly larger in relation to body weight in males than in females (16). The same was true for the exchangeable K and it was inferred from these results that lean body mass and cell mass occupied a larger body fraction in the male group.

Analyses of newborn animals and fetuses have shown a higher concentration of Na and Cl per kilogram body weight than that in adults of the same species (17). Since the concentration of Na in interstitial fluid

is the same, this has been taken to indicate that they contain more ECF per kilogram body weight than do adults.

2. REGULATION OF BODY WATER:

a) Internal transfers:

Movement of water among the various body fluid compartments is governed mainly by the hydrostatic pressure and the osmotic pressure on the two sides of the compartment boundary, and by the movement of ions from one side to the other (18). The difference in composition of plasma and interstitial fluid, as stated earlier, is due to the presence in the plasma of a greater amount of protein which accordingly exerts a greater osmotic pressure. Edelman et al. showed that serum osmolarity correlated closely with serum Na concentration when corrections were made for the osmotic contributions of glucose and non-protein nitrogen (19). The serum osmolarity thus corrected also exhibited a high degree of correlation with $(Na_E + K_E)/$ TBW (Na_E = exchangeable sodium, K_E = exchangeable potassium, TBW = total body water). These authors concluded that body water is passively distributed in proportion to osmotic activity.

The difference in composition of intracellular and interstitial fluid is due to the presence inside the cell of materials which cannot pass through the cell wall, and to the differential distribution of ions on the two sides of the cell membrane, notably Na being in much higher concentration in ECF, while K is higher in ICF. Several theories to explain this differential distribution of ions have been proposed but it is not well understood (20). Evidence that aldosterone is an important factor regulating cell permeability has been presented by Woodbury and Koch (21) who demonstrated that administration of this hormone in rats

caused increases in $\frac{\text{extracellular Na}}{\text{cellular Na}}$ and $\frac{\text{cellular K}}{\text{extracellular K}}$ in both brain and muscle.

b) External transfers:

External transfers of water and ions, effected through ingestion and through excretion via skin, lungs, kidneys, and intestine allow the organism to have a constant osmotic pressure in the body fluids. The osmotic pressure is maintained at the expense of volume, although in health the total body water varies but little, as evidenced by the constancy of body weight (1).

(i) Gastrointestinal tract: Fecal water loss is normally small, amounting only to about 100 ml. per day. Differential exchanges of electrolytes occur between the plasma and gastrointestinal tract and the factors controlling these are not well understood. Thus Visscher has shown that Na^{24} moves across the blood-intestinal lumen barrier at higher rates under comparable conditions at the oral than at the aboral end of the bowel (22).

It has been postulated that in sodium deprivation the adrenocortical hormones regulate to some extent the net absorption of Na and K from the gut in a manner similar to their regulation of the excretion of these electrolytes in the urine, sweat, and saliva (23). Estrogens have also been shown to affect intestinal electrolyte exchanges (24).

As much as 14% of the exchangeable sodium was found in the gut of rabbits by Edelman and Sweet (25). The same authors found that total gastrointestinal potassium approximated 7.2% of the exchangeable potassium of rabbits (26).

In absorption studies using radioactive tracers, the absorption of water from the stomachs of healthy persons was rapid, 95% of the dose

administered being absorbed in 50 minutes; but the rate of absorption from the small bowel was consistently much faster, 95% absorption occurring in ten minutes (27). Dual isotopes have been used for such studies (28).

(ii) Lungs and skin: Water loss occurs via lungs and skin by evaporation in appreciable amounts, as much as 800 to 1200 ml. per day in the non-febrile adult. Na, K, and Cl are also excreted in the sweat. Adreno-cortical hormones are known to inhibit excretion of Na and Cl and enhance that of K in the sweat (29).

(iii) Kidney: The kidney is the chief organ of regulation of the body fluids, the adjustments being effected primarily through alterations in tubular transfers. About 80 to 85% of water reabsorption is obligatory while the remaining 15 to 20% is subject to facultative reabsorption in the distal tubule and possibly the collecting ducts (1).

Reabsorption of water is enhanced by antidiuretic hormone. Data concerning this hormone have been reviewed by Thorn (30). Stimulation of osmoreceptors in the hypothalamus by hypertonicity of the ECF causes an increased production of antidiuretic hormone (ADH) in the supraoptico-hypophyseal system, in turn correcting the hypertonicity. In addition, the cellular dehydration resulting from the hypertonicity stimulates thirst and thus increases intake of water as well. ADH production is also affected by emotion, painful stimuli and alcohol.

c) Volume regulatory mechanisms:

Evidence is accumulating that there exist in the body receptors sensitive to sodium and to some phase of fluid volume. This material has been reviewed by Smith (31).

Several cases of mediastinal tumours have been associated with hyponatremia (32) suggesting that sodium receptors may be present in this portion of the body.

Receptors sensitive to blood volume are probably located in the left atrium and are responsible for the Henry-Gauer reflex, i.e. the inhibition of antidiuretic hormone by vagal impulses caused by distention of the left atrium (33). Stimulation of such receptors may account for the diuresis induced by changing position from sitting or standing to supine, and by infusion of isotonic saline, isooncotic, and hyperoncotic albumin solutions in the supine position. Conversely, the antidiuresis associated with hemorrhage, orthostatic circulatory insufficiency, occlusion of venous return by pressure cuffs or venacaval obstruction, and the sitting position may be explained on the basis of a decreased distention of the left atrium and therefore lessened inhibition of antidiuretic hormone (31).

Receptors sensitive to extracellular fluid volume may be an important factor in controlling sodium excretion. The site of such receptors is unknown. Possibly such receptors are part of a volume-regulating mechanism which involves aldosterone as the effector since there is some evidence that a decrease in ECF acts as a stimulus to aldosterone production and vice versa (34, 35, 36, 37, 38). Such a mechanism would account for the increased aldosterone production which occurs on vasopressin withdrawal (39).

An intact hypothalamus appears to be necessary for aldosterone secretion, and while the pituitary does not seem to be essential in the regulation of aldosterone, some effects on aldosterone excretion have been reported following hypophysectomy and ACTH (40). Increased ECFV (extracellular fluid volume) following ACTH administration has also been reported (41).

Increased aldosterone production was observed after human and monkey growth hormone administration (191), but not after non-primate growth hormone (42). Total body water, extracellular water and mean intracellular Na were found to be increased in acromegaly (43).

Other stimuli to aldosterone production which have been suggested are the serum K (44, 45), the Na:K ratio (46, 47), and some function of the total body Na(34).

Recently, acute constriction of the inferior vena cava above or below the hepatic veins was shown to increase aldosterone production within 30 minutes in dogs, irrespective of changes in vascular volume (48).

3. DIURNAL (24-HOUR) RHYTHMS:

a) Types of 24-hour rhythms:

Harker, in a comprehensive review (49), defined two types of 24-hour rhythm: exogenous - those which occur as immediate responses to environmental change and which do not continue in constant conditions; and endogenous - those which persist when an environmental condition is held constant, where this condition can, when fluctuating, affect or determine the form of the rhythm. Such rhythms, expressed as changes in locomotor activity, oxygen consumption, colour, ovulation, blood constituents, excretory products, body temperature and other indices, have been shown to occur in most phyla of animals and plants.

b) Cause of endogenous 24-hour rhythms:

The way in which persistent rhythms are maintained is unknown. A number of theories have been postulated but none appears to cover all the facts. The accumulation of toxic depressants, the elaboration and exhaustion of reserve products, and hormones have been suggested as exerting some type of control. Since persistent rhythms do not seem to

be related to feeding times in some animals, the first two methods of control seem unlikely. There is evidence that hormones are involved in certain rhythms (50), but it would seem in the case of the 24-hour rhythms of Protozoa and tissue cultures that hormones could not be responsible. The nervous system has also been suggested as the centre of rhythmicity but again it cannot account for the 24-hour rhythms occurring in tissue cultures.

Isolated plant tissue was shown by Enderle (51) to have a rhythm of turgor and weight change which persisted in darkness for several months. He interpreted this as evidence for a basic 24-hour rhythm present at the cellular level.

Harker suggests that there is a "basic 24-hour rhythm present in the cells of all animals, that this rhythm is inherited, that it continues unchanged even when it is not superficially evident, and that it may be concealed through the immediate influence of the environment, although environmental factors serve on the whole only to position the phases of the rhythms within the 24-hour cycle".

c) 24-hour rhythms in humans:

In man, many 24-hour rhythms have been described. The blood eosinophil count, the red blood cell count, clot retraction time, cholesterol and blood sugar levels, pulse rate and blood pressure all have 24-hour rhythms (49). The blood chloride may have a 24-hour rhythm although this is not clearly established (52).

The rhythms of water and electrolyte excretion have been studied extensively in man (53). Water, sodium, potassium and chloride are all excreted in greater amounts during the day than during the night regardless of intake. Urinary pH is lower at night and the excretion of ammonia and titratable acid is increased at night. The excretory

cycle is absent in the newborn but becomes apparent by the fourth to sixth week of life.

Reversal of the urinary diurnal rhythm has been reported to occur in habitual night workers, some cases of Addison's disease, and cases in which generalized edema occurs: cardiac failure, renal failure, malnutrition, cirrhosis, and the nephrotic syndrome (54). It has also been reported in some cases of head injury (55).

The cause of the diurnal rhythm of urinary excretion is unknown. Stanbury and Thompson concluded that small diurnal changes in glomerular filtration rate may help to produce the cyclic changes in electrolyte excretion, but changes in tubular function are probably of greater importance. They suggested that the changes might result from cyclic metabolic changes in the renal tubule cells produced by the alternation of sleep and wakefulness.

When the urinary rhythms were investigated in subjects living on 22, 21, and 27 -hour time schedules in the Arctic (56, 57, 58), the 24-hour rhythms persisted in most and there was some dissociation of the water and potassium rhythms. The body temperature rhythm on the other hand adapted rapidly and completely to the new time schedule.

Other evidence that the excretory rhythms are to some extent independent of each other is the observation that in normal individuals who carry out normal activities the sodium excretory peak is delayed by as much as 10 hours while the potassium peak occurs in midday as it does in recumbent normal individuals (59).

In recumbent normal fasting individuals there is a net movement of potassium out of resting forearm muscle into plasma in the late morning hours (60). From 1 a.m. to 10 a.m. no net movement occurred. Potassium is also known to leave muscle during exercise, anoxia, potassium depletion,

and under the influence of acidosis and of adrenocortical hormones.

Diurnal variations in hormone levels have been suggested as factors. The blood level of 17-hydroxy corticoids is lowest between 2:00 and 4:00 a.m., rises rapidly between 4:00 and 8:00 a.m., falls gradually through the day and becomes fairly steady during the evening hours (61, 60). Doe, Flink and Goosell (63) found that eosinophil variation and potassium excretion were closely related to variation in plasma 17-hydroxy corticoid level, but urinary sodium was not. Although striking changes occurred when large amounts of cortisone were given, Rosenbaum et al. concluded that the normal diurnal rhythm of renal excretion did not depend on rhythmic changes in endogenous adrenocortical secretion (64).

A diurnal variation in urinary aldosterone has also been reported (65, 66). Aldosterone excretion was highest in 6:00 to 12:00 a.m. collections, lower in the afternoon, and lowest at night, being fairly similar to corticoid and 17-ketosteroid patterns. Muller, Manning and Riondel (67) related aldosterone production to position and activity. Their urine collections were made from 7:00 a.m. to 7:00 p.m. and from 7:00 p.m. to 7:00 a.m. No difference was found whether food was taken during the day or at night. Aldosterone was found to be higher always during the day. With recumbency aldosterone excretion fell. Sodium excretion increased markedly during recumbency, followed by sodium retention on getting up again. With sitting the fall in aldosterone excretion was less marked. On changing position from sitting to lying down, aldosterone excretion fell the first day. A diurnal variation of aldosterone excretion was lacking in patients with pituitary insufficiency but a rhythm was established when the patient was placed on cortisone, provided the patients were up and active. These authors concluded that the vertical position and muscular activity cause the

diurnal variation of aldosterone, provided cortisone is present.

On assuming the erect position, there is a decrease in renal excretion of water, Na, K, and Cl (68). The decrease in water excretion is blocked by alcohol, suggesting that antidiuretic hormone is responsible, but the decrease in Na, K, and Cl excretion is unaffected by alcohol.

No data were found on diurnal variation of blood volume, but it is known that the blood volume rises on going from the vertical to the horizontal position, a change which is accompanied by a fall in serum proteins and haemoglobin levels (69). Widdowson and McCance (70) found that this change was maximal after two hours; and if bed rest was continued for as long as three days, the blood volume fell below its intial value, the hematocrit and haemoglobin rising accordingly. After protracted recumbency, a change to the vertical position for a few hours returned the individual to his original state. In the vertical position a brief bout of exercise was shown to be accompanied by the shift of more than one-half litre of fluid from the plasma into the extracellular fluid and/or tissues (71). Following exercise the pre-exercise values were reached in about 25 minutes.

Two abstracts were found which dealt with variation in extracellular fluid volume during the day. In the first (72), Peterson, Kirkendale, and O'Toole, in 40 studies using sucrose infusions, found that large fluctuations, as much as 60%, occurred in the sucrose space within a few hours. They concluded that the sucrose space was so variable as to render it of doubtful value as a biologic reference point.

In the second abstract (73), by the same authors, similar studies were described. Sucrose levels were followed hourly over 24-hour periods in man in the fasting state and supine position. Despite rigidly controlled infusion rates ($\pm$ ½%), the plasma sucrose level varied within $\pm$ 15% of the

mean. Although some fluctuations were attributable to variations in renal sucrose clearance, many wide fluctuations were independent of the clearance changes and were concluded to represent physiologic variations in the space, implying that striking variations in gastrointestinal water, in cellular hydration, or in other body fluid stores might occur during a "steady" state. In four studies, cardiac output, glomerular filtration rate, and renal blood flow were also measured and no correlation was observed with changes in sucrose space. Time of day, fluid ingestion, and body weight changes also appeared to be unrelated.

B. METHODS OF MEASURING EXTRACELLULAR FLUID VOLUME

1. GENERAL REVIEW OF THE MEASUREMENT OF BODY FLUID COMPARTMENTS:

Volumes of the total body water and its compartments have been estimated by a variety of means, and these have been reviewed by several authors (76, 77, 78, 79, 80).

a) In vitro methods:

Attempts to measure total body water date back to the mid-nineteenth century when von Bezold enunciated the fundamental concept that every animal possesses a normal water, organic matter, and salt content which is characteristic of its species and age (15). Obtaining his data by desiccation and chemical analysis, he showed this to be true in mammals, birds and amphibians.

The earliest determinations of human total body water were also made by desiccation of cadavers. Since this is a technically difficult procedure, and human material is rarely available, it has been done only a few times on humans (81, 82, 83), one of the earliest studies being reported by Bischoff who, in 1863, obtained a value of 58.8% body weight of cadaver (77).

Extracellular fluid volume was first estimated at the turn of the century, by measuring the width of tissue spaces in frozen cross-sections of frog sartorius muscle, values of 14% to 20% being obtained. Similar studies were reported by Fenn in 1936, a value of 17.5% being obtained (74).

In the same year, the extracellular fluid volume was estimated by determining the total electrolyte concentrations and assuming chloride to be limited entirely to the ECF except for that in red blood cells. Using this method, the ECF was found by Harrison, Darrow and Yannet to compose

about 27% body weight (7). Allowing also for the chloride contained in the gut lumen, the "excess" chloride of connective tissue and the chloride intracellular in liver and other tissues, Cheek, West and Golden (84) obtained a value for ECF volume of 22% body weight. These authors obtained a closely similar value for ECF volume when they calculated it from the total sodium, correcting for bone sodium and the intracellular sodium of muscle.

The above methods are applicable only to autopsy material and give no information as to how body fluid compartments may alter during life.

b) In vivo methods:

In vivo methods include specific gravity studies, balance studies and dilution studies.

(i) Specific gravity studies: Total body water has been determined from an empirical equation derived from data for total body water, specific gravity and body fat in sacrificed animals (85). The body specific gravity should be corrected for air in the gut and lungs. Lean body mass has been calculated to have a constant specific gravity of 1.099, fat 0.92. Thus the fatter the subject the less body water he has in relation to weight. This method is limited in its application to human subjects because it requires total submersion in water long enough to measure the amount of water displaced.

(ii) Balance studies: These comprise the accurate determination of input and output over a period of time. They have been used to follow changes in body water, but do not provide absolute measurement of fluid phases. ECF changes are calculated from the gain and loss of chloride (assuming chloride to be entirely extracellular), the change of concentration of chloride in ECF, and an initial ECF volume either assumed or measured.

Thus: $$E_2 = \frac{\left(E_1 \ [Cl]_{E_1} + b\ Cl\right)}{[Cl]_{E_2}}$$

where E_1 = initial ECF volume
E_2 = final ECF volume
b Cl = chloride balance
$[Cl]_{E_1}$ = initial } Cl concentrations
$[Cl]_{E_2}$ = final } in ECF (1)

$[Cl]_{E_1}$ and $[Cl]_{E_2}$ are calculated from their corresponding concentrations in serum, using a Donnan factor of 0.95.

If done very carefully, Wilson et al.(86) conclude that for short term studies in body composition, balance studies provide the most useful method.

(iii) Dilution studies: On the other hand, dilution techniques are most suitable for serial sequential observations over a long period of time and also provide a measure of ~~starting~~ total body composition (87, 76). The principle of this method is that the extent to which a substance is diluted in a solvent constitutes a measure of the volume of the solvent. Thus if the volume and concentration of a solution are known and the volume is altered, the new volume can be determined by measuring the new concentration of solute.

In practice this method has intrinsic errors when applied to the determination of body fluid compartments since a) the material added for measurement may metabolize or itself alter the volume being measured; b) the volume being measured is constantly being added to and lost from; c) the volume being measured may not be truly homogeneous.

Ideally the material to be used as a tracer must be readily diffusible but confined to the compartment being measured, stable, be only slowly excreted or metabolized, non-toxic, and lend itself to accurate assay.

The dilution theory was first applied to body composition studies in 1915 to determine plasma volume (88). This method using T1824 is still in wide use although the values obtained are thought to be slightly in

excess of the plasma water since some of the dye becomes bound to protein. More recently radio-iodinated serum albumin (RISA) has been used with similar results (89, 90).

