EXPERIMENTAL AND CLINICAL STUDIES ON

TOTAL BODY WATER AND WHOLE BODY POTASSIUM

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PROLOGUE

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In recent years the distribution of urea and creatinine in the human body has acquired clinical importance with the development of haemodialysis in the treatment of renal failure. Measurement of their volumes of distribution would allow the total body load of these metabolites to be calculated and a more accurate estimate to be made of their removal during haemodialysis. Although urea dilution has been established for many years as a method for measurement of the total body water, the reports on its volume of distribution, and that of creatinine, have been conflicting and only a few attempts have been made to compare them with the total body water as measured by the modern chemical and isotopic tracers.

In the present series of studies, tritiated water was first developed as a suitable method in our hands for measurement of total body water. Particular attention was paid to the use of a low dosage of tritiated water, thus allowing repeated estimations in the same individual, and to its preparation for intravenous injection. In the next study the rates and volumes of distribution of the endogenous metabolites, urea and creatinine, were compared with those of the more generally accepted tracer substances, N-acetyl 4-amino phenazone and tritiated water. However, the presence of normal renal function complicates the measurement of the volumes of distribution of endogenous metabolites and more accurate observations can be made on the nephrectomized animal or the oliguric patient.

In these experiments the nephrectomized dog was shown to be a suitable preparation. From the information obtained by the studies on the experimental animal, a third series of investigations was undertaken. In these, an attempt was made to measure the total body water of the patient in renal failure by the extraction of urea and creatinine at haemodialysis and to assess the value of this in the management of the patient. The results obtained were monitored against the total body water as measured by N-acetyl 4-amino phenazone and tritiated water.

With the measurement of total body water by the dilution of tritiated water established as a routine procedure, the next investigation was directed to the measurement of whole body potassium using the total body water as a parameter. The techniques of whole body counting were pioneered in the Medical Physics Department of the University of Leeds and thus apparatus of a type not usually found in hospitals was available. It was the intention to develop this apparatus as a clinical tool in the investigation of the potassium deficient patient. The problems of calibration are fundamental to whole body potassium measurements and these were examined first. Particular attention was paid to the advantages and disadvantages of the various methods of calibration in relation to their use in clinical studies on patients. Thereafter base-line values were obtained for a group of normal males and the studies were then extended to the potassium deficient patient, especially

patients suffering from potassium deficiency associated with uretero-colic anastomosis. As the measurement of total body water by tritiated water is a time-consuming procedure, the studies on whole body potassium were limited to males. Future work will extend to females. Every attempt has been made to explain and discuss whole body potassium counting in terms understandable to the clinician, rather than to present a rigorous discourse of the physical principles involved.

This thesis is presented in the order of the logical steps of investigation as described above.

I. THE MEASUREMENT OF TOTAL BODY WATER

I. 1. THE MEASUREMENT OF TOTAL BODY WATER

I. 1. Historical Review:

In the second half of the 19th Century the concept of body measurement dominated the work of the German anatomists. Until recently, the data from this period were all that was known about the gross composition of the human body. Credit for the earliest estimation of total body water is usually given to Bischoff (1863). He investigated the relative and absolute weight of various parts of the human body in six cadavers, ranging from a premature stillborn infant to an adult man. Desiccation procedures were used to study the water content of two of the subjects, one a 33 year old man executed by the guillotine and the other a female neonate. Bischoff concluded that the whole body comprised 58.5 per cent water and 41.5 per cent solid matter - values which are still acceptable.

The measurement of body water by desiccation is laborious and cadavers in which the water content has not been affected by the mode of death, are difficult to obtain. The method is suitable for tissue samples and small animal experiments, but obviously has no clinical application. The few published results are summarized in Table 1. Only the 46 year old male who died a week after a cerebral injury can be considered as reasonably normal.

The use of the dilution technique in the measurement of total body water in living organisms was first suggested by observations on the rate of turnover of water with deuterium as a tracer substance (Hevesy and Hofer, 1934; McDougall, Verzar, Erlenmeyer and Gaertner, 1934).

Table 1: Measurement of Body Water Content from Direct Analyses of Cadavers

Author	Year	Age	Sex	Weight (kg)	Body Water % Total Weight	Body Water % Fat-Free Weight	Mode of Death
Bischoff	1863	33	M	69.7	58.5	-	Guillotine
Mitchell et al.	1945	35	M	70.6	67.9	77.6	Decompensated mitral valve disease.
Widdowson and McCance	1951	42	F	45.1	56.6	73.2	Drowning
"		25	M	71.8	61.8	72.6	Uraemia
		48	M	63.8	81.5	82.4	Infective endocarditis with gross oedema
Forbes et al.	1956	46	M	53.8	55.1	68.4	Cerebral injury.
"		60	M	73.5	51.4	70.4	Unknown ? coronary artery disease

Since then many substances have been used as test solutes, including urea, ^{15}N-labelled urea, creatinine, sulphanilamide, antipyrine and N-acetyl 4-amino phenazone (N.A.A.P.) as well as the isotopic tracers, deuterium and tritiated water. The values obtained with the last four substances are generally accepted as measures of total body water.