In 1934 (91) and subsequently (92), the method was applied to the measurement of total body water using deuterium. Tritium has also been used (93, 94, 95, 96). Both heavy hydrogen and radioactive hydrogen exchange to the extent of about 5% with the hydrogen of substances other than water during the determination, but if this error is taken into account the values obtained by this method are in fairly good agreement with those obtained by desiccation (81).

Numerous other substances have been tried which are thought to diffuse throughout the water of the body: urea, thiourea, sulfanilamide, antipyrine (97), and aminopyrine (98).

No method has been devised for the measurement of intracellular fluid and this has been calculated as total body water less extracellular fluid. The exchangeable potassium showed only slight correlation with urinary creatinine and body weight when determined in normal women (99).

2. APPLICATION OF THE DILUTION PRINCIPLE TO ECF VOLUME DETERMINATION:

In applying such a technique to measurement of extracellular fluid volume, there are several difficulties. Thus far no substance has been identified which is a small molecule, readily diffusible into all the extracellular areas, non-metabolized, and exclusively extracellular in distribution.

Two principal methods have been used to determine the apparent volume of distribution of an injected substance, the single injection method and the constant infusion method. Both have been subject to criticism.

In the single injection method, the plasma concentration is followed

until equilibrium is attained and the volume of distribution at equilibrium then extrapolated back to zero time. This method has been used for rapidly diffusing, slowly excreted substances such as radiosodium.

If a constant infusion is used, the material is injected until equilibrium is attained; then the infusion is stopped and the total amount of solute excreted from that time to the point of complete disappearance of solute from the serum is measured. This method is used for slowly diffusing, rapidly excreted substances such as inulin.

For the type of study comtemplated, i.e. several independent determinations of ECF over 24 hours, all of the available substances had some disadvantages. The larger molecules diffuse too slowly for independent determinations and sucrose had been tried by Peterson et al.(72, 73) with the results described above (p.13). The smaller molecules used, Cl, Br, Na, SO4, SCN, S2O3, achieve rapid equilibrium but all enter cells to some extent, and also the transcellular fluids. Sodium exchanges to a significant degree with bone.

a) Sodium:

Despite its exchange with bone, sodium has been considered to be one of the more reliable substances for measuring ECF volume (100), although one criticism has been that in high fever and after massive trauma, the volume of distribution approaches as much as 40% body weight due presumably to widespread alterations in permeability to cations.

Radiosodium exhibits a concentration-time curve consisting of an initial phase of mixing in extracellular fluid requiring about 30 minutes followed by a slower phase of penetration of cells, transcellular fluids and bone apatite (101, 102, 103, 104, 105). By determining the zero-time extrapolation curve of the second phase results were obtained in good agreement with those obtained using inulin and sucrose. If the second

curve is followed to equilibrium at about 24 hours, the "exchangeable sodium" is obtained, an index of the total body pool of available sodium (106, 107).

Two isotopes of sodium are available: Na^{24} with a half-life of 15 hours and Na^{22} with a half-life of three years. Permission to use Na^{22} in humans in Canada has not as yet been granted.

A constant infusion technique using Na^{22} has been described by Hlad and Huffman (108) who obtained a value of 22.7 $\pm$ 3.5% body weight in normal subjects. The authors claim that this technique overcomes the disadvantages of previous methods for "Na space", namely that values obtained are a function of time, that 3 to 40 hours are required for a determination, and the short half-life of Na^{24}. However it is difficult to know in this case just what these authors are measuring. Using this method they found that the "Na space" increased an average of 27% after the administration of 9α-fluoro-hydrocortisone (109).

b) Thiocyanate:

Thiocyanate diffuses rapidly throughout the ECF (110). It has frequently been used as an index of ECF volume in the past but has been generally discarded because large increases in cellular permeability were noted in certain circumstances such that the volume of distribution approached that of the expected total body water (111). Simultaneous studies have shown the SCN space to be consistently larger than the chloride space (112).

c) Thiosulfate:

Thiosulfate is very rapidly excreted in the urine, about 50% in the first hour but slowly thereafter. Both constant infusion and single injection techniques have been used. The thiosulfate space is somewhat larger than the inulin space (110)(113) but smaller than the chloride

space (114). This method has been severely criticized on the grounds that altering the renal excretion markedly altered the calculated volume of distribution (115). When infusions of one litre of normal saline were carried out in nephrectomized dogs, increments in thiosulfate space were widely scattered (116).

d) Chloride and Bromide:

Since they are thought to behave similarly in the body, these two ions are grouped together (117). The isotopes of chloride and bromide have rather short half-lives except for Cl^{36} (10^3 years). Br^{82} is the most commonly used (half-life 34 hours). Chloride and bromide have an apparent volume of distribution smaller than that of Na^{24} and larger than those of sucrose and inulin (118) (119). They are known to enter the gastrointestinal tract, tendon, liver, red blood cells and other tissues (84).

Br^{82} has been used simultaneously with Cr^{51}, deuterium, Na^{24} and K^{42} to determine several aspects of body composition (120). Although equilibration times as short as three hours have been used, three to five days has been suggested as more suitable (121)(122).

e) Inulin:

Inulin , a lipoid-soluble carbohydrate, was chosen as a substance to measure ECF volume because its molecular weight is too large for it to enter cells. However its large size also hinders its mobility and it is slow to equilibrate with abnormal fluid depots. As mentioned above its volume of distribution is smaller than that of Na, Br, Cl, and thiosulfate (102, 110, 113, 114, 118).

f) Sucrose:

Sucrose behaves similarly to inulin and its volume of distribution is approximately the same (119). The increment in sucrose space following a one-litre expansion of ECF was 0.72 litre (116). In 24-hour studies, the sucrose space underwent large unexplained variations (72, 73).

g) Mannitol:

Another carbohydrate, mannitol shares the advantages and disadvantages of sucrose and inulin, and measures a similar space. It was reported to show greater accuracy than sucrose in measuring volume changes (116).

h) Sulfate:

While it shares many of the disadvantages of the substances just described, radiosulfate has been considered to have several advantages: its convenient half-life of 87 days; its short biological half-life of four to nine hours which makes repeated determinations feasible; the serum sulfate level is essentially unaltered by the small dose given, therefore the effective osmotic pressure of the extracellular fluid remains unchanged: although it is excreted rapidly and this is in many ways a disadvantage, the excretion is almost entirely in the urine so that it may be measured; radiosulfate diffuses very rapidly, attaining an equilibrium in about 18 minutes; it is readily assayed by virtue of its soft β-radiation which however required the use of a gas flow counter to enable the radiation to penetrate a very thin window.

3. THE SULFATE SPACE:

The sulfate space is thought to be an index of the "physiologic" ECF. Radiosulfate is known to equilibrate with peritoneal fluid (164) and is therefore assumed to equilibrate with pleural and pericardial fluid. Data are lacking for lymph, synovial fluid and the ocular humors. Very little has been found in gastric juice, sweat and cerebrospinal fluid and it has been assumed to exclude transcellular water (130); however, as stated below, sulfate has been shown to enter bile and other gastro-intestinal fluids fairly rapidly;

Nevertheless its volume of distribution does give a value similar to those of inulin, sucrose and other substances which are considered to measure the same physiologically distinct compartment of body water, which may be termed the "functional" ECF.

a) Fate of intravenously injected sulfate:

(i) Blood: After intravenous injection of radiosulfate, the blood level falls rapidly for the first 20 minutes, then more slowly over the next 6 or more hours. Walser (123) interpreted this to mean that S3504 becomes distributed throughout the ECF in the first 20 minutes and slowly penetrates the cells thereafter, since the slow fall in plasma level exceeds that which would be accounted for by excretion into the urine.

Passage of S35 into red and white blood cells was reported by Sheatz and Wilde (124); and also by Swan, Feinstein and Madisso (125) who calculated that equilibrium with red blood cells was established within one hour and corresponded to that predictable on the basis of the Donnan theory. They found that the ratio of concentration of S3504 in serum water to that of dialysate was 0.95.

Association of sulfate with protein has been reported by several authors (126, 127, 128). Dziewiatkowski (126) showed that in rats,

after intraperitoneal injection of S3504, an increasing fraction of the isotope remaining in the blood was associated with serum protein, about 10% after six hours, 70% after 72 hours. Similar results were obtained with precipitation with 5% trichloracetic acid, precipitation with 75% ethanol, electrophoresis in a starch block and equilibrium dialysis against water. Electrophoretic analysis of sera showed that most of the S35 was associated with the α_1-globulins and the albumins, and to a lesser extent with the γ-globulins. Chromatography on Dowex 50 resin of hydrolysates of sera removed 24 hours after injection revealed that about 5% of the isotope associated with the proteins was present as cystine and methionine and the remainder as sulfate, about 10% of which could be released upon acid hydrolysis.

(ii) Urine: Urinary excretion is rapid, about 50% in the first six hours; in a study by Walser et al. (129), 93.9% of the injected dose was recovered in the urine, 0.6% in the feces, over five days.

Urinary loss in the first 20 minutes was reported by these authors (130) to amount to 4-8%, and later by Ryan et al., to 1-19%.

Most authors have obtained clearance values for sulfate of about 35 cc/minute. The work of Hyman and Johnson (132) suggested that there was extensive reabsorption of sulfate by the kidney. Cope claimed that sulfate was excreted by filtration plus tubular secretion. Several groups noted that the sulfate clearance increased on elevation of the plasma sulfate level in both dog and man. Bjering and Ollgaard (133) obtained similar figures for sulfate clearance but attributed the apparent rise with elevation of the plasma level to protein binding. They calculated the amount of bound sulfate to be about 0.8-0.9 mg.%, and when this value was subtracted from the values for serum sulfate, the clearances were found to be constant at about 90cc/minute in two subjects. However

this correction is said by Smith (134) to be unwarranted in view of the evidence of Hayman and Johnson (132), and of Goudsmit, Power and Bollman (135) that endogenous sulfate is completely ultrafiltrable in man and dog, using collodion sacs.

Letonoff and Reinhold (128) found that the choice of a protein precipitant may definitely influence the results of sulfate determinations. They found higher values when trichloracetic acid was employed as compared with uranium acetate. Deproteinization by heat and acetic acid gave even higher results, ranging 10% above the trichloracetic acid figures. They concluded that the increase of 25 to 35% in inorganic sulfur caused by the use of acid precipitating agents represented liberations of sulfate from a constituent of serum, probably from protein. They also found that sulfate added to serum could be recovered satisfactorily only provided analysis was started at once after mixing the added sulfate with the serum. Delay longer than 10 minutes led to losses of 40 to 50%. This effect was observed regardless of the protein precipitant used and similar results were obtained when trichloracetic acid, alcohol, or heat was employed for removal of protein. These findings suggested that sulfate added to serum becomes firmly bound, and is thus rendered non-precipitable by benzidine. They concluded that a similarly bound fraction preexists in serum.

According to Macy (136), when a person is following a regimen of fasting, the excretion of inorganic sulfate is fairly constant throughout the day, whereas when he is receiving a normal diet there is considerable variation, excretion being lowest during the morning with a peak at 6:00 to 9:00 p.m. with low excretion at night. Blood concentration varied very little.

(iii) Gastrointestinal fluids: Very little radiosulfate has been found in gastric juice (137).

It has been reported by Everett and Simmons (137) to be excreted into the bile and upper gastrointestinal tract in rats, and subsequently reabsorbed in the large gut. Fourteen per cent of the dose was found in the gastrointestinal tract at one hour and decreased thereafter, indicating reabsorption. Four per cent was excreted in the feces and ten per cent in the bile of bile-fistula rats in 24 hours.

In rabbits fecal excretion was found to be very rapid, ½% being excreted within three hours of ingestion of S3504 (138). Blood peak level occurred about six hours after dosing. These results suggested that part of the dose was rapidly absorbed and then excreted into the lower gastrointestinal tract.

(iv) Tissues: After intraperitoneal administration of radiosulfate into rats, Dziewiatkowski (139) noted that S35 concentration increased until about the 8th hour in bone and until the 24th hour in bone marrow, and then fell more slowly than that of blood, liver and brain.

In baby pigs (140) the highest uptake occurred in ear cartilage, red bone marrow and aorta, the lowest in brain and muscle, after four days of dosing.

Uptake by mast cells, bone and bone marrow, cartilage, skin, pancreas exocrine cells, red spleen, kidney tubules, pyramidal cells of the central nervous system, hair and lachrymal glands of various laboratory animals have been described by Belanger (141, 142) and others (143, 144, 145, 170) using autoradiographic techniques.

Incorporation of S35 into cartilage was shown to be affected by growth hormone (146), hypophysectomy (147), amino acids, cortisone and other steroids (148, 149, 150). Some of the S35 of cartilage is incor-

porated into chondroitin sulfate (151, 152, 153, 154).

The concentration of S35 in dermis of rat was maximal at four hours (155) and was affected by hydrocortisone (156). Fixation of S35 in wound tissue was found to be influenced by thyroxine and scurvy (157).

S3504 uptake in the eye and in corneal grafts was studied by Bohlman and Claes-Henrik (158, 159, 160).

Although S35 is only slowly excreted into the gastric juice, it is readily fixed by the mucus-secreting glands of the stomach and this fixation was found to be significantly reduced by cortisone and hydrocortisone (161).

Ingested sulfate was found to be rapidly absorbed, some of the urinary sulfur being present as ethereal sulfate sulfur (162, 163, 165).

Small amounts of S35 were shown to become incorporated into cystine of rats (166), and the laying hen (167), and into taurine of chicks(168, 169).

S3504 was shown to be taken up rapidly by chondrosarcomas in man (171) and the differential sulfate fixation of tumor tissue, normal tissue and muscle tissue was suggested as a method for confirming cartilaginous cancers and their metastases (172).

b) Radiosulfate space as an index of the physiologic ECF:

Walser et al.(130) obtained a mean value for the sulfate space in 28 normal men and women of 15.1% (11% - 21%) body weight, the variation being attributed to wide variation in body fat content. The ratio of the sulfate space to simultaneously determined inulin space in nine subjects was 0.95 ± 0.11. Repeated determinations in a single individual differed by 0.26 - 0.30 litre. They considered that urine collection could be omitted without serious error since in 15 normal individuals the proportion of the administered dose recovered in the urine during equilibration varied from 4 - 8%, the variation being as great in a single individual

as for the entire group, assumed to be due to variation in the amount of injected material which circulated through the kidney during mixing. They also made no correction for cellular penetration during this interval.

Ryan et al. (131, 173, 184) chose to extrapolate the plasma concentration back to zero time. They also determined the excretion rate following equilibration and extrapolated this line back to zero as well to correct for the "excessive" urinary loss before equilibrium was achieved. By this method a mean value for ECF volume in 11 young men was 19% body weight. The ratio of S3504 space to simultaneously determined inulin space was 1.05. In five elderly subjects who had a second determination of sulfate space 1 to 30 days after the initial measurement, the mean difference was 0.12 litres. They obtained values of 1-19% for urinary excretion during the equilibration period. Lower values were obtained in elderly individuals, a result considered to be due to a decrease in glomerular filtration with advancing age. The mean value in young men was 8%, in elderly individuals 5%.

Becker and Heinemann (174) determined distribution volumes of S3204, S3504, and inulin obtaining a mean value for Vol.S35/Vol.in. of 1.04. However since the correlation in any one individual was poor, they concluded that inulin and sulfate are not always distributed in the same volume of fluid.

In nephrectomized dogs the volumes of distribution of S3504, thiosulfate, mannitol, sucose, raffinose, and inulin were compared by Swan, Madisso and Pitts (175). The S3504, thiosulfate and mannitol volumes were equal when measured simultaneously in the same animal. Volumes of sucrose, raffinose and inulin were smaller. Concentrations of these substances in cerebrospinal fluid six hours after infusion were less than 10% of simultaneous plasma concentrations but in bile and

pancreatic juice approached those of plasma.

Walser, Seldin and Grollman also used nephrectomized dogs in their studies of the radiosulfate space of muscle (176). The ratio of the sulfate space of muscle to the chloride space of muscle was found to be 0.6 to 0.8 and did not increase after the first half-hour. They concluded that radiosulfate space is a valid measure of the readily diffusible extracellular space of muscle and that about 30% of the chloride in muscle is in a relatively indiffusible space.

In nephrectomized dogs given infusions of isotonic saline (116), the volume of distribution of sulfate reflected increments in ECF with fair accuracy and was comparable to mannitol in this regard.

Portwood, Gwynne, and Seldin (177) measured radiosulfate, chloride and sodium spaces in man during acute changes in ECF volume induced by thiomerin and rapid saline infusion. The ratio of change in weight to change in sulfate space was 0.97 ± 0.26. The ratio of change in chloride space to change in sulfate space was 0.98 ± 0.19. They concluded that $S^{35}O_4$ was an ideal reference substance for determining changes in volume of functional ECF.

A method for the simultaneous determination of radiosodium, radiopotassium and radiosulfate spaces was described by Burrows, Hines and Ross (178) whereby Na^{24} and K^{42} were counted with a γ scintillation counter and the sulfate S^{35} was counted with a β counter after the short-lived Na and K had decayed to negligible amounts.

Following the 18-minute equilibration period the volume of distribution increases slowly with time. Walser (123) noted this, and calculated that this increase could not be accounted for by urinary excretion, fecal loss nor penetration of the muscle chloride space. Ryan et al. (131) attempted to study the slowly-exchanging space in more detail. They calculated that the maximal amount of $S^{35}O_4$ entering the slowly exchanging pool was 10 to

15% of the injected dose. They found this maximum to be reached 6-8 hours after injection. They calculated the volume which this might represent but found that such volumes would vary from 0.3 to 22 litres and were not reproducible. They concluded that the slowly exchanging pool was not due to simple diffusion from physiologic extracellular fluid to some other fluid compartment but might be the net result of several simultaneously occurring phenomena.

c) Radiosulfate space in disease:

Few studies have been done of the changes which occur in the sulfate space in disease states. All determinations of body fluid volumes involving dilution methods are made much more difficult in the presence of edema since diffusion of the tracer into edema fluid is slow.

The 20-minute radiosulfate space was shown by Walser et al. (179) to be normal in hypertension, increased in heart failure without palpable edema (17 - 36% body weight), increased in acute rheumatic fever (17- 35%) but unchanged in other acute arthritides. Equilibrium in gross ascites and edema required five to eleven hours. It was calculated that a patient in mild heart failure might have an increase of five litres in extracellular fluid without palpable edema (180).

In a study of the volume of fluid compartments in human and experimental hypertension, Teng, Shapiro and Grollman (181, 182) found that in humans the mean ECF volume of a hypertensive group as measured by the 20-minute sulfate space (assuming 5% excretion) was significantly higher than that of a matched normotensive group if referred to % body weight but not to lean body mass.

Walser (183) also studied the effects of cortisone and ACTH (adrenocorticotrophic hormone) on blood and ECF volume in normotensive subjects without renal or cardiac disease. He found that without salt restriction

blood volume did not change but S35 space increased slightly, the increment being considered to be derived from internal sources. When salt was supplied the ECF expanded considerably but venous pressure and plasma volume remained constant or fell slightly. It was concluded that the stimulus which promotes renal adjustment to an excess of interstitial fluid need not involve blood volume, venous pressure nor plasma oncontic pressure.

d) Methods of determining the radiosulfate space:

The usual procedure is to inject a convenient volume of sodium sulfate-S35 solution (about 10 cc) over a period of one minute. If only one plasma sample is to be used it is often taken at 20 minutes (123), or serial samples an hour or so apart may be taken and the straight line obtained by plotting the logarithm of the concentration against time extrapolated back to zero time (131).

Since it is difficult to obtain an accurate urine collection over a period as short as 20 minutes, some authors have omitted the urine collection during the period of equilibration (123) although it has been suggested (131) that this may result in a fairly large error. If serial samples of urine are collected, the urinary excretion line may be extrapolated back to zero time, the amount at zero time representing the excess sulfate which is excreted before mixing is completed. This excess sulfate, obtained by recalculating the data of Ryan (131), amounted to $4.5 \pm 4.5\%$ of the administered dose in young men, and to about $1.0 \pm 1.0\%$ in elderly individuals. These figures suggest that one-sixth of the values for excess sulfate are less than zero which seems very unlikely; possibly this discrepancy is due to the urinary dead space.

Activity of S35 in blood and other body fluids has been measured

in several types of sample. Precipitation of the benzidine or barium salts after removal of protein with trichloracetic acid has been used (123, 139, 124, 185). Walser (123) collected the precipitates on filter papers which were then permanently mounted on aluminum discs. This author also determined the activity of urine using liquid samples covered with a thin layer of tinfoil (129). Ryan (131) assayed evaporated samples of serum, and of urine with added discard plasma containing no activity, after drying at 50° C.

C. EXPERIMENTAL PROCEDURES

1. SULFATE SPACE:

As described in section B, the sulfate space method involves a) injection of a known amount of radiosulfate, b) collection of serial blood and urine samples and samples of other body fluids as required, c) determination of the amount of activity in the collected samples, d) estimation of ECF volume from these data by use of the dilution formula.

a) Injection of radiosulfate:

(i) Preparation of solution to be injected: Radiosulfate was received from Abbott Laboratories as approximately one ml. of buffered sodium sulfate containing one millicurie S35. The contents of the vial containing this material were transferred to 100 ml. of sterile normal saline and the vial washed five times with the solution thus obtained. When account was taken of the decay from time of shipment, a close approximation of the activity of the solution was obtained, sufficiently accurate for safe dosage.

(ii) Dosage: The safe level of S35 which may be maintained continuously in man has been estimated to be 100 microcuries (186). For short periods this dose may be increased. In the following studies 100 microcuries (uc.) was the usual dose employed for single determinations. When studies were repeated during the day, 25 - 50 uc. were used for each injection.

(iii) Preparation of standard solution: A standard was made up by diluting 2.00 ml. of the injection solution up to 2000 ml. with distilled water in a volumetric flask.

(iv) Injection procedure: Labelled syringes, calibrated to hold 5, 10, or 20 ml. were used for injection. The syringe was filled to the

desired volume from the vial of stock solution containing approximately 10 uc. per ml. The contaminated needle was replaced by another needle and the material injected into an arm vein, drawing back only enough blood to ensure that the needle was in the vein. The material was injected as evenly as possible over one minute. The time of beginning and ending the injection, the volume injected, and the number of the syringe were recorded.

The volume delivered by the syringe was found by injecting the same amount of water into a weighed flask using a technique as closely similar as possible to the above. Each volume was checked by at least three weighings. All measurements were carried out with solutions at room temperature.

b) Collection of samples:

(i) Blood: From 8 - 10 ml. blood was withdrawn before injection and at intervals after injection from the arm not used for injection, and, where this was not feasible, distal to the site of injection. A tourniquet was used but was applied for periods usually no longer than 15 seconds. In some studies heparinized syringes were used. From 1 to 24 hours after being drawn and refrigerated, the samples were centrifuged at 2000 RPM for 15 minutes. The serum was then separated, the tubes of serum covered with Parafilm and refrigerated until plated.

In some cases a short spinal needle with trochar was inserted into an arm vein to avoid extra needle punctures. Such needles remained open for three to ten hours without the use of anticoagulant.

(ii) Bile: Samples of bile were collected from post-operative cholecystectomy patients in whom the bile duct had been explored and a T-tube left in the common bile duct. Specimens were obtained by

allowing the bile to drip from the end of the plastic tubing into a test tube. The volume of the tubing was measured and the results corrected accordingly.

(iii) Urine: Catheters were used only in neurological patients where these were required as part of the nursing care. In all other cases the individual was asked to empty the bladder as completely as possible.

c) Determination of the amount of radioactivity in the collected samples:

(i) Characteristics of radiosulfate: S35, a radioactive isotope of S32, has a physical half-life of 87 days and a biologic half-life of about ½ day, varying with the individual. It emits only beta, of 1.167 mev.

As obtained from Abbott Laboratories, it has a specific activity of 10,000 millicuries per gram S.

Since S35 emits only "soft" or weak beta particles, its radiation will not pass to any appreciable extent through glass, rubber, or metal, making it easy to handle in the laboratory. While this property is an advantage in handling, it is a disadvantage in detection since special equipment is required.

(ii) The counter: The radiation detector used in these studies was a model D47 gas flow counter (Nuclear Instrument and Chemical Corp., 223 West Erie St., Chicago, Ill.). It was operated as a Geiger Muller counter. The gas used was a mixture of 98.7% Helium and 1.3% Butane called Q-gas (obtained also from Nuclear-Chicago). In most counters, other gases, including air, are used but at lower pressures. Helium is used in this case, because having a low starting potential with a low specific ionization it may be used at comparatively high pressures (187)(188). Butane is added in small amount to make the counter self-quenching, i.e. to decrease the time required to terminate the discharge. In this counter, which is operated at atmospheric pressures, the gas flows into the chamber continuously,

enabling a very thin window to be inserted between the sample and the chamber. Small holes at the sides of the chamber allow gas to escape so that the pressure on both sides of the window is equal, preventing rupture of the window, while the continuous flow prevents air from diffusing into the chamber.

A micromil window weighing less than 120 micrograms per cm.^{2} was used. This is of such thinness as to allow about 90% of the soft beta radiation of S35 to pass through it. Although higher counts can be obtained by operating the counter without the window, the ease of operation is greatly decreased since chamber contamination, charge effect, and vapour effect are frequent sources of error in windowless counting.

A model M5 sample changer was employed. This consisted of a manually rotated table with three recesses containing reversible stainless steel adapters for holding sample pans 3/32" deep by 1¼" diameter, or 5/16" deep by 1" diameter. While one sample was counted the next was preflushed. The gas leaving the counter flowed through a hole in the base plate to the preflush area, and was finally exhausted through a bubbler.

The counter was operated with an applied voltage in the Geiger region, i.e. that part of the characteristic curve of pulse size versus voltage in which the pulse size is independent of the number of ions produced in the initial ionizing event. To determine the optimum voltage frequent curves were obtained. The usual operating voltage was 1250 volts.

A preamplifier, model 216E (Atomic Instrument Co., Cambridge 39, Mass.) was interposed in the circuit between the counter and the scaler, which combined a model 312 superstable high voltage power supply with a model 131 discriminator.

(iii) Background: With no radioactive sample in the chamber, the count averaged 15 to 20 counts per minute (cpm.). Background counts in

this type of counting may be due to alpha contamination inside the chamber, cosmic radiation, and natural radioactivity of the surroundings (187). Since the volume of the chamber was very small, the effects of the latter two factors were minimized.

(iv) Sample preparation: Dry samples rather than liquid samples were used, since counting efficiency was about 50 times greater and the likelihood of contaminating the micromil window was decreased. In dry form the samples could be stored, and counted and weighed at a later date.

Two types of stainless steel planchette were available, a smaller deeper one and a larger shallower one. The shallow type (1¼" diameter) was used since it gave increased counting efficiency and was easier to clean.

Using volumetric pipettes, 2 ml. serum was diluted with 2 ml. distilled water and thoroughly mixed in a small flask. One ml. of the diluted serum was pipetted into each of three weighed planchettes placed on an aluminum tray for easy handling. The tray was then placed under an infra-red lamp at a temperature of about 50°C. until the samples were dry. They were then allowed to cool to room temperature.

Where the activity of other body fluids was being determined, an amount giving a weight of 20 to 50 mg. was pipetted into the planchettes and treated in a similar fashion. In the case of urine, 2 ml. of urine were diluted with enough distilled water to reduce the activity below 5000 counts per minute, and 2 ml. of the diluted urine added to 2 ml. of discard plasma, mixed, and 1 ml. pipetted into each of 3 weighed planchettes.

(v) Counting procedure: The scaler was turned on at least two hours, and the Q-gas at least one hour prior to counting. After checking the voltage, the background count for each of the three sample holders was determined for three minutes, and if this exceeded 20 cpm. in any one

it was cleaned and recounted until the count was 20 or less. Then each of 10 samples of the standard was counted to about 4000 counts. Each of the test planchettes was counted to a minimum of 2000 counts. The counts from the three planchettes made up from an individual sample were averaged and corrected for self-absorption from a weight-activity curve. Since counts were always referred to the standard, no decay corrections were required. At intervals of several hours, the standard counts were repeated and the background checked in the above manner.

(vi) Correction for self-absorption: The observed counting rate of a sample containing S35 depends on several factors (189): the distance of a sample from the sensitive volume of the counter; the amount of backscatter; the absorption of beta particles by the window of the counter and by the air between the sample and the window; the dead time of the counter; and self-absorption by the source itself.

If the first three factors are kept constant, and if the number of counts per minute is kept below 5000 (at 6000 cpm. there is a loss of 1%) so that there is no appreciable loss due to the dead time of the counter, then the observed counting rate will depend on the strength of the source and the internal absorption by the source.

Hence as plasma or serum is added to a fixed amount of S35, the observed radioactivity decreases as the grams/cm.^{2} of material increases. This relationship is expressed by the equation:

$$\frac{R}{R_o} = \frac{1}{mx}\left(1 - \epsilon^{-mx}\right)$$

where R = measured counting rate
R_o = true counting rate
m = absorption coefficient cm.^{2}/mg.
x = sample thickness mg./cm.^{2}

(vi) Decontamination: After counting and weighing of samples was completed, the planchettes were placed in a beaker containing household detergent in tap water, and left to soak for at least 48 hours. The layer of dried serum separated fairly readily. The planchettes were then washed again with fresh detergent, dried and polished. Several hundred

planchettes were counted after cleaning to ensure a count within background count. Since in no case was the count elevated, this precaution was discarded.

Syringes, needles, pipettes, flasks and beakers were decontaminated while still wet with running water, then allowed to soak in detergent before routine washing was carried out.

d) Calculation of sulfate space from data obtained:

According to the dilution formula:

$$\text{Sulfate space (litres)} = \frac{\left(\text{Amount injected} - \text{cc}\right) \times \left(\frac{\text{Activity of standard}}{\text{cpm/cc}}\right)}{\text{Serum activity cpm/cc} \times \frac{100}{93} \times \frac{100}{90}} \qquad (180)$$

where 100/90 is the Donnan factor, and 100/93 is the correction for the water content of plasma.

2. BALANCE STUDIES:

A number of experiments was carried out in which the balance of Na, Cl, K, and water was determined. Most such studies were carried out in cooperation with Dr. J. Dossëter who conducted the balance studies as part of a project designed to elucidate the nature of the diurnal variations in electrolyte excretion.

Requirements for the diets were submitted to the research dieticians who planned the diets so that each meal contained the same amount of protein, fat, carbohydrate, Na, K, Cl and water. The meals were made up in the research diet kitchens.

Such diets are referred to as "controlled" diets in contrast to "ad libitum" diets in which the subject chose his meals from the hospital menus. Where subjects were on controlled diets, the diet was begun three

days before any determinations of sulfate space were made.

All urine was collected and sent to the research biochemistry laboratories where the volumes were noted and analyses for Na, K, and Cl carried out. No corrections were made for losses from lungs, skin or feces.

3. BLOOD VOLUMES:

Blood volume determinations were done by Dr. Marvin Feldstein in the Haematology department using radioiodinated serum albumin, according to the routine hospital procedure. This included the hematocrit determinations, and from these data the red blood cell volumes were derived. Samples of radiosulfate taken during such experiments were not counted for six weeks or longer until the radioiodine count was negligible.

4. BODY WEIGHTS: Where body weights were recorded daily these were done on the same counter-balanced scales each time and where possible by the same individual, either the author or the same ward nurse.

C. EXPERIMENTS

1. DETERMINATION OF THE RELATIONSHIP OF ACTIVITY TO WEIGHT FOR S35 IN PLASMA AND SERUM:

Solutions of serum were made up containing the same concentration of radiosulfate and varying concentrations of serum or plasma. Five 1 ml. samples of each were pipetted into planchettes, dried, counted and weighed. The results are shown in Figure 1.

2. EVALUATION OF TECHNICAL SOURCES OF ERROR:

a) Volume injected:

A calibrated syringe with an identifying number etched on the side and barrel top was filled to the 10 ml. mark with distilled water, and then emptied into a weighed volumetric flask using a technique similar to that used in injecting subjects. This procedure was repeated at least three times to determine the injected volume for each syringe, and 10 times in several syringes to determine the probable error of injection volume.

b) Pipetting:

Volumetric pipettes were used throughout, and the same pipette was always used to make up the standard. The probable error of the volume delivered by this latter pipette was determined by weighing the volume of distilled water delivered by it ten times.

c) Counting:

The statistical error caused by the random nature of the decay process is inversely proportional to the square root of the total count (190). Other sources of error in counting are (i) change in the position of the sample with respect to the counter; (ii) change in backscattering or in self-absorption; and (iii) lack of uniform distribution of radio-

active material over the planchette surface. The probable error of counting was determined experimentally by studying the variations in observed counts of 20 identical samples of serum and 20 of inorganic sulfate solution. Each sample was counted for the same length of time and the background count subtracted. Serum counts were corrected for self-absorption according to their weights. Inorganic sulfate solutions weighed less than 1 mg./ml. and therefore required no correction for self-absorption. Results are shown in Table 1.

3. FATE OF INJECTED RADIOSULFATE:

In this and the following studies subjects are referred to by their initials and information regarding age, weight, diagnosis, etc., is given in Table 7 .

a) Serum disappearance curves:

Following injection of S35 samples of serum were taken at intervals and their activities determined.

Figure 2 A,B shows the same data for subject M.B. plotted in two ways. In Figure 2A the concentration of S35 is plotted linearly against time; while in Figure 2B the concentration of S35 is plotted on a logarithmic scale and time is plotted linearly. Subsequent data are also shown on a semi-logarithmic scale.

Figure 3 shows serum disappearance curves followed for 24 hours.

In most studies the serum disappearance was followed after serial injections every six hours, as for example in Figure 4.

In four subjects, A.McC., L.M., P.M., and N.W., multiple studies were carried out in which six serum samples were obtained during the six-hour period following injection. The equations of the best straight lines were calculated by the method of least squares. The slopes obtained, the volumes obtained, and the time of injection are shown in Table 2.

b) Excretion of S35 in transcellular fluids:

(i) Urine: Although $S3504$ is excreted rapidly in the urine, this route accounts for only part of the loss from plasma after equilibration with the physiologic ECF is established. This is shown in Figure 5 where the amount remaining in the body (dose minus urinary excretion) as % dose is plotted along with the plasma disappearance rate. Plasma concentration is expressed as % of the zero-time extrapolation value.

Urinary excretion was determined on 15 occasions as shown in Table 3. In order to obtain a reasonably large volume and thus reduce the error due to bladder dead space in the 20 minute collections, the subjects passed no urine for several hours prior to the test. No catheters were used and the subjects were requested to empty the bladder as completely as possible.

In subject V.Y., six serial injections of S35 were given at 12-hourly intervals and urines collected throughout. Since 30 minutes is too short a period for urine collection, the last urine prior to injection was passed about 1½ hours before injection and the amount in the 30-minute collection following injection corrected for that due to the previous injection by extrapolating the slope of urinary excretion. Since this latter amount was of the order of 2% of the injection dose, only a small error was involved. Although urine was collected 30 minutes after injection in this subject, the 20-minute excretion was calculated so that the results could be compared with those of other subjects.

Zero-time urinary excretions for young men were calculated from the data of Ryan *et al.*(131). A histogram showing the distribution of these results is given in Figure 6.

(ii) Bile: Bile was collected from bile fistula patients at various times after injection of radiosulfate, and the concentrations compared with those of serum as in Figure 7. Such patients were about one week post-cholecystectomy and had no known liver dysfunction.

(iii) Duodenal content: Samples of duodenal content were collected in only one human subject and in one rabbit. Concentrations are compared with those of serum in the human subject in Figure 8. In this case the contents were aspirated through a Levine tube which was assumed to be duodenally located when the aspirate was clear and alkaline.

(iv) Gastric juice: At various times after injection, gastric juice was collected in two human subjects (via Levine tube) and the one rabbit (via syringe at sacrifice), and the activity compared with that of serum in Table 4.

c) Change of plasma and serum activity with time (Entry of S35 into red blood cells):

Since S35 is known to enter red blood cells (124, 125), it was felt that an experiment should be carried out to determine if plasma counts would change with the length of time the plasma was in contact with the red blood cells, since, if there were such a change, the sulfate space calculation would be altered proportionally.

Sixty ml. of blood was collected from a normal subject 20 minutes after injection of S35 by means of two syringes, one heparinized and one not heparinized. Five or six ml. was placed in each of 11 tubes, and these were left at room temperature. The tubes were centrifuged at the times indicated in Table 5, the plasma separated and its activity determined.