Urea was noted to be widely distributed in the body by Rees (1850). Marshall and Davies (1914) found the concentrations in whole blood, body tissues and viscera were similar and Leifer, Roth and Hemplemann (1948) confirmed these results using ^{14}C-labelled urea. Panter (1940) found a significant correlation between the volumes of distribution of urea, sulphanilamide and the body water measured by desiccation in dogs. Urea infusion was used by Steffensen (1947), McCance and Widdowson (1951) and Pawan (1956) and ^{15}N-labelled urea by San Pietro and Rittenberg (1955) to measure the body water of humans. While the urea method produced satisfactory results there is biochemical evidence that urea may be bound to protein in the body (Ralls, 1943; Pasynskii and Chernyak, 1950; Murdaugh and Doyle, 1961). Clinical studies during haemodialysis in the treatment of renal failure suggested that in some patients a proportion of the urea was bound and non-diffusible (Blackmore and Elder, 1961; Shackman, Chisholm, Holden and Pigott, 1962). The validity of the results obtained by urea and ^{15}N-labelled urea were reviewed by Keys and Brozek (1953).

The volume of distribution of creatinine has been variably reported as 37 per cent of body weight (Sapirstein, Vidt, Mandel and Hanusek, 1955) 48 per cent (Greenberg, Schwartz, Spinner, Silver and Starr, 1952) and 63 per cent (Dominguez, Goldblatt and Pomerene, 1937).

Edwards (1959) reported the distribution of creatinine in the body water phase in ureter-ligated rabbits when compared with measurement of body water by antipyrine dilution and by desiccation and Schloerb (1960) found similar volumes of distribution for creatinine, urea and tritiated water in nephrectomized dogs. Although urea has been superseded by modern tracers in the measurement of body water, its volume of distribution and that of creatinine have become important in the treatment of renal failure by haemodialysis.

Antipyrine, N-acetyl 4-amino phenazone (N.A.A.P.), deuterium and tritiated water are generally accepted as suitable indicators for body water measurement. Antipyrine, an antipyretic drug, was first used by Soberman, Brodie, Levy, Axelrod, Hollander and Steele (1949) to measure body water. More recently, radio-iodinated antipyrine (Talso, Lahr, Spafford, Ferenzi and Jackson, 1955) and amino-antipyreme (Huckabee, 1956) have been introduced. N.A.A.P. was used by Brodie, Berger, Axelrod, Dunning, Porosowska and Steele (1951) and unlike antipyrine which may be bound to proteins by an appreciable extent, N.A.A.P. is almost free from this theoretical disadvantage.

Deuterium, discovered by Urey in 1931, was suggested by Hevesy and Hofer (1934) as an ideal test substance to estimate body water on the dilution principle. The falling drop method for the estimation of deuterium was developed by Schloerb, Friis-Hansen, Edelman, Shelden and Moore (1951) and has been widely used. Tritium was discovered by Alvarez and Cornog (1939) and Pace, Kline, Shachman and Marfenist (1947) introduced it as a tracer for body water. It is a weak

beta-emitter and its assay presents technical problems. The development of liquid scintillation counting (Langham, Eversole, Hayes and Trujillo, 1956) made possible the assay of a large number of samples though the apparatus required is expensive. Isotopic tracers have the disadvantage that the isotope label exchanges with labile hydrogen in organic compounds in the body. Detailed comparison of the chemical and isotopic tracers is discussed later.

Apart from desiccation or the dilution technique, two other methods are available for the measurement of body water. Firstly, Lavietes, D'Escpo and Harrison (1935) used balance studies of electrolytes and water. Secondly, the body water may be calculated from the total body fat. The relationship of lean tissue to water is fairly constant within the body and the corollary also applies - namely that the amount of non-aqueous tissue in the body (fat) varies inversely with the total volume of body water. Analysing the carcasses of shaved eviscerated guinea pigs, Rathbun and Pace (1945) found an experimental formula relating excess fat percentage to total body density.

$$\%F = 100 \left(\frac{5.548}{D} - 5.044\right) \qquad (1)$$

where %F is fat as a percentage of total body weight and D is total body density in g/ml. Using body densitometry, antipyrine and the Rathbun-Pace formula, total body water can be correlated with body specific gravity and a regression formula obtained (Osserman, Pitts, Welham and Behnke, 1950).

$$\%TBW = 100 \left(4.317 - \frac{3.260}{Sp.Gr.}\right) \qquad (2)$$

where %TBW is total body water as a percentage of total body weight.