Two other similar experiments using serum were carried out allowing longer times to elapse before centrifuging. The results of these are also shown in Table 5.

d) Association of S35 with protein in serum:

Because it seemed desirable to carry out plasma volume determinations in conjunction with ECF volume studies and since the method used in the Royal Victoria Hospital employs radioiodinated albumin, it was thought that

a simple way of separating the radiation of S35 and I^{131} might be the precipitation of the protein containing the I^{131}, leaving the S35 in solution. It was not clear from the literature whether or not this would be feasible for the short time required - minimum of 45 minutes after the time of injection before the protein could be precipitated. An alternative method was to allow the I^{131} to decay to negligible amounts, a process requiring about two months since the ½-life of I^{131} is 8 days. There are several other possibilities such as separating the radiations with a pulse-height analyser but this would involve counting all the samples twice, increasing the time and the counting error.

Trichloracetic acid was tried first as a precipitant, but on drying tended to spread over the edges of the planchettes giving inconsistent counts. Ethanol 75% was then used as described by Dziewiatkowski (126).

To 0.50 ml. of each of the sera obtained in Exp. 3 c) was added 1.5 ml. of 95% ethanol in a centrifuge tube. This was thoroughly mixed and allowed to stand 30 minutes in the refrigerator, then centrifuged and the supernatant decanted into a weighed planchette which was then placed under an infra-red lamp to evaporate the ethanol. The precipitate was extracted again with 1.5 ml. 75% ethanol and the supernatant added to the first. When evaporation of the ethanol was complete, the planchette was allowed to cool and the activity determined.

A short <u>in vitro</u> experiment was also carried out. Fresh normal serum containing no activity was mixed with an equal amount of a solution of S35 of known activity for 3 minutes; then two 5 ml. aliquots were combined with ethanol as described above. This was repeated at five minutes. Results are shown for both experiments in Table 6.

4. FATE OF INGESTED RADIOSULFATE:

A solution containing 100 uc. S35 in normal saline was given by mouth to six subjects during or just after the midday meal and the activity of the

serum determined at various times after ingestion. Results are shown in Figure 9.

On a different day, 100 uc. S35 was injected intravenously into two of these subjects. One of these studies is compared with that obtained after ingestion in Figure 10.

5. EQUILIBRATION TIME IN NORMAL AND EDEMATOUS SUBJECTS:

Equilibration studies of Walser, Ryan and others showed that the time required for the plasma activity to give a straight line when plotted semi-logarithmically against time in normal individuals is 18 to 20 minutes, and in grossly edematous patients with ascites several hours (131).

The 18-minute equilibration in normals was again confirmed in these studies and an example is shown in Figure 11.

Since in several instances studies were done on patients who were paralysed or mildly edematous, equilibration studies were carried out on these subjects to ensure that adequate time had been allowed for equilibration.

Subject N.W. was comatose, and paralysed completely on her left side. Because of the paralysis, her left hand and left leg were slightly swollen. Samples were taken from both arms and the arm used noted. The results are shown in Figure 11. Similar studies were carried out in two other mildly edematous patients with similar results.

6. EXTENT OF THE SULFATE SPACE IN HUMANS:

The results of 100 determinations of ECF volume carried out in humans are tabulated (Table 7) along with the sex, age, weight of the individual and the conditions under which the experiment was carried out.

Since the slopes of serum disappearance plotted semi-logarithmically were found to be straight for at least the first six hours, later studies were carried out using only two serum samples, one at 30 minutes and one at

three to six hours. Values of ECF volume determined by either method gave closely similar results (Table 9).

Table 8 gives an analysis of the data for well-nourished subjects with no known abnormalities of water and electrolyte metabolism, along with the values obtained in obese subjects.

7. REPRODUCIBILITY OF THE SULFATE SPACE UNDER THE SAME CONDITIONS OF DIETARY INTAKE, ACTIVITY, AND TIME OF DAY:

In a number of instances, sulfate spaces were determined under approximately the same conditions of time of day, activity and dietary intake on two occasions in the same individual. These are compared in Table 10. In all these cases, the ECF volume was calculated using a zero-time serum concentration derived from two points as mentioned above. No correction was made for "excess" zero-time urinary excretion.

8. FLUCTUATION OF THE SULFATE SPACE UNDER VARIOUS CONDITIONS, TIME OF DAY AND DIET CONSTANT: CORRELATION WITH WEIGHT AND BALANCE DATA:

In two patients in whom complete balance studies were being carried out for other reasons, the sulfate space was determined at intervals. In one patient with advanced kidney disease, P.M., two determinations were carried out while she was receiving human growth hormone. Sulfate spaces and the pertinent balance data are shown in Figure 12. Although this subject had mild pitting edema of her ankles on all occasions, no change in slope of serum disappearance was noted after 30 minutes.

The other patient, L.M., had essential hypertension. Sulfate spaces were determined on high and low sodium diets, and on one occasion following the administration of adrenocorticotrophic hormone. Sulfate spaces and the pertinent balance data are shown in Figure 13.

9. DIURNAL FLUCTUATION IN SULFATE SPACE: CORRELATION WITH BALANCE DATA:

a) Ordinary diet, activity during the day:

Two studies were done in which the subject ate a diet of known protein, fat and carbohydrate, Na, K, and Cl content, at ordinary meal-times, approximately 8 a.m., 12 noon, 6 p.m., with an 8 p.m. snack. Injections for sulfate spaces were carried out at approximately 5:30 and 11:30. Results are shown in Figure 14. Balance data for Na, K, and Cl are also shown, as well as for water.

The subjects continued their usual activities; both were medical students. In the case of A.McC. the studies were carried out during the summer while he was doing sedentary work in a nearby laboratory. N.B. was attending classes at the time of the other study.

b) Ad libitum diet, no activity:

Two studies were carried out on J.K., the patient with a spinal cord transection at the level of C3-4, while he was on an ad libitum diet. Meals were taken at approximately 8 a.m., 12 noon, 5 p.m. with a snack at 8 p.m. Sulfate spaces were determined at 5:30 and 11:30.

c) Six-hourly diet, activity during the day:

In five cases, studies were done in which the subjects were on a controlled 6-hourly diet, and carrying out their usual light activities. The diet was divided into four equal portions which were taken at 6:00 and 12:00. Sulfate spaces were determined at 5:30 and 11:30, thus maintaining a constant relationship to meals.

Three of the subjects were normal medical students (A.McC., N.B., G.G.). K.M. was a medical student admitted for investigation of hypertension, in whom no cause for his hypertension was found and a diagnosis of essential hypertension made. V.Y. was admitted for investigation of idiopathic edema. At the time of the study she had no pitting edema although on admission one

week before there was a slight pitting edema of her ankles, and some puffiness about the eyes. No cause for her edema was found although it seemed to be related to psychological stress.

Balance studies are shown in Figure 15. Correlation of sodium balance with change in ECF is shown in Figure 16A. Day and night values are compared in Table 11.

d) Six-hourly diet, uniform activity:

Two subjects were studied on a controlled 6-hourly diet, whose activity was necessarily uniform; J.K., the subject with spinal cord transection, and N.W., a subject with a brain tumour.

N.W. had been comatose and hemiplegic for some months before the study. Her state remained steady for some months afterward until she died of a pulmonary infection. During all of this time her serum electrolytes remained normal.

Both subjects had a mild edema of the paralysed limbs, but no sacral edema.

The study on J.K. was complicated by the fact that at 11:00 p.m. of the day of study the tongs inserted into his head for traction were removed causing him severe discomfort for some hours.

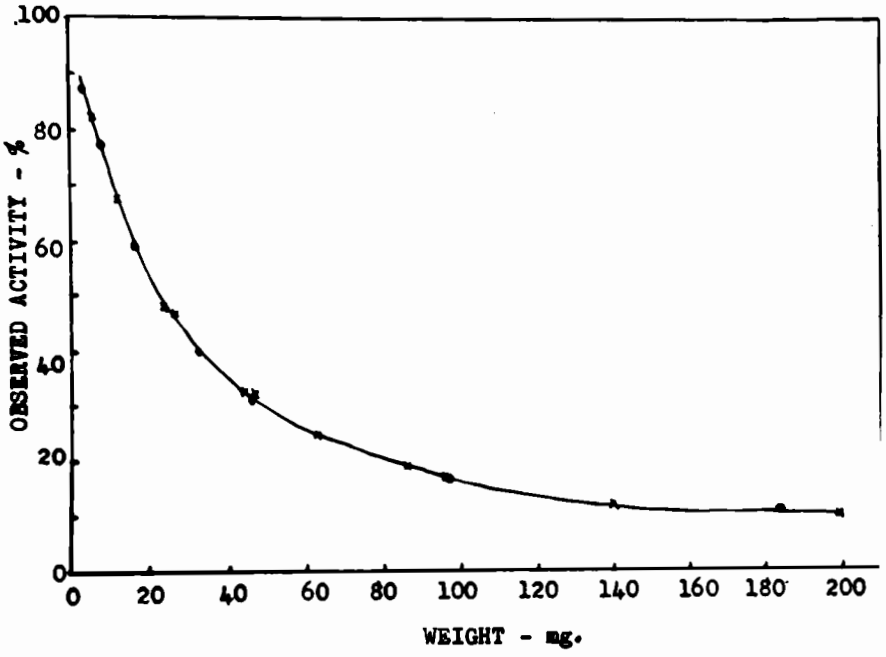
e) Six-hourly diet, prolonged bed rest:

To eliminate the effect of activity on any possible diurnal variation of extracellular fluid volume, it was decided to see what effect complete bed rest might have in subjects without neurological lesions.

Three subjects were studied. Only one of these, N.B., was normal, the others being V.Y., the subject with idiopathic edema, and K.M., the subject with essential hypertension. Blood volumes were also done in K.M. and N.B. Results of sulfate spaces, blood volume, and balance studies are shown in Figure 16.

RESULTS

1. The relationship of activity of radiosulfate to weight of plasma and serum:



Serum x ; Plasma o .

2. Technical sources of error:

   a) Probable error (i.e. average standard deviation) of volume injected (5.0 ml.) = 0.5%; (10 ml.) = 0.3%.

   b) Volume delivered by pipette used for standard = 1.993 ± 0.002 (0.02%)

c) Probable error of counting:

TABLE 1

SERUM					INORGANIC SULFATE
Observed Count	Observed Count - Background	Weight	Self-Abs. Factor	Corrected Count	Observed Count - Background
cpm	cpm	mg.		cpm x 10^{-1}	cpm
2045	1905	32.4	2.45	467	4417
2030	1890	33.4	2.50	473	4267
2030	1890	33.4	2.50	473	4345
1948	1808	33.6	2.52	455	4227
2092	1952	32.4	2.45	478	4513
2070	1930	34.2	2.54	491	4342
2061	1921	33.2	2.49	478	4345
2136	1996	33.2	2.49	496	4256
1926	1786	33.7	2.52	450	4298
2044	1904	34.0	2.54	483	4467
2028	1888	32.3	2.44	462	4482
2075	1935	32.5	2.46	475	4345
1996	1856	31.7	2.42	448	4328
2065	1925	31.7	2.42	465	4351
2012	1872	31.6	2.41	451	4179
2145	2005	32.5	2.46	494	4408
2097	1957	31.0	2.38	466	4407
2078	1938	31.1	2.38	461	4322
2125	1985	32.6	2.46	489	4271
2120	1980	31.2	2.39	472	4268
M 2056	1916			471	4360
SD	58			15	106
SD(%)	3.0			3.2	2.4

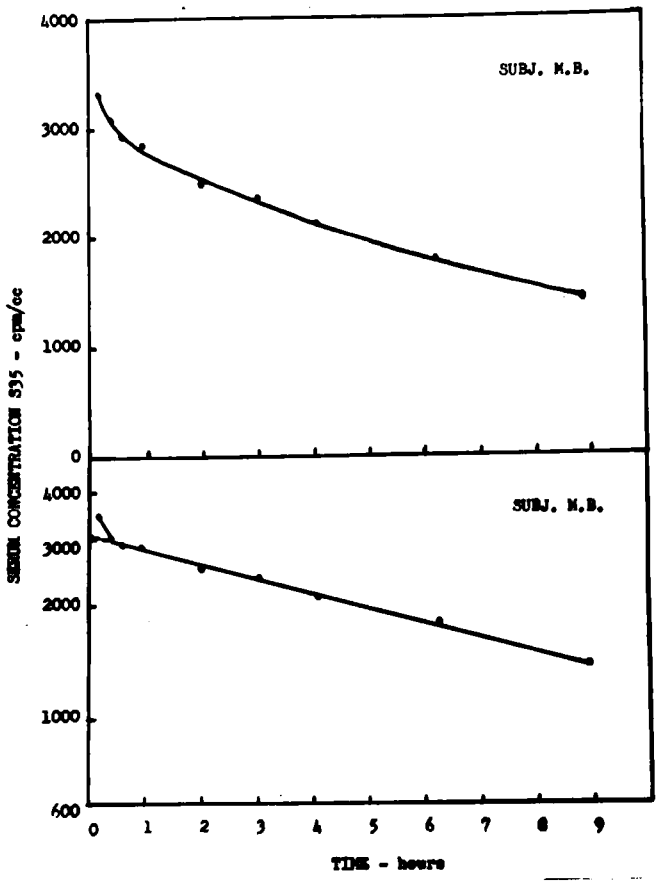
3. Fate of Injected Radiosulfate

a) Serum disappearance curves: in the following figures: N - noon
MN - midnight
- injection
- meal

Figure 2

Upper: Serum concentration (cpm/cc) plotted against time in hours using conventional graph paper

Lower: Serum concentration (cpm/cc) plotted against time in hours using semi-logarithmic paper



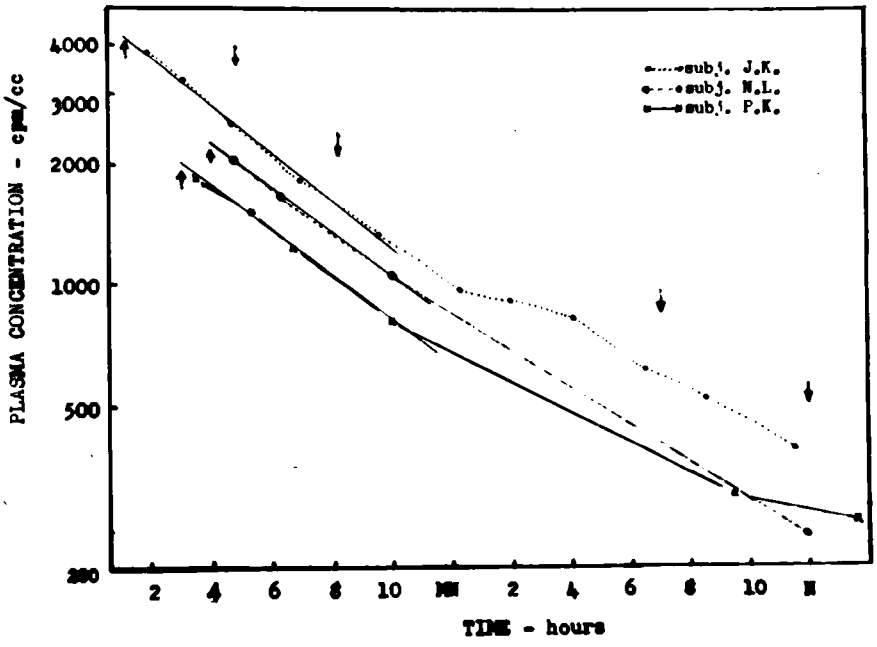


Figure 3

Serum disappearance curves followed for 24 hours: plasma concentration (cpm/cc) vs time (shown according to the clock)

TABLE 2

Subject	Time	Volume litres	Slope △ log/hour
A. McC.	10:37 a.m.	12.1	-0.0985
	4:12 p.m.	11.3	-0.0904
	10:14 p.m.	11.8	-0.0940
	4:08 a.m.	13.0	-0.0640
	11:07 p.m.	13.7	-0.0598
	5:02 a.m.	10.9	-0.0702
	11:06 a.m.	12.1	-0.0814
	5:10 p.m.	14.0	-0.0816
	12:13 a.m.	13.4	-0.0585
	6:32 a.m.	10.6	-0.0662
	12:17 p.m.	14.4	-0.0677
	6:11 p.m.	14.8	-0.0630
			M-0.0745 ± 0.0137
L.M.	8:30 a.m.	9.3	-0.1010
	8:30 a.m.	10.8	-0.1051
	8:30 a.m.	9.2	-0.0829
	8:30 a.m.	8.9	-0.0825
	8:30 a.m.	9.5	-0.1122
	8:30 a.m.	9.5	-0.0700
			M -0.0961 ± 0.0133
P.M.	8:30 a.m.	10.2	-0.0112
	8:30 a.m.	9.9	-0.0224
	8:30 a.m.	11.2	-0.0060
	8:30 a.m.	12.1	-0.0057
	8:30 a.m.	9.8	-0.0245
	8:30 a.m.	11.3	-0.0151
			M -0.0143 ± 0.0078
N.W.	10:50 p.m.	9.6	-0.0960
	5:07 a.m.	10.9	-0.0906
	11:26 a.m.	10.0	-0.0988
	5:12 p.m.	9.4	-0.1014
			M -0.0960

3. a) continued: Serial determinations of sulfate space: plasma concentration (cpm/cc) vs time according to clock

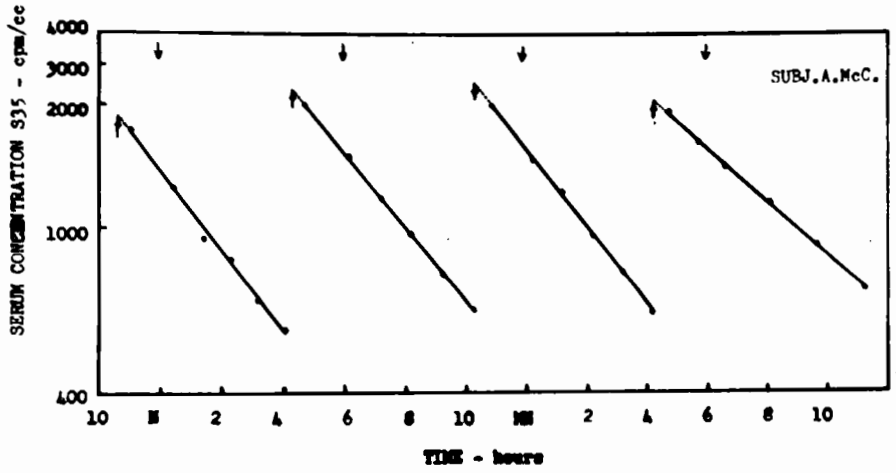


Figure 4

3. b) Urinary excretion:

(i) Comparison of urinary excretion rate with serum disappearance rate

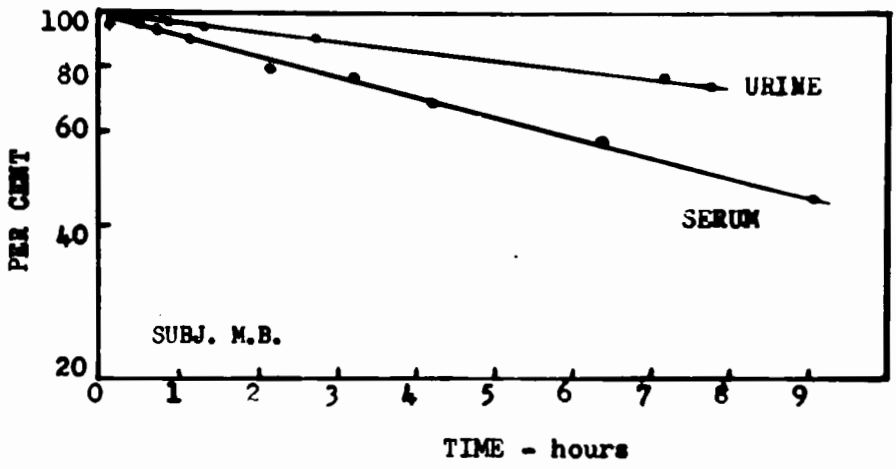


Figure 5

The fall in serum concentration is plotted taking zero time value as 100%. The cumulative urinary excretion is plotted from above downwards so that the amount below the line represents the total dose administered minus the amount excreted in the urine - i.e. the amount of S35 remaining in the body as a % of the original amount injected.