Thus body water in man with a normal degree of hydration can be estimated by Body densitometry. The uncertainties involved in such an indirect estimation of total body water makes its accuracy less than direct estimation by the dilution technique. Body densitometry involves weighing the subject in air and again totally submerged in water, and is therefore unsuitable for clinical use.

Of the various methods of body water measurement which have been discussed, the dilution technique using antipyrine, N.A.A.P., deuterium or tritiated water is the most suitable for clinical studies. Desiccation is inapplicable, water balance is prolonged and not suited to changing states of hydration and body densitometry with underwater weighing is useful in physiological experiments but cannot be applied to the diseased patient.

I. 2. Anatomical Distribution of Body Water:

Water constitutes about two-thirds of the total body weight of mammals. It is the medium in which biochemical reactions take place and deviation from the normal is associated with disturbances of physiological function. In the living organism it is usually thought that water is not free and unattached, but as water of hydration it forms part of complex ionic equilibria in cell substance and body fluids; much of the water probably exists in loose combination as a gel. The total quantity of water in the body is related to the total quantity of cations, especially sodium and potassium, which in their turn govern the total content of anions and their associated water.

Anatomically, the body water can be divided into extracellular and intracellular fluid compartments on the basis of their relationship to the cell membrane. The extracellular fluid is about 28 per cent of the total body water and forms the environment of the cells. It comprises plasma, interstitial fluid, lymph and several confined fluid compartments such as the cerebro-spinal fluid, synovial fluid and aqueous humour as well as fluid in serious cavities. The total volume of fluid in these confined compartments is small in health. The fluid in the gastro-intestinal tract is often considered as being outside the body and not strictly a part of the extracellular fluid. Edelman, Olney, James, Brooks and Moore (1952) used the term "transcellular fluid" for the fluids in the body cavities and the gastro-intestinal secretions. The volume of the extracellular fluid can be measured by the dilution of a substance which passes freely through the walls of the blood capillaries but does not enter freely into the cells of the body. Different results are obtained in the measurement of extracellular fluid depending on the test substance used, for these vary in their penetration of the interstitial tissues found in dense connective tissue, bone and cartilage (Edelman and Leibman, 1959). Some substances (like thiocyanate) can penetrate this connective tissue but also enter the intracellular compartment to some extent; other substances (like inulin) do not enter cells but are slow to penetrate connective tissue and some injected material is excreted before equilibrium is reached.

Intracellular water is about 70 per cent of total body water and is associated with potassium, the most important intracellular cation, and with phosphate, the main intracellular anion. The volume of the intracellular fluid can only be measured indirectly, as the difference between the total body water and extracellular fluid.

The fluid exchange between adjacent body compartments is rapid and the measured volume of these compartments by the volume of distribution of a test substance, represents a steady state rather than a static figure. While a two-compartment body fluid system exists anatomically, Schloerb (1960) emphasized that many variable factors, such as different blood flow rates in different organs and even at different sites with the same organ, lead to an almost infinite number of intracellular and extracellular fluid units. It follows that the plasma disappearance curve of any injected substance represents an infinite number of intercompartmental movements which cannot be resolved mathematically into the two-compartmental, anatomical body fluid. The final volume of distribution of a substance represents the average of these infinite number of body fluid units of varying concentrations.

I. 3. Measurement of Body Water by the Dilution Technique:

(a) Basic Concept: Dilution techniques are used to measure the volume of the body fluid compartments in clinical studies. A known quantity, Q, of a test substance or indicator is introduced into a fluid compartment the volume, V, of which is to be measured. After uniform distribution a sample is taken to measure the concentration, C. of the test substance. If the test substance is neither formed nor

destroyed in the body then the quantity administered equals the product of its concentration in the fluid compartment and the volume of the latter:

$$Q = C \times V$$

$$\text{or} \qquad V = Q/C \tag{3}$$

In the application of this formula it is assumed that the volume of the test substance administered, Vs, is negligible when compared with that to be measured, V. If not, then the increment of the latter Vs must be considered and the equation used is

$$V = (Q/C) - Vs \tag{4}$$

(b) Requirements for Test Substances: Test substances used to measure body fluid volumes should themselves have no influence on the volume of the fluid compartment being measured. Also, they should be distributed rapidly and uniformly throughout the compartment being measured, be non-toxic and readily assayed quantitatively. Corrections must be made if the test substance is formed in the body or if it is lost from the fluid compartment by diffusion, excretion or metabolic alteration. Blank determinations are necessary if the indicator is also a natural constituent of the plasma or it its method of analysis is not specific. If the indicator is lost from the fluid compartment and excreted in the urine then the total amount excreted must be measured and subtracted from the total amount given. In the extrapolation method, which can be used to correct for an exponential loss from a fluid compartment, the logarithm of the concentration is plotted against time and when the linear portion of

this curve is extrapolated to zero time, the intercept on the concentration axis gives the concentration which would have existed if there were no loss.