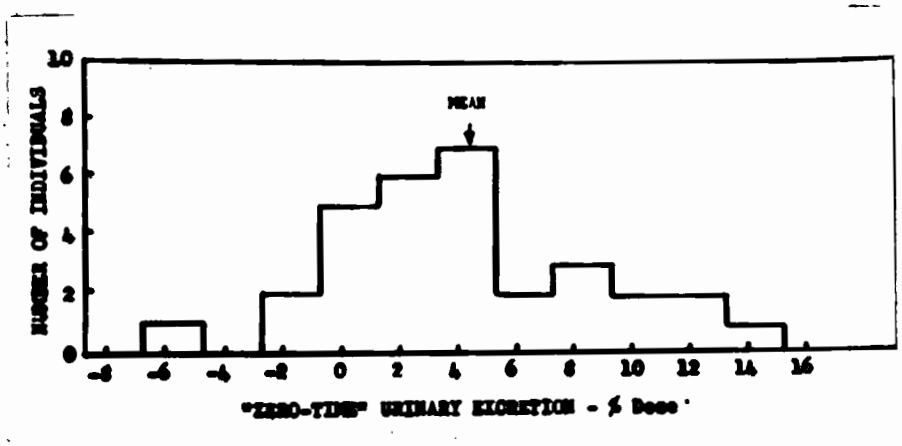
TABLE 3

(ii) Urinary Excretion in Normal Young Men:

Subject	Age yrs.	Time	Diet	Activity	%Dose excreted in 20 min.	Zero-time urinary excretion % Dose	Δlog/hour
1	29	5 p.m.	Uncontrolled	UP	8.9	1.0	-0.1080
2	23	"	"	"	4.6	-2.0	-0.0876
3	26	"	"	"	5.0	1.0	-0.0526
4	24	"	"	"	6.5	1.4	-0.0654
5	25	"	"	"	7.3	1.6	-0.0774
6	24	"	"	"	4.9	0.0	-0.0648
1	29	9 a.m.	Fasting	"	3.8	-1.0	-0.0630
2	23	"	"	"	6.7		
3	26	"	"	"	3.5	-2.0	-0.0690
4	24	"	"	"	4.5	0.5	-0.0552
5	25	"	"	"	9.8	4.5	-0.0732
6	24	"	"	"	7.5	0.0	-0.1030

(iii) Urinary Excretion in Middle Age:

N.K.	60	4 p.m.	Uncontrolled	Recumbent	5.0	2.5	-0.0334
M.B.	47	10 a.m.	Fasting	"	1.3	0.0	-0.0225
J.K.	45	11 a.m.	Uncontrolled	"	8.2	-1.0	-0.0551
V.Y.	43	11 a.m.	Q6h 6-12	Up	7.9	0.8	-0.0960
		11 p.m.	"	Recumbent	8.1	3.0	-0.0816
		11 a.m.	"	"	9.7	2.2	-0.1074
		11 p.m.	"	"	10.0	2.3	-0.1068
		11 a.m.	"	"	10.0	2.8	-0.1002



← The number of individuals is plotted against the urinary excretion expressed as % of the dose given. The mean zero time urinary excretion was 4.5% with S.D. 4.5%

Figure 6

Zero-time urinary excretion in young men; recalculation of data of Ryan (131).

3. c) Appearance of S35 in other transcellular fluids:

(i) Bile

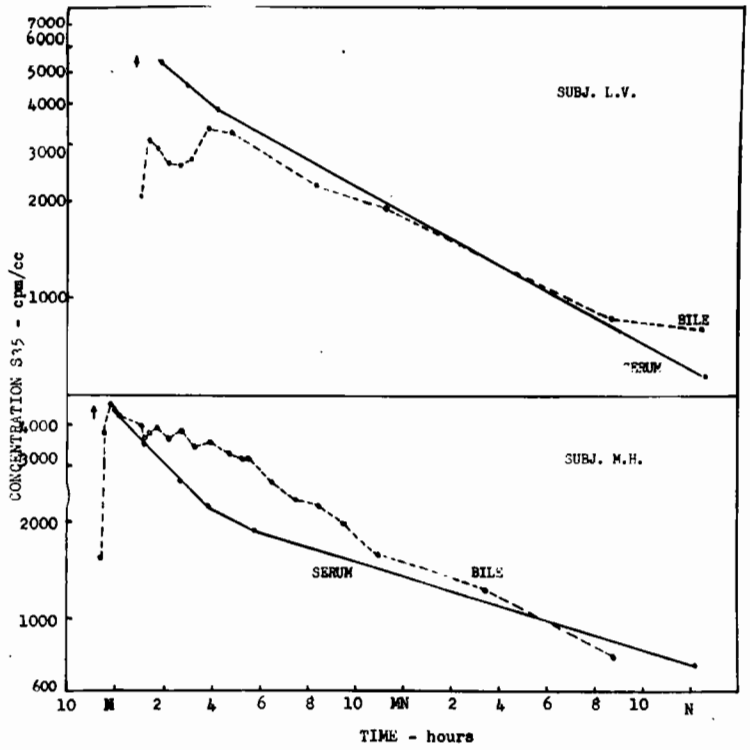
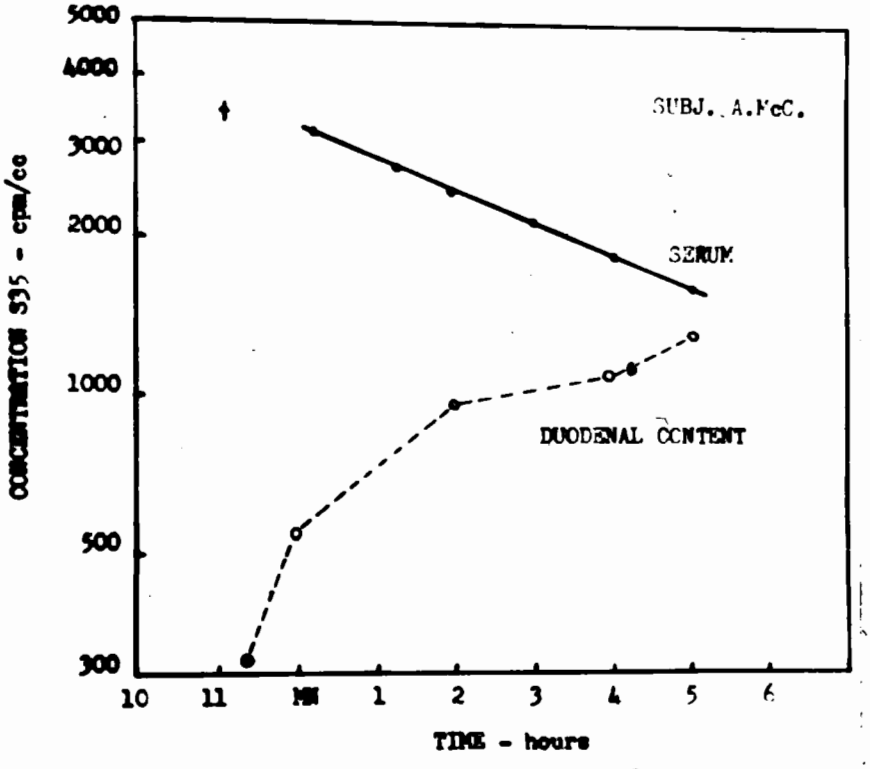


Figure 7

The concentrations of S35 in serum and bile (cpm/cc) are plotted vs time (in hours according to the clock). The concentration of S35 in bile rapidly approaches that of serum, at times exceeding it, but in general remaining at approximately the same level.

(ii) Duodenal content: appearance of S35.



Concentration of S35 in serum and duodenal content (cpm/cc) are plotted vs time (hours by the clock). The concentration of S35 in duodenal content approached that of serum only after 4 - 5 hours.

Figure 8

(iii) Gastric juice: appearance of S35.

TABLE 4

Subject	Time after injection hours	Concentration S35 in gastric juice % serum concentration
N.W.	4	38.2
N.W.	10	34.4
A.McC.	½	24.3
Rabbit	1	28.7

3. d) Change of serum and plasma activity with time before centrifugation:

TABLE 5

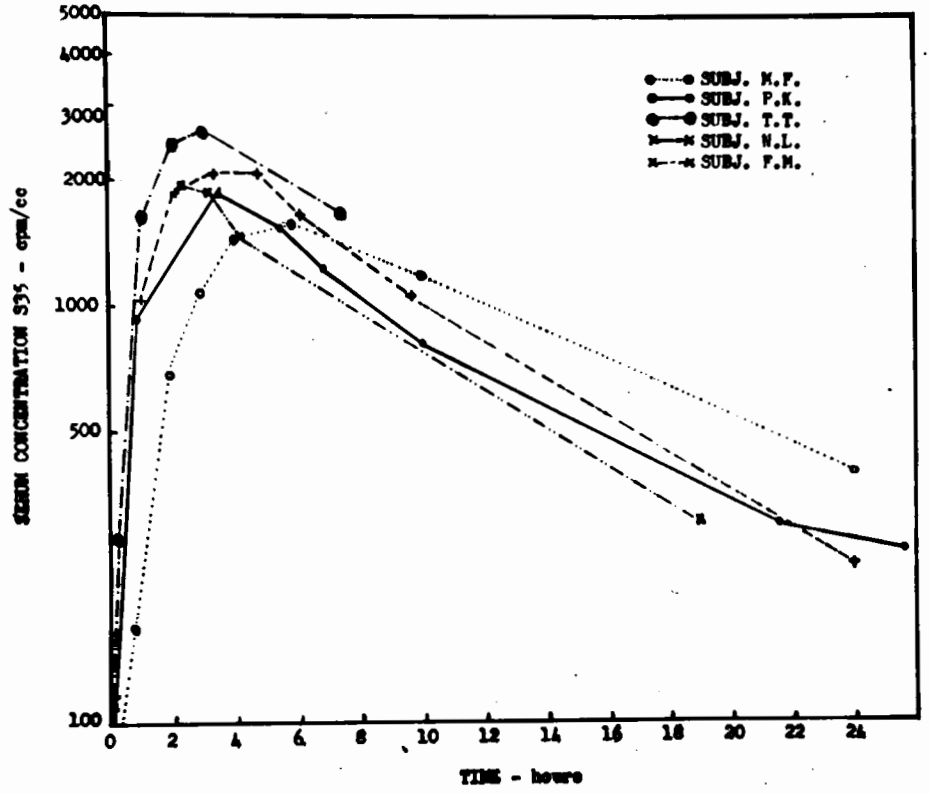
Time after injection blood drawn	Time after injection centrifuged	Serum activity cpm/cc	Plasma activity cpm/cc
15 min.	23 min.	3470	3475
	43 "	3435	3435
	58 "	3435	3490
	86 "	3493	3255
	144 "	3440	3365
	240 "	3455	3475
70 min.	1.5 hours	4950	
	3 '	4810	
	8 '	4610	
	24 '	4780	
90 min.	3 '	2030	
	6 '	2080	
	24 '	2000	
	29 '	2070	

3. e) Association of S^{35} with protein:

TABLE 6

Time ethanol added	Serum Activity cpm/cc	Protein-free filtrate activity cpm/cc	Protein-free filtrate activity % total
44 min. after inj.	3430	1645	48.0
86 " " "	3430	1667	48.6
196 " " "	3430	1687	49.1
406 " " "	3430	1700	49.6
3 min. after	5189	4030	77.7
5 activity	5189	4180	80.6
85 added	5189	3640	70.2

4. Fate of ingested radiosulfate:



Serum concentration of S35 (cpm/cc) shown vs time after ingestion (hours). S35 appeared very quickly in the serum, reaching peak levels in 2 - 4 hours.

Figure 9

The curve of subject MF in Fig 9 is replotted (dotted lines), adding the curve obtained after injection of S35 (solid line) under similar circumstances on a different day. Completeness of absorption is suggested by the fact that the peak serum concentration after ingestion approaches that obtained after injection after which the serum level falls at the same rate.

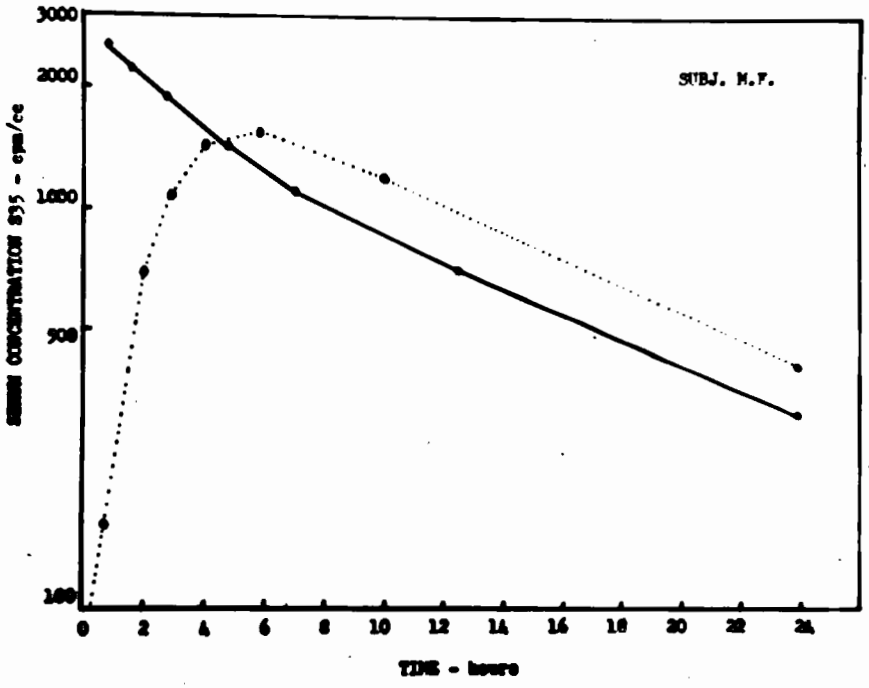


Figure 10

5. Equilibration time in normal and slightly edematous subjects:

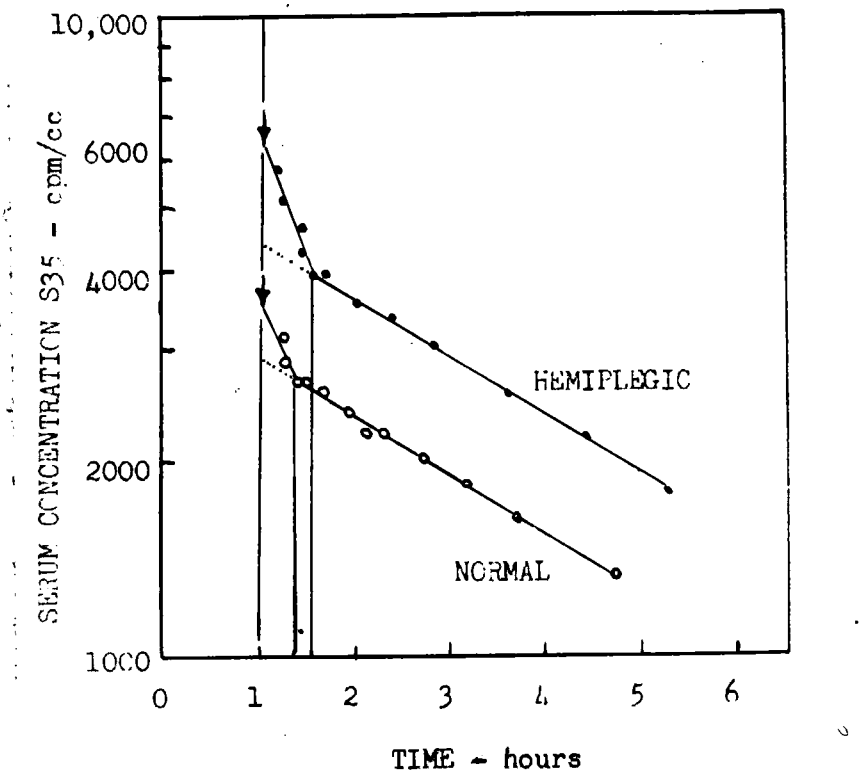


Figure 11

Concentration of S35 in serum (cpm/cc) plotted vs time (hours). The early parts of the curves obtained following injection are plotted to show the rates at which equilibration was achieved in 2 subjects. In the normal subject, equilibrium was achieved by 20 minutes, in the hemiplegic by 30 minutes.