(c) Chemical Tracers: Several test substances have been used in clinical studies to measure total body water. These include urea, thiourea and sulphanilamide. (I.l.p.2). Antipyrine and N.A.A.P. are more commonly used and generally accepted as suitable indicators for body water measurement.

Antipyrine, originally used as an antipyretic and analgesic, was introduced by Soberman et al. (1949) to measure body water. It is freely soluble in water and reacts with sodium nitrite to form 4-nitroso-antipyrine, this reaction being the basis of its chemical determination and spectrophotometric assay. About 10 per cent of it is bound to plasma proteins but any error this introduces is apparently balanced by binding with tissue proteins (Soberman, et al. 1949), though this may not be true in abnormal states. It has been found to penetrate the gastro-intestinal lumen. Although antipyrine is metabolized slowly in man an extrapolation technique is still required. In the dog it is more rapidly metabolized. It is relatively non-toxic, can be accurately determined in plasma and measured values for total body water agree with those obtained by desiccation (Soberman, 1950). In oedematous subjects, Soberman et al. (1949) found that the total body water as measured with antipyrine was usually smaller than the value obtained with isotopic tracers.

Part of this difference may be due to the slower rate of diffusion of antipyrine with failure to obtain uniform mixing.

N.A.A.P. was first used by Brodie et al. (1951) to measure total body water. It can be hydrolyzed to form 4-amino-antipyrine. When the latter has been diazotized and coupled with alpha naphthol, the dye formed can be assayed in a spectrophotometer. In dog tissues N.A.A.P. has been found to be distributed in proportion to the water content with negligible binding to plasma proteins (Brodie and Axelrod, 1950). Reid, Balch and Glascock (1958) found that N.A.A.P. was slow to enter the gastro-intestinal water of rabbits. They reported that total body water measured with N.A.A.P. by the extrapolation technique agreed with that obtained by desiccation of the animal body after removal of gastro-intestinal content, while antipyrine and tritiated water gave results which agreed with those of the whole animal, including the gastro-intestinal content. Reid, Balch and Head (1957) had performed similar experiments with cattle. Thus, it appeared that the difference in the volumes of distribution of N.A.A.P. and antipyrine provided an estimation of the water content of the gastro-intestinal tract. However, Whiting, Balch and Campling (1960) also compared these volumes of distribution in cattle and found that the difference was not a reliable indication. In twelve human subjects, the average total body water volume measured by N.A.A.P. was 1.9 litres less than that with antipyrine (Brodie et al. (1951) and Prentice, Siri, Berlin, Hyde, Parsons, Joiner and

Lawrence (1952) found a difference of about 3 - 4 per cent of body weight when they compared it with the volume of distribution of tritiated water.

Antipyrine and N.A.A.P. are reliable test substances for the measurement of body water but the latter is slower to reach its volume of distribution and is thus not suitable for subjects with abnormal fluid depots.

(d) Isotopic Tracers: Deuterium, D or ^{2}H, is a stable isotope of hydrogen with approximately twice the mass of H. Deuterium oxide, or heavy water, is about 10 per cent heavier than ordinary water. Deuterium may be measured by mass spectrometry or, more commonly, by the falling drop method which depends on its physical difference from ordinary water. The exchange of deuterium, and tritium, with labile hydrogen ions in body tissues leads to slight over-estimation of body water. Deuterium oxide equilibrates rapidly except in cases of abnormal fluid accumulation when a prolonged sampling time is required. Nevertheless, it is still a better test substance than either antipyrine or N.A.A.P. under such abnormal conditions. It enters the gastro-intestinal tract and is non-toxic in the quantities used.

Tritium oxide was first used by Pace et al. (1947) to measure total body water. Tritium, T or ^{3}H, is a radioactive isotope of hydrogen with approximately three times the mass of H. In the investigations to be described, the dilution of tritiated water was

developed as a method for measurement of body water in experimental and clinical studies. In particular, an attempt was made to obtain accurate measurements of body water using small quantities of the tritiated water in order that repeated measurements could be made on an individual within the permitted dose. Its physical properties and the problems associated with its use in clinical medicine are desribed in detail.

(e) Intercomparison of Test Substances: Numerous intercomparisons of the results obtained with the various test substances used for measurement of body water have been made and the subject was reviewed by Keys and Brozek (1953) and Chien and Gregersen (1962). In animal experiments the results obtained with some test substances have been compared with those obtained by direct measurement of body water by dessication. The isotopic tracers exchange with the hydrogen on organic molecules and thus have a larger volume of distribution than the chemical tracers. It was reported that N.A.A.P. had a volume of distribution about 3 - 4 per cent of body weight less than that of tritiated water (Prentice et al. 1952) and a similar result was found by Faller, Petty, Last, Pascale and Bond (1955) using antipyrine and deuterium oxide.

The volumes of distribution of urea and creatinine have became important clinically in the treatment of renal failure by haemodialysis (I.5.p.17) but few attempts have been made to compare these volumes with those of the body water as measured by the accepted methods of the modern chemical and isotopic test substances.

Such comparisons as have been made (Edwards 1959, Schloerb 1960, Bradbury 1961) have not been in complete agreement and this, together with the considerable biochemical and clinical evidence that urea may not be distributed through the total body water, suggest that further studies on these comparisons are required. This subject is discussed in detail in Chapter IV.

I. 4. Parameters for Total Body Water:

It is desirable to compare the measured body water of a subject with normal values. A direct comparison of absolute values is of little significance as there are considerable individual variations in body size and the body water of an individual has a marked dependence on body size. Therefore, if the body water is normalized in terms of body size a better comparison among individuals will be obtained and deviation from the normal more readily detected.

(a) Body Weight: Body weight is the parameter most commonly used to express body size. Although body water described in terms of body weight reduces the range of normal values, it is still far from an ideal parameter. The water content of adipose tissue is low (about 20 per cent) and with increasing obesity of an individual the gain of fluid is small in proportion to the gain in body weight (Keys and Brozek 1953). Therefore, a fat subject has a smaller amount of body water per unit of body weight than a thin person. Similarly, women generally have more fat and less body water per unit body weight than men (Osserman et al.1950; and Allen, Welch, Trujillo and Roberts 1959).

(b) Lean Body Mass: To eliminate the effect of the leanness/fatness ratio on the expression of body water, other parameters have been suggested. The lean body mass is defined as the body weight less all except indispensible fat (Behnke,1943). From an analysis of body composition in a large number of men, Behnke, Feen and Welham (1942) postulated a basic lean body mass of relatively constant composition which comprised 70 per cent. water, 20 per cent solids and an irreducible minimum of 10 per cent fat, though more recently a value of 2 per cent indispensible fat has been suggested (Behnke, Osserman and Welham 1953). It has been demonstrated that this type of constant composition is not peculiar to the human species and that in several species of small animals, although the fat content varied widely, the lean body mass was fairly constant. In chemical analysis of shaved, eviscerated guinea pigs, Pace and Rathbun (1945) found that water accounted for a mean of 72.42 per cent (standard deviation = 2.11 per cent) of the fat-free mass. Osserman et al. (1950) reported that the mean percentage of water in the lean body mass of man was 71.8 per cent with a standard deviation of 2.99 per cent, a result which leads to the equation:-

$$\%TBW = 0.718\ (100 - \%F) \qquad (5)$$

where %TBW is total body water as a percentage of total body weight, and %F is body fat as a percentage of total body weight. Since (100 - %F) is the lean body mass, the equation can be written:

$$LBM = \frac{0.718 \times \text{Total body water}}{100} \qquad (6)$$

Formulae (5) and (6) have been used extensively in clinical studies to estimate the lean body mass from the more readily measured total body water. However, they can only be applied when the state of hydration is normal.

Distinction should be made between the terms "body fat" and "adipose tissue". Body fat (or lipid) refers to the chemical fat in the body, whereas adipose tissue (or anatomical fat) is lipid plus the water and solids associated with it as cellular connective tissue. Adipose tissue contains about 20 per cent water and lean tissue about 70 per cent water.

The lean body mass can also be estimated from measurement of body fat which is then subtracted from body weight. When the total body weight is divided into two components of different densities, namely lean body mass and excess fat, the density of the whole body will be determined by the relative proportion of the two components. If the fat content of the body increases, the average density of that body decreases. By weighing a body in air and when submerged in water, its specific gravity can be directly measured and, assuming a constant composition and density for the lean body mass, the fat content of the body can be calculated. This densitometry is unsuitable for clinical studies and the variable quantity of residual air in the lungs during submerged weighing produces inaccuracies (Brozek 1962). Body fat can also be calculated from measurement of skinfold thickness at multiple sites in the body if the subject is in a healthy state of nutrition and hydration.

(c) Variations in the Concept of Lean Body Mass: The definition of lean body mass includes indispensable fat of uncertain quantity and Keys and Brozek (1953) suggested that the fat-free body should be used instead in densitometric experiments. Allen, Peng, Chen, Huang, Chang and Fang (1956) derived formulae for the essential body mass representing the difference between total body weight and total adipose tissue. Division of the body into fat and lean tissue is an over-simplification and takes no account of the skeletal mass of the body. Allen et al. (1959) combined body densitometry, bone mineral estimation by anthropological measurement and body water measured by the isotopic tracers, to produce equations for the estimation of body fat and total tissue solids.