TABLE 7

Subject	Sex	Age yrs.	Weight kg.	Date	Time	Diet	Activity	ECFV l.	ECFV %B.W.	Comments
N.K.	F	60	71.0	5- 2-58	9:00 a.m.	Ad lib.	Recumbent	12.5	17.6	Diabetic; well-controlled
N.W.	F	63	45.4	21- 3-58	10:50 a.m.	Fasting	Comatose	9.5	20.9	Brain tumour; hemiplegic
				27- 4-58	10:50 p.m.	Q6h 2-8	"	9.6	21.2	Normal water and
				28- 4-58	5:07 a.m.	"	"	10.9	24.0	electrolyte balance
				28- 4-58	11:26 a.m.	"	"	10.0	22.0	
				28- 4-58	5:12 p.m.	"	"	9.4	20.7	
N.G.	M	20	71.0	3- 4-58	8:17 a.m.	Fasting	Recumbent	11.4	16.1	Acute glomerulonephritis after recovery
L.V.	F	34	76.6	21- 5-58	1:00 p.m.	p.c.	Recumbent	9.0	11.8	Post-cholecystectomy; obese
M.B.	F	47	139.0	30- 4-58	10:00 a.m.	Fasting	Recumbent	19.0	13.7	Obese; one kidney
A.McC.	M	29	67.4	17- 7-58	10:37 a.m.	Q6h 6-12	Up	12.1	17.9	Normal medical student
				17- 7-58	4:12 p.m.	"	Up	11.3	16.7	
				17- 7-58	10:14 p.m.	"	Up	11.8	17.5	
				17- 7-58	4:08 a.m.	"	Recumbent	13.0	19.3	
				6- 8-58	11:07 p.m.	8a.m.,12N	Up	13.7	20.2	
				7- 8-58	5:02 a.m.	5-6p.m.,	Recumbent	10.9	16.2	
				7- 8-58	11:06 a.m.	8 p.m.	Up	12.1	17.9	
				7- 8-58	5:10 p.m.	"	Up	14.0	20.8	
				8- 8-58	12:13 a.m.	"	Up	13.4	19.9	
				8- 8-58	6:32 a.m.	"	Recumbent	10.6	15.7	
				8- 8-58	12:17 p.m.	"	Up	14.4	21.9	
				8- 8-58	6:11 p.m.	"	Up	14.8	22.0	
			74.4	24- 4-59	5:00 p.m.	a.c.ad lib	Up	12.1	16.3	
M.F.	F	17	142.5	9- 9-58	9:48 a.m.	p.c.ad lib	Recumbent	18.6	13.1	

Subject	Sex	Age yrs.	Weight kg.	Date	Time	Diet	Activity	ECFV l.	ECFV %B.W,	Comments
P.M.	F	17	49.1	15- 9-58	8:30 a.m.	Fasting	Recumbent	10.2	20.7	Chronic renal failure
			49.1	22- 9-58	8:30 a.m.	"	"	9.9	20.2	
			49.7	3-10-58	8:30 a.m.	"	"	11.3	22.6	
			51.4	17-10-58	8:30 a.m.	"	"	12.1	23.5	
			50.6	24-10-58	8:30 a.m.	"	"	9.8	19.4	
			50.9	3-11-58	8:30 a.m.	"	"	11.3	22.2	
L.M.	F	40	50.1	27-10-58	8:30 a.m.	Fasting	Recumbent	9.3	18.6	Essential hypertension
			50.5	30-10-58	8:30 a.m.	"	"	10.8	21.4	
			49.7	3-11-58	8:30 a.m.	"	"	9.2	18.5	
			48.7	7-11-58	8:30 a.m.	"	"	8.9	18.3	
			48.3	19-11-58	8:30 a.m.	"	"	9.5	19.7	
			47.8	8 -12-58	8:30 a.m.	"	"	9.5	19.9	
J.K.	M	45	100	22- 1-59	5:21 p.m.	Q6h 6-12	Immobile	17.0	17.0	Traumatic division of spinal cord C3-4
				22- 1-59	11:15 p.m.	"	"	17.4	17.4	
				23- 1-59	5:15 a.m.	"	"	16.2	16.2	
				23- 1-59	11:23 a.m.	"	"	14.3	14.3	
				3- 2-59	11:03 a.m.	Ad lib.	Rocking	18.4	18.4	
				3- 2-59	4:01 p.m.	8:30 a.m.	bed	17.9	17.9	
				3- 2-59	11:14 p.m.	12:00 N	"	17.2	17.2	
				4- 2-59	8:11 a.m.	4:30-5 p.m. 8:00 p.m.	"	18.5	18.5	
				12- 2-59	11:10 a.m.	"	"	17.5	17.5	
				12- 2-59	4:15 p.m.	"	"	20.4	20.4	
				12- 2-59	11:10 p.m.	"	"	18.1	18.1	
				13- 2-59	9:20 a.m.	"	"	17.7	17.7	
				13- 2-59	11:10 a.m.	"	"	19.1	19.1	
N.B.	M	22	88.5	3-11-58	12:05 a.m.	Q6h 6-12	Recumbent	15.8	17.9	
				4-11-58	6:02 a.m.	"	Recumbent	15.9	18.0	
				4-11058	1:14 p.m.	"	Up	13.7	15.5	
				4-11-58	8:05 p.m.	"	Up	13.4	15.2	

Subject	Sex	Age yrs.	Weight kg.	Date	Time	Diet	Activity	ECFV l.	ECFV %B.W.	Comments
N.B. (continued)			85.5	8-11-58	6:29 a.m.	8-N-6	Recumbent	13.2	15.4	
				9-11-58	12:14 p.m.	"	Up	14.2	16.6	
				9-11-58	5:19 p.m.	"	Up	14.5	17.0	
				9-11-58	12:18 a.m.	"	Recumbent	15.3	17.9	
				10-11-58	7:28 a.m.	"	Recumbent	15.1	17.7	
			85.2	22-11-58	6:20 p.m.	Q6h 6-12	Up	14.1	16.6	
				23-11-58	12:30 a.m.	"	Recumbent	16.1	18.9	
				23-11-58	6:23 a.m.	"	Recumbent	15.7	18.5	
				23-11-58	12:14 p.m.	"	Recumbent	16.0	18.8	
				23-11-58	6:12 p.m.	"	Recumbent	15.0	17.7	
				24-11-58	6:47 a.m.	"	Recumbent	13.2	15.5	
				24-11-58	12:15 p.m.	"	Recumbent	13.3	15.6	
				25-11-58	12:29 a.m.	"	Recumbent	11.5	13.5	
				25-11-58	1:33 p.m.	"	Up	12.6	14.8	
P.K.	M	29	171.5	19- 9-58	9:27 a.m.	p.c.ad lib	Recumbent	21.0	12.5	Obese
G.G.	M	25	70.4	12- 3-59	11:47 a.m.	Q6h 6-12	Up	12.5	17.8	Normal
				12- 3-59	5:42 p.m.	"	Up	12.5	17.8	
				12- 3-59	11:57 p.m.	"	Recumbent	13.4	19.0	
				13- 3-59	5:58 a.m.	"	Recumbent	13.2	18.8	
				13- 3-59	11:35 a.m.	"	Up	12.7	18.0	
J.deC.	M	22	66.6	12- 3-59	5:35 p.m.	Q6h 6-12	Up	13.1	19.7	Normal
				13- 3-59	11:30 a.m.	"	Up	13.0	19.5	

Subject	Sex	Age yrs.	Weight kg.	Date	Time	Diet	Activity	ECFV l.	ECFV %B.W.	Comments
K.M.	M	29	81.8	8- 4-59	12:29 p.m.	Q6h 6-12	Up	14.1	17.2	Essential hypertension medical student
			81.9	9- 4-59	12:30 a.m.	"	Recumbent	14.3	17.5	
			81.2	9- 4-59	12:29 p.m.	"	Up	14.3	17.6	
			81.2	10- 4-59	12:15 a.m.	"	Recumbent	14.8	18.2	
			80.0	10- 4-59	12:22 p.m.	"	Recumbent	13.6	17.0	
			80.5	11- 4-59	12:45 a.m.	"	Recumbent	14.6	18.1	
			80.5	11- 4-59	12:40 p.m.	"	Recumbent	14.3	17.8	
			80.5	12- 4-59	12:28 a.m.	"	Recumbent	13.4	16.7	
			80.5	12- 4-59	12:20 p.m.	"	Recumbent	12.6	15.7	
			80.5	13- 4-59	1:00 p.m.	"	Up	13.6	16.9	
			80.5	14- 4-59	12:30 a.m.	"	Recumbent	12.2	15.2	
			80.5	14- 4-59	12:53 p.m.	"	Up	12.0	14.9	
V.Y.	F	43	58.7	8- 5-59	11:57 a.m.	Q6h 6-12	Up	8.93	15.2	Investigation for ideopathic edema; no edema at time of study
			58.9	8- 5-59	11:33 p.m.	"	Recumbent	10.0	17.0	
			58.9	9- 5-59	11:40 a.m.	"	Sitting	10.2	17.3	
			58.8	9- 5-59	11:30 p.m.	"	Recumbent	9.84	16.7	
			58.8	10- 5-59	11:35 a.m.	"	Recumbent	10.3	17.5	
			58.7	10- 5-59	11:35 p.m.	"	Recumbent	9.92	16.9	
			58.7	11- 5-59	11:40 a.m.	"	Recumbent	10.3	17.5	
			58.7	11- 5-59	11:52 p.m.	"	Recumbent	10.0	17.1	
			58.5	12- 5-59	11:53 a.m.	"	Recumbent	9.88	16.9	
			58.7	12- 5-59	11:40 p.m.	"	Recumbent	9.70	16.5	
			58.4	13- 5-59	11:40 a.m.	"	Recumbent	10.2	17.5	
			58.4	13- 5-59	11:40 p.m.	"	Recumbent	9.96	17.1	
			58.0	14- 5-59	11:40 a.m.	"	Up	8.58	14.8	

TABLE 8

WELL-NOURISHED SUBJECTS:					OBESE SUBJECTS:	
Subject	No. of Det.	Sulfate space range % B.W.	Mean % B. W.	S.D.	Subject	Sulfate space % B.W.
A.McC.	13	15.7 - 22.0	18.8	2.17	L.V.	11.8
G.G.	5	17.8 - 19.0	18.3	0.60	M.B.	13.7
J.deC.	2	19.5 - 19.7	19.6		M.F.	13.1
N.B.	14	13.5 - 18.9	16.7	1.53	P K.	12.5
V.Y.	13	14.8 - 17.5	16.8	0.72		
K.M.	12	17.9 - 18.1	16.9	1.10		
L.M.	6	18.3 - 19.9	19.0	0.70		
			M 18.0			M 12.8

Mean of all values for well-nourished subjects: 17.5±3.0 (10.8%).

TABLE 9

Subject	ECFV: line calculated from 6 points	ECFV: line calculated from 2 points	Difference l.
A.McC.	12.1	11.8	0.3
	11.3	11.3	0
	11.8	11.8	0
	13.0	13.0	0
	13.7	13.7	0
	10.9	11.1	0.2
	12.1	11.5	0.6
	14.0	13.7	0.3
	13.4	12.5	0.9
	10.6	10.8	0.2
	14.4	14.7	0.3
	14.8	14.0	0.8
L.M.	9.3	9.3	0
	10.8	10.8	0
	9.2	9.2	0
	8.9	8.6	0.3
	9.5	9.5	0
	9.5	9.5	0

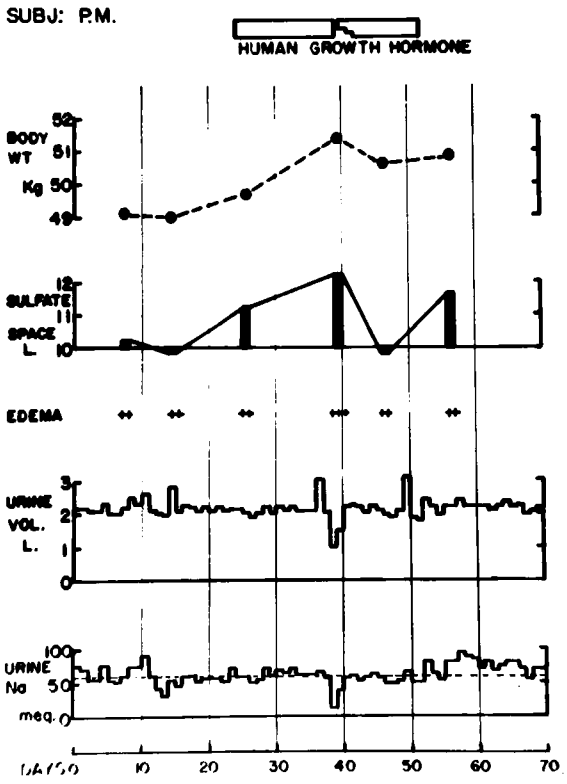
7. REPRODUCIBILITY OF THE SULFATE SPACE UNDER THE SAME CONDITIONS of DIETARY INTAKE, ACTIVITY AND TIME OF DAY: (Normal urinary excretion)

TABLE 10

Subject	ECFV#1	ECFV#2	ECFV#3	ECFV#4	Difference from mean
	%B.W.	%B.W.	%B.W.	%B.W.	%B.W.
A.McC.	20.2	19.9			0.15
	16.5	16.0			0.25
	17.5	21.5			2.0
	20.3	20.8			0.25
L.M.	18.6	18.5	19.7	19.9	0.6, 0.7, 0.5, 0.7
J.K.	17.5	19.1	18.4		0.8, 0.8, 0.1
	17.9	20.4			1.25
	17.2	18.1			0.45
	18.5	17.7			0.40
N.B.	15.4	17.7			1.15
	18.0	18.5			0.25
	17.9	18.9			0.50
G.G.	17.8	18.0			0.10
K.M.	17.2	17.6			0.2
	17.5	18.2			0.35
V.Y.	17.0	16.7			0.15

Coefficient of variation: 5.66

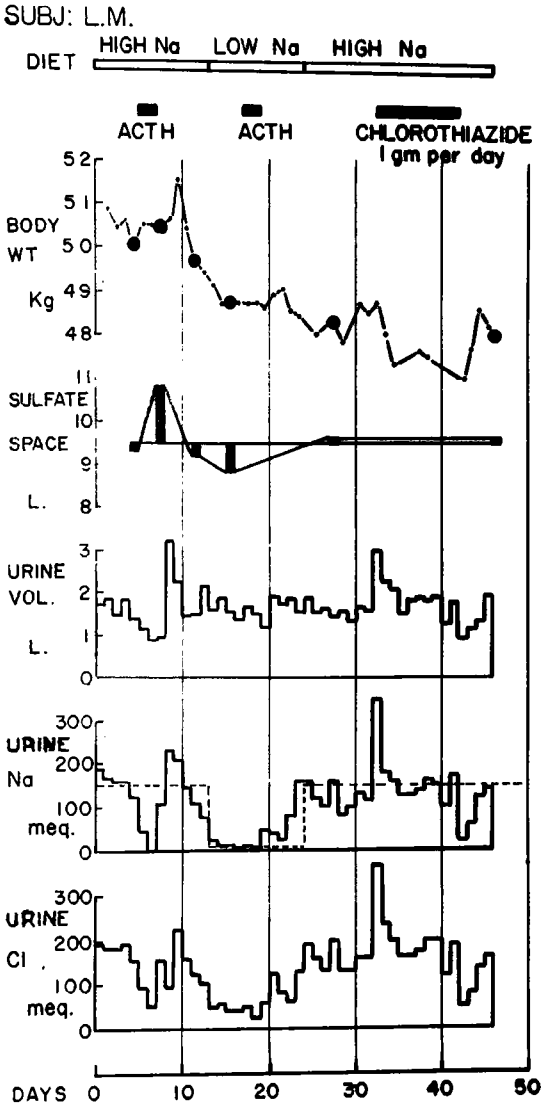
8. Fluctuation of the sulfate space under various conditions, time of day and diet kept constant; correlation with weight and balance data.



←Figure 12: Subject P.M.: Plotted against time in days are various ordinates -abbreviated for the sake of space- body weight in kilograms with the values determined on the same days as sulfate spaces indicated by large black dots; sulfate space in litres, the mean control value indicated by a horizontal line with individual values shown as vertical bars above and below; edema designated according to a 0 to ++++ system; daily urine volume in litres; daily urine sodium in meq. with the daily sodium intake shown as a dotted line.

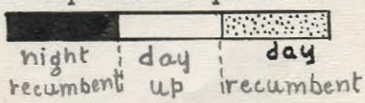
Figure 13: →

Subject L.M.: The material is plotted in a fashion similar that of Figure 12. Dietary sodium is indicated across the top of the graph while underneath this line is shown the times of administration of ACTH and Chlorothiazide. Urine Cl in meq. is also shown.

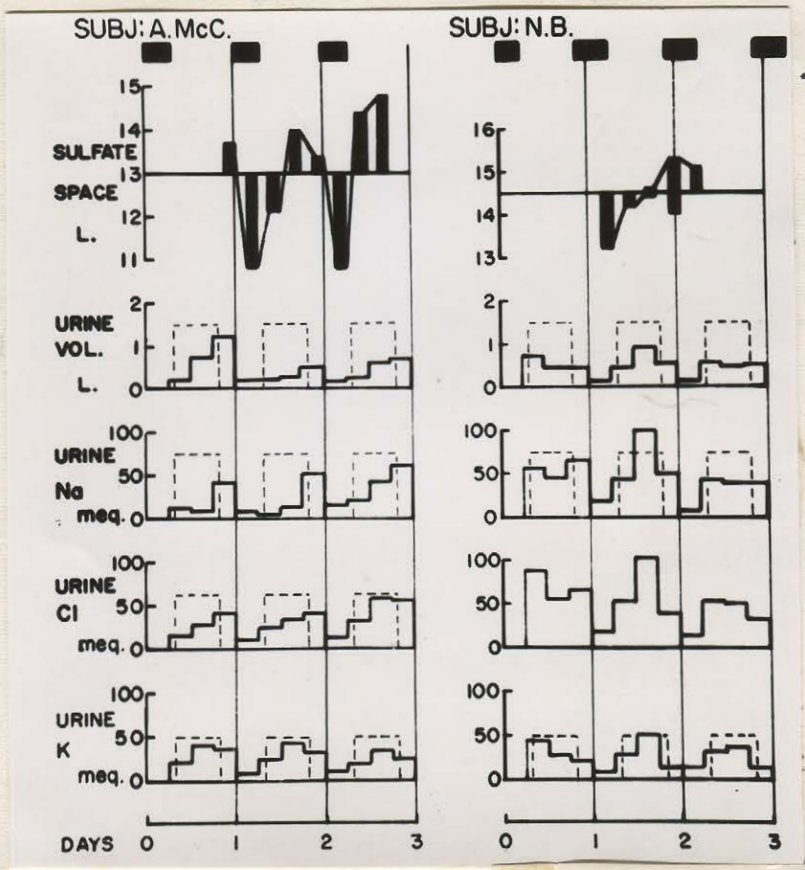


9. Diurnal fluctuation in sulfate space: correlation with balance data.

In these and the following figures, the top line represents activity or recumbency as follows:



a) Ordinary diet, activity during the day:



← Figure 14:

The ordinates are similar to those of Figures 12 and 13. The time scale is different, each day being shown by a vertical line, and excretion plotted for six-hour periods.