(d) Potassium: The correlation between the total body water and the cellular mass of the body has been discussed (I.A.e.p. and as 98 per cent of the body potassium is intracellular a high correlation would be expected between it and body water. Crooks, Bluhm and Muldowney (1959) correlated exchangeable body potassium with the total body water. Talso, Miller, Carbello and Vasquez (1960) compared the values for exchangeable potassium with other parameters of body composition, including body weight, body surface area, total body water, lean body mass, fat-free body solids and 24-hour creatinine excretion. Coefficients of correlation between the values for exchangeable potassium and these parameters were highest for total body water, lean body mass and body solids. Allen, Anderson and

Langham (1960) showed that the whole body potassium estimated by gamma ray spectrometry, was proportional to the body mass minus bone mineral, fat and water and that this in turn correlated with the total body water.

I. 5. The Importance of the Volumes of Distribution of Urea and Creatinine in Renal Failure.

The volumes of distribution of urea and creatinine have acquired clinical importance with the development of haemodialysis in the treatment of renal failure. Reduction in the plasma concentration of these substances by dialysis depends on the total load present (plasma water concentration multiplied by the volume of distribution) and this varies widely among patients. Attention has been drawn to the few comparisons that have been made between the volumes of distribution of urea and creatinine and those of the modern test substances, to the lack of agreement obtained, and to the biochemical and clinical evidence that urea may not be freely diffusible throughout the body water in some circumstances. Information on the volume of distribution of these metabolites in renal failure would allow the total body load carried by the patient to be calculated and a more accurate prediction made of the results of dialysis assessed in terms of fall in the plasma urea and creatinine concentrations.

Assuming urea and creatinine to be distributed in the total body water, if the volume of the latter is small the total load of these metabolites in renal failure will be correspondingly small. In such

circumstances, a shorter dialysis may be adequate to lower these plasma concentrations and the patient thus saved from a more prolonged and exhausting dialysis. However, while a shorter dialysis may be sufficient for removal of urea and creatinine, the same is not necessarily true for those metabolites which distribute more slowly in the body. Again assuming the distribution of urea and creatinine in the body water phase, it may be possible to estimate the total body water from the fall in plasma water concentration of urea and creatinine during haemodialysis and the amount of these substances removed by the dialysing bath fluids.

Summary: The various methods of measurement of total body water have been reviewed, the anatomical distribution of body water has been described and its measurement by various test substances has been discussed. There is evidence that further investigation into the volumes of distribution of urea and creatinine are indicated, particularly with reference to the modern test substances. Helpful information for the treatment of the patient in renal failure may be gained from a study of total body water and its measurement during haemodialysis.

II. THE USE OF TRITIUM IN THE MEASUREMENT OF TOTAL BODY WATER

II. The Use of Tritium in the Measurement of Total Body Water

II. 1. Introduction:

Tritium, the radioactive isotope of hydrogen of mass three, was discovered by Alvarez and Cornog (1939) and has been developed as a tracer for hydrogen in biological experimentation. Pace and co-workers (1947) introduced it as a tracer for body water. Langham et al. (1956) developed the technique of liquid scintillation counting of tritium and Vaughan and Boling (1961) described the vacuum distillation of plasma samples to obtain tritiated plasma water. Using the principles described by these workers, tritiated water has been used by many investigators to measure body water of human beings and experimental animals.

Although the principles of liquid scintillation counting of tritium are well-established, considerable research work must be done by the individual investigator to obtain optimum results from the particular type of counting apparatus used. At the start of the present study there was no published report available to provide details on the use of our apparatus. This basic research problem was solved by Mr. A. H. Smith, Department of Medical Physics, University of Leeds. After he established the necessary physical data, it was then our object:-

(1) to prepare tritiated water for intravenous administration in order to measure the body water content of patients with both normal and abnormal states of hydration. In

particular, an attempt was made to obtain accurate measurements using small quantities of tritiated water in order that repeated measurements could be made on one individual within the permitted dose.

(ii) to compare these results with those of other tracers used for measurement of body water

(iii) to examine the total body water in relation to haemodialysis in the treatment of renal failure.

In the present section the theoretical aspects of the use of tritium are discussed, followed by a description of the materials and apparatus used. Experimental results relating to the use of tritium are described in Section III.

II. 2. Properties:

Tritium has a physical half-life of 12.4 years and decays to helium^3 with emission of beta-particles of about 18 keV maximum energy. No gamma radiation is emitted and consequently it presents no radiological working hazard as long as it is prevented from entering the body. The biological half-life of tritium in the body at normal rates of water intake is 8 - 14 days for man (Schloerb et al. 1950) with an average about 10 days. Experiments using tritium are controlled in the United Kingdom by the Medical Research Council who permit a maximal dosage of 1 mC in human subjects.