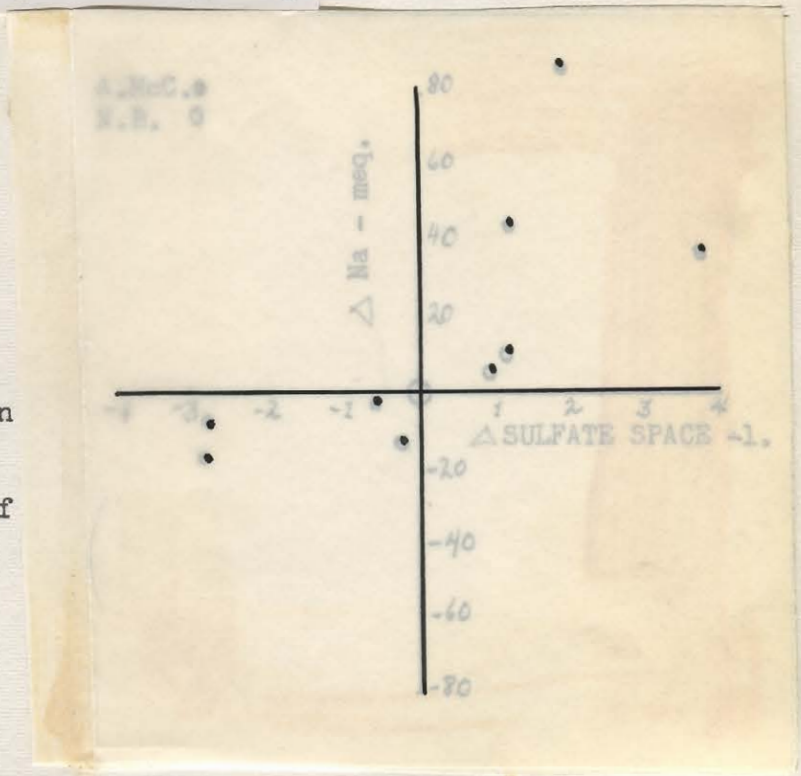
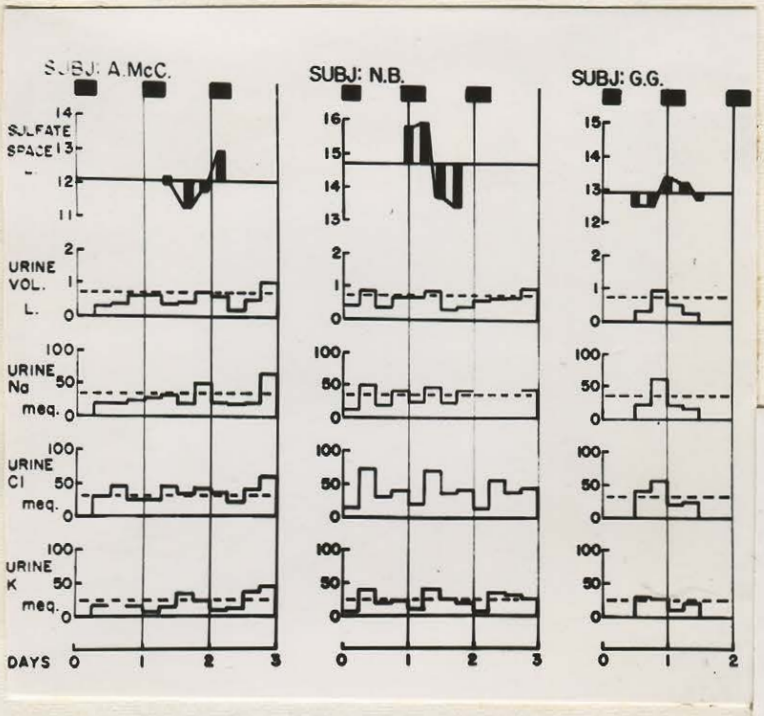


Figure 14a: →

Correlation of change in sulfate space (litres) with change in sodium balance (meq.) - data of Figure 14.

9. c) Six-hourly diet, activity during the day:



← Figure 15:

The mode of presentation in these graphs is similar to that of Figure 14.

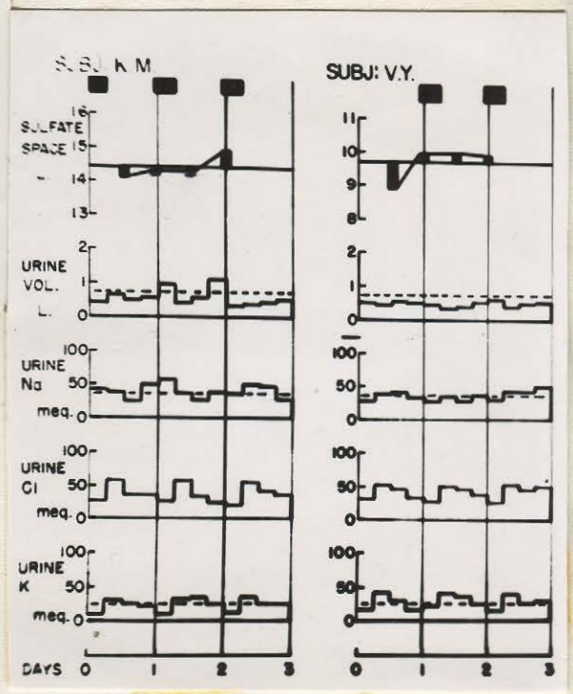
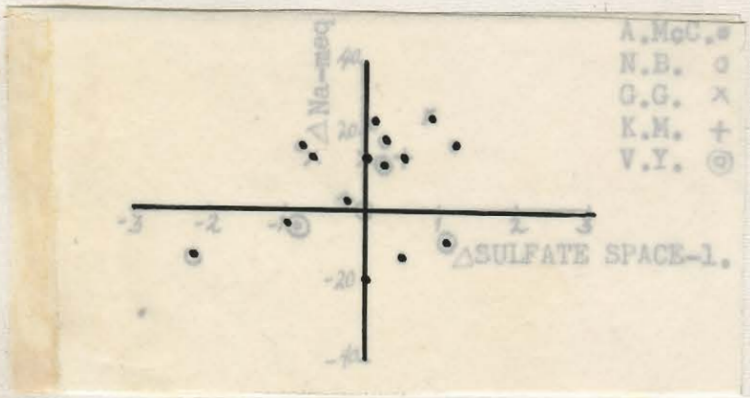


Figure 16: →

The mode of presenation in these graphs is similar to that of Figure 14.



← Figure 16a:

Correlation of change in sulfate space (litres) with change in Na balance (meq.) - data of Figures 15 and 16.

9. DIURNAL FLUCTUATION IN SULFATE SPACE: six-hourly diet
activity during the day

TABLE 11

		DAY				NIGHT			
Subject	Mean Sulfate Space % B.W.	Noon Sulfate space % B.W.	Diff. from mean	6 p.m. Sulfate space % B.W.	Diff. from mean	Midnight Sulfate space % B.W.	Diff. from mean	6 a.m. Sulfate space % B.W.	Diff. from mean
A.McC.	17.8	17.9	0.1	16.7	-1.1	(17.5)	-0.2	19.3	1.5
N.B.	17.2	15.5	-1.7	15.2	-2.0	17.9	0.7	18.0	0.8
				16.6	-0.6	18.9	1.7	18.5	1.3
G.G.	18.3	17.8	-0.5	17.8	-0.5	19.0	0.7	18.8	0.5
		18.0	-0.3						
K.M.	17.6	17.2	-0.4			17.5	-0.1		
		17.6	0			18.2	0.6		
V.Y.	16.6	15.2	-1.4			17.0	0.4		
		(17.3)	0.7			16.7	0.1		
	M 17.5		M -0.4		M -1.1		M 0.5		M 1.0

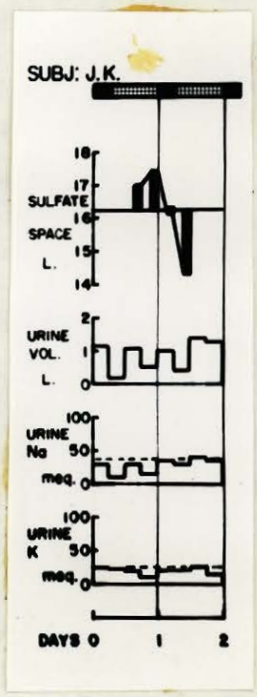
Combined day values: Difference from mean: -0.64
Combined night values: " " " : 0.67
} 1.31 (7.5 % of mean sulfate space)

Values of sulfate space marked ( ) refer to values obtained when the subject was still active at MN (A.McC.) or resting during the day (V.Y.).

9. d) Six-hourly diet, uniform activity:

Figure 17: →

The mode of representation in this figure is similar to that of Figure 14.



9. e) Six-hourly diet, prolonged bed rest:

Figures 18 and 19:

The mode of representation is similar to that of Figure 14 but further ordinates are added: TBV - total blood volume in litres; PV - plasma volume in litres; RCV - red cell volume in litres; HCT - hematocrit; serum Na, K, and Cl are shown in meq./ litre.

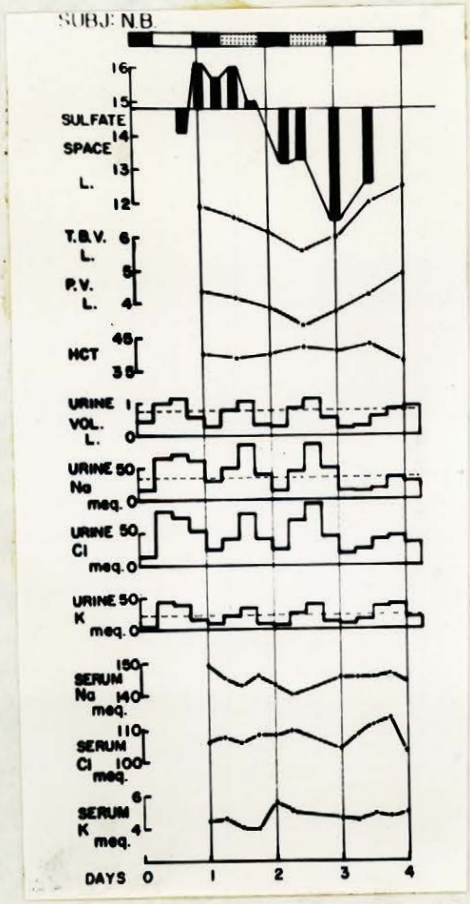


Figure 18

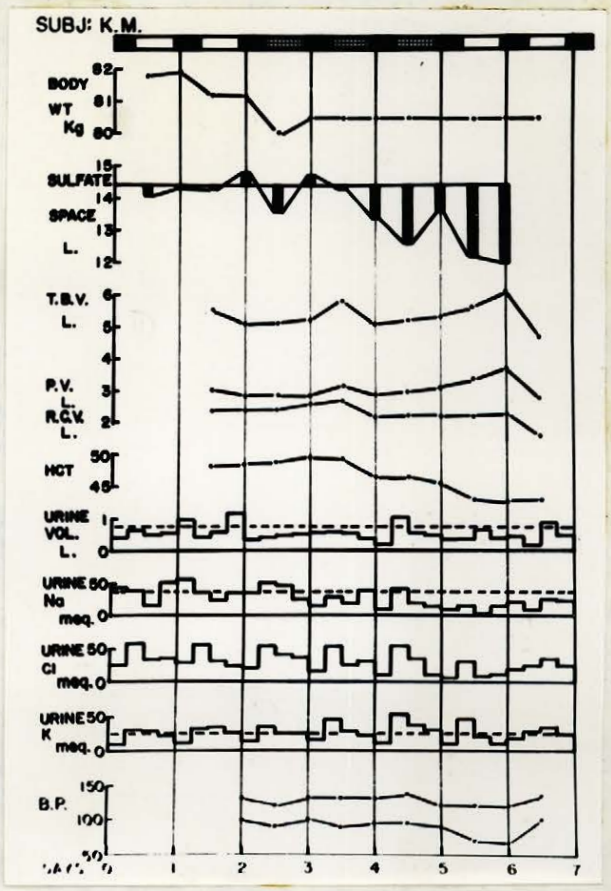


Figure 19

9. e) continued:

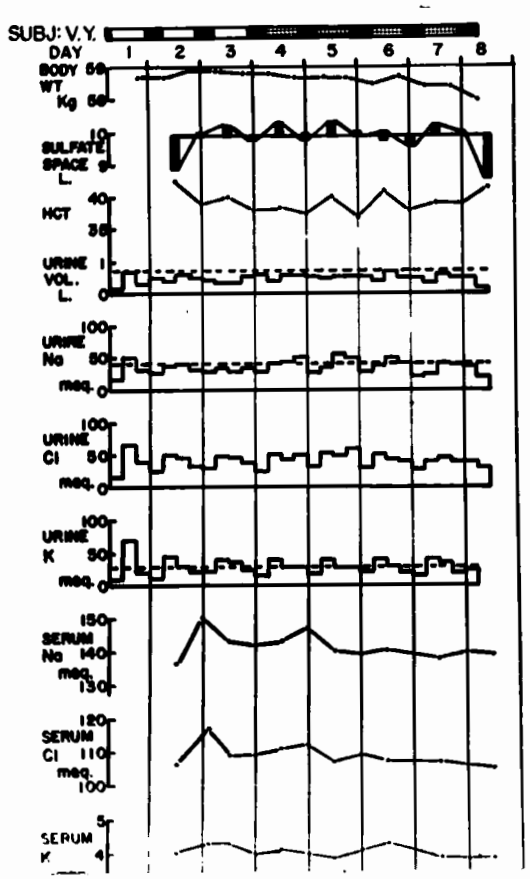


Figure 20

The mode of representation is similar to that of Figures 18 and 19 but lacking data for blood volumes.

D. DISCUSSION

1. RELATIONSHIP OF ACTIVITY TO WEIGHT FOR S35 IN PLASMA AND SERUM:

In Figure 1 it is seen that the greater the weight per unit area of sample, the lower is the observed activity since more of the β- particles are absorbed by the material in which they are dispersed. From the graph it may be seen that the absorption is negligible for weights less than 1 mg. per planchette area. The curve falls steeply at first, then levels off as the weight increases. Above 70 mg., samples tended to dry unevenly; below 30 mg., the rapid change of true count with small weight changes was such that a small error in weighing would cause a large error in the estimated count. Therefore weights in the range 30 to 70 mg. were used.

2. TECHNICAL SOURCES OF ERROR:

Errors due to pipetting and injection were relatively small.

The coefficient of variation of the observed counts of the 20 identical samples of serum was 3.0%. When corrected for self-absorption, the coefficient of variation was 3.2%. Since three samples were counted for each specimen of plasma or serum the probable error of the average count was $3.2/\sqrt{N} = 3.2/1.73 = 1.8\%$ or about 2%.

The observed counts of 20 inorganic sulfate solution samples, corrected for background gave a mean of 4360 with a standard deviation (S.D.) of 106 or 2.4%. Since 10 samples of the standard were counted before and after each series of serum counts, the probable error of each control count was $2.4/\sqrt{N} = 2.4/3.16 = 0.76\%$ or about 0.8%.

If the errors were due only to the random nature of the decay process, the probable errors would equal $\sqrt{1916} = 43.7$ (2.28%) for serum, and $\sqrt{4360} = 66$ (1.5%) for inorganic sulfate solution. Since the measured variation

exceeded these values, the increase was attributed mainly to the lack of uniform distribution of material occurring on drying.

Other sources of error are:

(i) Timing of samples: when available an automatic timer was used; at other times a wrist watch with a second hand was used. Accuracy of timing samples was estimated at 15 seconds. An error of 30 seconds would introduce an error in serum concentration of about 0.5%, negligible in these studies.

(ii) Urinary excretion: this error, which is probably the largest involved, is discussed in the section "fate of injected sulfate".

3. FATE OF INJECTED RADIOSULFATE:

a) Serum disappearance curves:

After injection the serum concentration falls rapidly for the first 20 minutes and then more slowly as shown in Figure 2A. Since the rate of fall is proportional to the concentration remaining in the serum, this second portion of the curve becomes a straight line when the logarithm of the serum concentration is plotted against time, as shown in Figure 2B. If the straight line thus obtained is extrapolated back to zero-time, the serum concentration is assumed to be that concentration which would have been achieved if all the S35 injected had been instantly mixed throughout the physiological extracellular fluid volume, providing the straight-line relationship were valid during the period of extrapolation. This latter provision is not really true however since urinary excretion is greater during the equilibration period than later.

The serum disappearance curves remained straight within the limits of experimental error (± 2%) for 6-9 hours, then, as in the examples shown in Figure 3, tended to flatten out somewhat by 24 hours.

There are five factors which might reasonably be expected to alter the second rate of fall of serum concentration:

(i) change in urinary excretion of sulfate

(ii) change in rate at which S35 penetrates the tissues

(iii) back-diffusion from the tissues

(iv) protein-binding of the S35

(v) change in the extracellular fluid volume.

When serial injections were carried out every six hours, as shown in Figure 4, the slopes obtained appeared similar to each other in the same individual. To obtain the serum zero-time value in the second and subsequent determinations, the serum activity immediately before the injection was subtracted from the zero-time extrapolated value of the serum activity following injection.

When slopes were calculated as shown in Table 2, the variation for the initial six-hour period was about twice that expected from the experimental error of counting.

The slopes of subject P.M. were much less steep than those of the others because this patient was suffering from chronic renal failure and therefore the urinary excretion of S35 was very small.

When small meals were given during the period following injection, no significant effect on the slope of serum disappearance was observed. However in most studies the relationship to meals was kept constant to exclude food absorption as a factor altering the ECFV.

b) Excretion of S35 into transcellular fluids:

(i) Urine: When sulfate in injected intravenously, it first of all mixes with the blood. Since the volume of the blood plasma is one-third that of the extracellular fluid volume with which the sulfate is equilibrated by 18 minutes, the plasma concentration is for a very short time about three

times that of its equilibration concentration. Hence the concentration in the blood reaching the kidneys is that much greater with the result that an "excess" is excreted in the urine. It is difficult to see how this amount could be less than zero.

Such results as those in Table 3 and Figure 4 may be attributable to the urinary dead space, since this may be considerable despite the fairly high urinary flow rates achieved by giving 200 ml. of water every two hours as Ryan et al. (131) did. A certain amount of the variation must also be due to technical error, although this is probably not very great.

In doing serial injections of radiosulfate an attempt was made to collect serial urine samples (V.Y.). Poor volumes were obtained in spite of collecting for 1½ hours before each injection since the subject was not given any extra fluid to drink. However the results which were obtained were consistent.

The effect of position on the urinary excretion of sulfate was not investigated in these studies as its possible importance was not at first appreciated. Nor did Ryan et al. state whether their subjects were lying down or standing. Since the serum disappearance curves remain so constant in these studies, it seems reasonable to suppose that the sulfate clearance does not change greatly under the conditions used, since a change in slope of urinary excretion would be expected to alter the serum disappearance rate as well. If the variation in the excessive excretion during equilibration is due only to mixing, then if equilibration were more rapid as in a more active subject, the excess sulfate excretion would be lower and vice versa. In the only study in which urinary excretion during equilibration was determined both after activity and recumbency no striking difference was noted although values during recumbency (8.1, 9.7 , 10.0, 10.0) tended to be higher than the one value obtained just following activity (7.9), as did the values for "excess" excretion, 3.0, 2.2, 2.3, 2.8 compared with 0.8.

(ii) Bile: So far as is known, the excretion of sulfate in bile has not been studied in humans although it was reported to occur in rats (137). From two studies shown in Figure 7, it may be concluded that the passage of S35 into bile is very rapid, that a substantial concentration is present in bile by the time the S35 has equilibrated with the "physiologic" ECF, and that the concentration in bile is comparable to that of serum for many hours after injection, in these studies somewhat exceeding it.

(iii) Duodenal content: In the only subject studied, the duodenal content took several hours to reach a concentration approaching that of serum (Fig.8).

(iv) Gastric juice: Very little S35 was found in gastric juice in the first few hours after injection (Table 4). This is in accordance with the findings in rats (137).

c) Entry of radiosulfate into red blood cells (change of serum activity with time):

From Table 5 it may be seen that there is no significant change in plasma or serum concentration for many hours after allowing samples to stand at room temperature. Similar results were found when samples were refrigerated.

No difference was apparent between plasma and serum concentrations of samples taken at the same time although in practice one or the other type of samples was used consistently throughout a study. Usually serum samples were used. Plasma samples were obtained in two studies in which blood volumes were also done.

It was concluded that the equilibrium between plasma and red blood cells is achieved by 20 minutes and that no further change occurs thereafter in either clotted or heparinized samples for 24 hours or longer.

d) Association of S35 with plasma protein:

After addition of a protein precipitant to S35 in contact with plasma for 44 minutes, about 50% of the activity was found in the protein-free filtrate (Table 6). When the S35 had been in contact with the plasma only a few minutes, about 75% of the activity was found in the protein-free filtrate.

These results are not evidence of protein-binding of sulfate but do indicate that the S35 does become in some manner associated with protein, making its separation from protein more difficult.

Consequently in studies where plasma volumes were also carried out, samples were plated, covered with Saranwrap and left standing for 6 weeks until the I^{131} had decayed to negligible amounts. By this time the activity of the S35 was reduced to about one-half of its activity at the time of injection.

4. FATE OF INGESTED RADIOSULFATE:

S3504 is rapidly and completely absorbed from the gastro-intestinal tract according to the studies shown in Figures 9 and 10. Completeness of absorption is indicated by the fact that peak serum concentration after ingestion approximates that attained with intravenous injection. From these studies the site of absorption is not established, although since the radiosulfate was given just after a meal the most likely site would be the stomach since stomach emptying would not likely occur for an hour or more. If this were so, then the rate of absorption and rate of secretion of radiosulfate in the stomach are greatly different.

An attempt was made to find a subject in whom the stomach had been completely removed but no suitable subject was found, and this problem was not further investigated. No data on the absorption of radiosulfate was found in the literature.

5. EQUILIBRATION TIME IN NORMAL AND EDEMATOUS SUBJECTS:

Studies conducted on normal subjects, as for example that shown in Figure 11, were in agreement with those of Walser and Ryan i.e. equilibration was achieved by 18-20 minutes.

In the subjects in whom edema was due to total inactivity, (N.W., J.K.), equilibrium was achieved by 30 minutes.

In subject P.M., who had ++ to +++ pitting edema of the ankles, slightly puffy eyes, but no evidence of pleural effusion or ascites, a straight-line relationship was achieved in all six studies by 30 minutes.

However such studies do not exclude the possibility that the edema fluid was not in equilibrium since no samples of it were obtained.

6. EXTENT OF THE SULFATE SPACE IN HUMANS:

Values of the sulfate space in well-nourished individuals with no known abnormality of water or electrolyte metabolism ranged from 13.5 to 22% body weight. Taken together the values had a mean of 17.5 ± 3.0 % B.W., with a coefficient of variation of 10%. The mean of the individual mean values was 17.8 ± 1.3%B.W., with a coefficient of variation of 7.3.

The mean value for sulfate space obtained by this method is somewhat higher than that obtained by Walser et al., i.e. 15.1%B.W. Their values ranged from 11 to 21%, and repeated determinations in a single individual differed by 0.16 - 0.30 litres.

Ryan et al. obtained a mean value of 18.1 ± 2%B.W. in normal young men; repeat values in five individuals differed by an average of 0.24 litres. Repeat values by both groups were done in elderly individuals.

In the obese subjects studied the sulfate space ranged from 11.8 to 13.7%B.W. with a mean of 12.8%B.W., significantly below the values for well-nourished subjects. Such values have been found by other investigators and are due to the fact that fatty tissue contains less water than other tissues.

7. REPRODUCIBILITY OF THE SULFATE SPACE UNDER THE SAME CONDITIONS OF DIETARY INTAKE, ACTIVITY, AND TIME OF DAY:

When values for sulfate space in the same individual, similar with respect to time of day, activity and relationship to meals, were compared, the coefficient of variation was 5.66. This value approximates the estimated error of the sulfate space determination if the error for urinary excretion is taken from Ryan's data. In this case the probable error would be equal to the square root of the sum of the squares of the probable errors in the technical procedure plus the square of the 4.6% error calculated from Ryan's data, giving $\sqrt{0.5^2 + 0.02^2 + 2.0^2 + 4.6^2} = \sqrt{25.45} = 5\%$.

8. FLUCTUATION OF THE SULFATE SPACE UNDER VARIOUS CONDITIONS, TIME OF DAY AND DIET KEPT CONSTANT: Correlation with weight and balance data.

(i) P.M.: The two control studies checked well with each other. On the 24th day of the study, a preparation of human growth hormone (10 mg. per day) was begun. Two days later the sulfate space had risen by about 1 litre. Weight had increased 0.6 kg. above the control values. On the 38th day, a second preparation of human growth hormone from the same company was given. So far as known, this preparation was identical with the first. On the second day of this preparation, a sudden gain in weight occurred, with a sudden increase in edema, and Na and fluid retention. There was a corresponding increase in the sulfate space. One dose of growth hormone was omitted, a reduced dose given for two days, and then the original dosage resumed. The sulfate space dropped to the control level on the 23rd day of growth hormone, but it had risen 5 days after stopping the growth hormone.

The values for sulfate space, while they do not always correspond exactly to the weight, changed in the same direction as the weight in each case, and corresponded approximately with the Na and fluid balance.

The error of the sulfate space determinations in this patient should be smaller, of the order of 3%, than that calculated in section 7 since the loss of S35 into the urine was very small.

(ii) L.M.: The data on Miss L.M. are difficult to relate to weight change since she lost weight gradually throughout the study; although if a line be drawn through the average weights of the control periods the sulfate spaces correspond fairly well with the deviations of weight above and below the line.

The four control studies agreed very well with each other. Eight hours after the last dose of ACTH (Acton X20 units q6h x 8) given on the 6th and 7th days of the study, the sulfate space increased by about 1.5 litres, corresponding to a period of Na and fluid retention. An increase of sulfate space after ACTH was reported by Walser, Seldin and Burnett (183).

9. DIURNAL VARIATION IN SULFATE SPACE: Correlation with balance data:

a) Ordinary (controlled) diet, activity during the day:

(i) A.McC.: The water, Na, K, and Cl rhythms are clearly defined with highest excretion occurring in the 6 p.m. - MN collections, lowest values MN - 6 a.m. The sulfate space showed a significant fluctuation, higher values occurring at 6 p.m. and MN, lowest values at 6 a.m.

According to the balance study, intake exceeded excretion from 8 a.m. to 8 p.m. corresponding to a net gain in this interval of about 100 meq. Na and 1.3 litres of water, 50 meq. K, and 50 meq. Cl. From 8 p.m. to 8 a.m. there was no intake and excretion amounted to 1 litre of water, 50 meq. $N\overset{a}{\underset{a}{}}$, 30 meq. K, and 45 meq. Cl. Thus the sulfate space pattern is similar to that of the water and electrolyte balance although the difference between day and night values (3 litres) somewhat exceeds that expected.

(ii) N.B.: The water, Na, K, and Cl rhythms are clearly defined in this case also, the peak excretion occurring in the N - 6 p.m. collections, the lowest occurring in the MN - 6 a.m. collections. The highest sulfate space value occurred at MN, the lowest at 6 a.m., but a second 6 a.m. value was higher than the mean value and the amount of fluctuation was not significant. In the balance study peak excretion coincided with peak intake and there was therefore little net gain in the Na balance during the day although there was a gain of 0.5 litre water and about 30 meq. K. At night there was a loss of 0.8 litre water, 40 meq. Na, 30 meq. K. The sulfate space values changed in the directions of the fluid balance. There is a significant correlation of change in sulfate space with change in Na balance on this regimen, as shown in Fig. 14a.

b) Uncontrolled diet, no activity:

No balance studies were carried out in these experiments. One high value for the sulfate space occurred at 4 p.m. but the fluctuation from the mean was not significant.

c) Six-hourly diet, activity during the day:

(i) A.McC.: Peak excretion of water, Na, K, and Cl occurred in the 6 p.m. - MN collections in this study also but the peaks were less well-defined. Highest sulfate space value occurred at 6 a.m., lowest at 6 p.m. but these values are not significantly different from the mean.

(ii) N.B.: The rhythms of water, Na and Cl excretion are poorly defined, but peak K excretion occurs from 6 a.m. to N, lowest from MN - 6 a.m. Highest sulfate space values occurred at MN and 6 a.m., lower values at N and 8 p.m.

(iii) G.G.: Highest excretion of water, Na, K, Cl occurred from

6 p.m. - MN. Highest value of sulfate space occurred at MN but was not significantly different from the mean.

(iv) K.M.: Excretory rhythms were very poorly defined and sulfate space values did not differ significantly from the mean.

(v) V.Y.: Excretions of K and Cl were higher during the day while no rhythm of water or Na excretion was apparent. One low value of sulfate space occurred at N but did not differ significantly from the mean.

When day (noon, 6 p.m.) and night (midnight, 6 a.m.) values were compared (Table 11), almost all day values were lower than the mean values while night values were higher. Day values averaged 3.7% B.W. below the mean values, while night values averaged 3.8% B.W. above the mean values. Such a difference might be due to time of day, activity or position.

It is interesting to note that almost the only values which did not fit into the above scheme were those in which the subject was either still active at midnight (A.McC.) or resting during the day (V.Y.) as marked () in the table.

The difference between day and night values was significant at the 5% level.[192] Possible technical errors which might produce such a difference could include a difference in "excess" zero-time urinary excretion in recumbent and upright subjects. This might occur as a result of slower mixing in the recumbent individual producing a higher blood level of S35 for a longer period, hence a greater urinary excretion, and an apparent increase in the sulfate space. Although a separate study was not done to rule this out, the values for urinary excretion obtained by other authors using recumbent subjects were similar to those obtained by the author using upright subjects, and, in a few cases only, recumbent subjects. Another possibility

is that the rate of entry of S35 into cells is different in recumbent and upright, mildly active subjects. One might have considered doing the sulfate space in recumbent subjects only, but then the problem arises as to how long the patient should be recumbent before injection; also this alters the condition of activity during the day.

If these two possibilities be disregarded, then the significant difference between day and night values suggests that under conditions of day activity, night recumbency, the extracellular fluid volume rises at night and falls during the day. This is the opposite to what one would expect if the ECFV were directly related to aldosterone secretion which is known to be higher during the day than at night (65, 66, 67).

If the ECFV were the controlling factor then possibly the rise in aldosterone during the day might occur in response to a falling ECFV, the drop at night to a rising ECFV, the aldosterone tending to offset the ECF changes and maintain a more constant ECFV.

If the Na balance were the controlling factor, one might expect that there would be a correlation between sulfate space change and sodium balance but no such correlation was found (Fig. 16a).

Possibly the lag of Na excretion during the day (59) which occurs in many individuals getting exercise during the day is due to aldosterone secretion in response to activity, and the natriuresis which occurs on lying down is due to the decrease in aldosterone secretion occurring in recumbency; but it is difficult to relate such changes to the observed diurnal variation in sulfate space. If the difference between day and night values of sulfate space is due to activity rather than to a true diurnal rhythm, then such results are in contrast to those of Collumbine and Koch (191) who found that the thiocyanate space increased with exercise while the plasma volume decreased with exercise.

d) Six-hourly diet, uniform activity:

(i) N.W.: No balance data were obtained. The highest sulfate space value occurred at 6 a.m., the lowest at noon but these values were not significantly different from the mean.

(ii) J.K.: No K or Na rhythm was evident. Greatest water excretion occurred from noon to 6 p.m., lowest from 6 a.m. to noon. Sulfate space value was highest at midnight, lowest at noon.

Little can be concluded from these two experiments. The study on J.K. suggests that significant changes may occur in the sulfate space value without activity; however this is based on only one point.

e) Six-hourly diet, prolonged bed rest:

(i) N.B.: The first two (control) sulfate spaces compared well with previous values under similar conditions (c - ii). There was little change in the first day of bed rest, but on the second day the values were significantly lower than control values and had not returned to normal four hours after getting up.

Whole blood, red blood cell mass, and plasma volumes all fell with prolonged bed rest, returned to normal four hours after getting up, and excee ded the normal values 16 hours after getting up. Hematocrits altered accordingly.

Na excretion exceeded intake during the period of bed rest, and, unaccountably, before it. Retention of Na, water and Cl occurred when the subject got up after the prolonged bed rest. No significant changes occurred in the serum electrolytes.

(ii) K.M.: Body weight fell during the first two days of the study, then remained constant during and after the period of prolonged bed rest.

Sulfate space values remained in the normal range during the first and second days of bed rest, then fell on the third day and on the first day after getting up. Thus the response seemed to be somewhat like that of N.B. but delayed.

Blood volumes rose slightly on the second day of bed rest, fell back to normal on the third day, and rose again after the subject got up.

Retention of water occurred on the first and second days of bed rest, and after getting up. Na and Cl retention occurred on getting up. There was no significant change in serum electrolytes.

(iii) V.Y.: Body weight remained constant throughout the study. Sulfate spaces were constant throughout except for the first and last values which were taken when the patient was up and active. The third value should have corresponded to the first but contrary to instructions the patient had lain down much of the morning and had been lying down for 30 minutes at the time of injection.

Hematocrits corresponded fairly well with sulfate spaces, the two highest values corresponding to the two lowest sulfate spaces.

There was no discernible rhythm of water excretion. No changes in electrolyte balance or serum electrolytes occurred throughout the study.

It is difficult to interpret the results of these three studies since only one of the three subjects was truly normal and the results are different in each. However in the two cases where there was a drop in ECF with prolonged bed rest, there was a definite water, Na and Cl retention when the subject got up. In the third case the balance study was not continued long enough to tell whether this might occur or not.

Blood volumes in both the first two cases rose above the control level 16 hours after the subject got up. The results of the blood volumes in N.B. correspond to some extent with the results of Widdowson and

McCance (70), who found that protracted recumbency had the opposite effect of short-term recumbency, i.e. it decreased the plasma volume instead of increasing it. Possibly this is true of the extracellular fluid volume as well, i.e. that with short term recumbency it rises but with prolonged bed rest it falls. However these results are too meager to draw any conclusions, and do not answer the question as to whether the rise in sulfate space at night might be due to the recumbent position or lack of activity.

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SUMMARY

1. The fate of injected radiosulfate was investigated and the work of other authors confirmed regarding plasma disappearance rates, appearance of S35 in gastric juice and urine. Appearance in the bile was shown to be very rapid. Since plasma activity was not altered by prolonged contact with red blood cells up to 24 hours, it was concluded that equilibrium with red blood cells was established within 20 minutes. Radiosulfate was found to become associated rapidly with serum protein, rendering its separation by protein precipitation methods difficult.

2. Ingested radiosulfate was shown to be rapidly and completely absorbed, peak blood level occurring at about one hour.

3. The sulfate space method was established, its sources of error assessed, and the probable error estimated to be 5%. Values among subjects with no abnormality of water or electrolyte balance gave a mean of 17.5 $\pm$ 1.3% body weight with a range of 13.5 to 22.0% body weight. Values among obese subjects averaged 12.8% body weight.

   When values for sulfate space were compared in the same individual under conditions similar with respect to diet, activity, and time of day, the coefficient of variation was calculated to be 5.66.

4. In two long-term balance studies, sulfate spaces were found to correlate fairly well with changes in body weight and balance data. In diurnal studies, sulfate space values were consistent with balance data except in studies of prolonged bed rest.

5. When intake was divided equally over the 24-hour period, and activity was normal, day values of the sulfate space averaged 7.5% lower than the

night values. This difference was significant at the 5% level. The possible importance of position in producing a diurnal fluctuation in extracellular fluid volume was not clarified by three studies of prolonged bed rest in which inconsistent results were obtained.

6. It was concluded that under conditions of uniform intake and normal activity there was a diurnal variation of sulfate space, higher values occurring at night, and that this represents a diurnal variation in extracellular fluid volume.

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