II. 3. Terminology Applied to Protium (hydrogen), Deuterium and Tritium:

Technically the terms deuterium and tritium refer only to hydrogen isotopes and not to water molecules, though they are often used interchangeably. The use of the terms DHO, THO, deuterium oxide (D_2O) and tritiated water may lead to confusion. Experimentally, deuterium oxide (D_2O) is actually injected and after dilution in water or body fluid it becomes deuteriated water (DHO). However, only a tracer quantity of tritium is present in the injected water and so tritiated water (THO) is being used throughout. In the present study, the term tritiated water will be used and THO will be used as an abbreviation or reserved for comparison with the formula T_2O.

II. 4. Isotope Effect of Tritium:

In tracer studies with tritium the possibility of an isotope effect must be considered for the mass of tritium is three times greater than that of protium and will affect equilibrium constants, distribution coefficients, diffusion rates, and binding. The effect of alteration of these characteristics in biological tracer experiments depends on the nature of the process being investigated. An isotope effect may be present when water is involved in metabolism and Sini and Evers (1962) reported that expired water vapour is subject to a large and unmistakable isotope effect. They found that in humans the specific activity of expired water vapour relative to that of urine and blood ranged from 0.78 to 0.96.

By contrast, they found that the respiratory apparatus of the pigeon was able to fractionate THO and H_2O with considerable efficiency reducing the relative specific activity to 50 per cent. Moore (1962) also found that breath vapour had a specific activity 88 to 98 per cent of that in plasma. It is unlikely that there is any isotope effect in the measurement of body water or water kinetics by tritium, or any effect that is present is not detectable. Numerous investigators have failed to find significant differences in the specific activity of tritium in blood, urine and other body fluids once equilibrium had occurred (Thomson and Ballou, 1953; Copper and Gibbons, 1960; Siri and Evers, 1962).

II, 5. Water Exchanges Within the Body:

When tritiated water is introduced into the body, tritium is diluted by exchange with H atoms in the body water and with the exchangeable H atoms in organic molecules. These latter include the hydrogen atoms in amino, carboxyl, hydroxyl, imino, sulphydryl and in other radicals where the hydrogen atom is not directly bound to a carbon atom. The readily exchangeable hydrogen atoms in the organic constituents of the body are estimated to correspond to a water equivalent of 0.5 to 2.0 per cent of the body weight in man (Hevesy and Jacobson, 1940; Schloerb et al. 1950). This result was calculated from assumed values of rapidly exchangeable hydrogen atoms in protein and carbohydrate; it was thought that the readily exchangeable hydrogen atoms in fat were negligible in amounts. In almost all reports giving the percentage of body weight

appearing as body water obtained from the dilution of tritiated water, the above equivalent has not been subtracted and the results reported are high to this extent. Udelavu, Kozoll and Meyer (1963) compensated for this error by extrapolation to zero time of their measured values.

Direct binding of hydrogen atoms to carbon atoms takes place very slowly as such atoms become labile during chemical reactions in the body metabolism. While these exchanges are important in metabolic studies they are too slow to have any effect in water balance experiments of short duration and can be ignored in such measurements.

Pinson and Langham (1957) showed that radiation hazard from tritium exchange with the hydrogen atoms of tissues is small compared with the amount of tritiated water which must be administered to induce that activity into tissue components. They found the highest tissue activity in brain, skin and muscle and the lowest in liver.

It is concluded that tritium exchange with hydrogen atoms of tissues is small and not a radiation hazard but it produces an over-estimation of body water by 0.5 to 2.0 per cent of body weight.

II. 6. Methods of Administration:

Tritiated water may be given orally or intravenously. It is rapidly absorbed following oral administration and the increase in concentration in the venous blood following ingestion is linear with time, equilibrium being obtained in 40 to 45 minutes (London and

Rittenberg, 1950; Pinson and Langham 1957). Prentice et al. (1952) quoted investigations by Race that show equilibrium of distribution of tritiated water in less than two hours following intravenous administration. This was confirmed by Schloerb (1960) who found equilibrium in one hour in the nephrectomized dog and by Udekwu et al. (1963) who reported equilibration in an average time of 90 minutes in man. Thus in patients with normal hydration, equilibrium should be shown by plasma samples taken 2 hours or more after administration of tritiated water.

In the present investigations tritiated water was given by intravenous injection. This route was chosen as many of the clinical studies were performed on patients in advanced uraemia who were often confused, uneo-operative or even comatose. Vomiting was not infrequent with these patients and some had uraemic intestinal ileus which might have affected the rate of absorption of tritium administered orally.

II. 7. Principles of Tritium Counting by Liquid Scintillation:

Tritium counting by liquid scintillator is the method of choice when there is a large number of samples and was used in the experiments to be described. The body fluid sample containing tritium is placed in a mirrored quartz ampoule along with dioxane-based scintillator and stored in darkness for at least 15 hours to minimize phosphorescence. For counting, the ampoule is placed over a photomultiplier tube specially selected for its low electronic noise. (Fig.1). The ampoule and the face of the photomultiplier tube are optically coupled by an oil-seal and both are enclosed in a 2" thick lead

shield which forms the Counting Head. Cold tap water is circulated in a cooling coil round the photomultiplier tube so that the temperature within the counting head is below room temperature and is maintained approximately constant. The reduction in temperature lowers the amount of electronic noise, the fluctuation of which is reduced by temperature stablization. Fig.2 shows a quartz ampoule, held in calipers, being placed within the counting head. Rotation and depression of the handle on top of the counting head moves the ampoule to its correct position for counting over the photomultiplier tube.

The beta-particles from the tritium contained in the ampoule are absorbed by the liquid scintillator and light photons are emitted. When a light photon strikes the photo-emissive surface of the photomultiplier tube, electrons are released and are multiplied in number by passage through the dynodes of the multiplier section of the tube. The group of electrons is finally collected at the anode where it is converted into a voltage pulse for subsequent amplification in a valve amplifier with a voltage gain about 10,000. The amplified voltage pulses are then analysed by a Pulse Height Selector (P.H.S.) and those between certain fixed pulse heights are counted to obtain optimum counting of pulses from tritium in the presence of background counts. By counting an aliquot of the activity of the administered solution of tritiated water after dilution, the principles of the dilution technique can be applied and the volume of distribution calculated.

II. C. Materials and Apparatus:

(a) Preparation of Injection Solutions: About 1 ml of the stock solution containing approximately 1 mC of tritium was vacuum distilled (Vaughan and Boling, 1961) and the pyrogen-free distillate added to 160 ml of 0.9 per cent sterile saline. This solution was filtered and 40 ml aliquots were measured into Pyrex ampoules. The ampoules were sealed by heat and immediately autoclaved at not less than 10 lbs/square inch for 30 minutes. The maximal radiation hazard from an accident in this technique was the liberation of 1 mC of tritium. As the breakage of an ampoule in the autoclave was the likliest accident, only two ampoules were autoclaved at one time.

Sealed Pyrex ampoules were used in preference to rubber stopped bottles ("Clinbritic") for in the latter, it was thought that small quantities of steam in the bottle might exchange with those in the autoclave (Bloom, 1962). Although when cooled the volume remained constant, some radioactivity could be lost during the process. Recently Smith (1963) has developed a copper container for "Clibritic" bottles. This has an airtight screw lid and the bottles are sterilised in an autoclave at a pressure of 10 lbs/square inch for 60 minutes. When cool, the copper containers are opened in a fume cupboard specially adapted for handling radioactive samples.

(b) Dosage: In the United Kingdom the use of radiation material with humans is controlled by the Medical Research Council. The maximal dose of tritium allowed for humans is 1 mC. In clinical

studies which involve changes in body composition, repeated measurements of body water are of value. A dose of 250 μC was chosen as experience showed that accurate results could be obtained by careful technique using this dosage and it was thus possible to repeat the measurement on four occasions on any one individual, provided a suitable time interval was allowed for the excretion of the previous dose of tritium. This varied with the water turnover of the patient but was usually about 20 days if fluid intake was high. Dosage was not correlated with body weight. At a dosage of 250 μc, the plasma sample distillate for assay (0.4 ml) gave about 700 counts per minute (c/m) for a total body water about 35 litres, against a background of 30-50 c/m. When a second measurement of body water was made while considerable activity was still present in the patient from a previous measurement, the dose was increased to 500 μC.

(c) Administration: Tritiated water was given by intravenous injection in doses of 250-500 μC in 40 ml 0.9 per cent saline from a 50 ml syringe which was weighed before and after injection. Loss of a few drops of solution during injection or the withdrawal of a few drops of blood to ensure venepuncture produced only minimal errors when the total solution injected from the syringe was 40 ml. About 1 ml of solution from which the injection was taken was retained to provide a standard solution against which to compare the radioactivity of blood samples. On a few occasions, tritiated water was given orally. A beaker was weighed empty and again when

the tritiated solution had been added. The patient drank the fluid through a straw and the beaker was rinsed three times with tap water, the rinsing fluid being sucked through the straw on each occasion. It was then assumed that all tritiated water present in the beaker had been imbibed.

(d) Blood Samples: After equilibration of the injected tritiated water, venous blood samples were taken and their tritium content assayed. In patients three samples were collected between two hours and six hours after intravenous administration of tritiated water (Prentice et al. 1952), with an interval of at least one hour between samples. In some patients, only two blood samples were taken. If abnormal fluid collections were present, three samples were taken between six hours and 24 hours with a minimal interval of three hours. Samples were collected in heparinised tubes, the plasma separated immediately and stored at 4°C until analysed.

(e) Vacuum Distillation: Following the technique described by Vaughan and Boling (1961), 1.5 ml of tritiated plasma was pipetted into a Thunberg flask, frozen in liquid nitrogen and placed under a vacuum for five minutes by a rotary oil pump (Fig.3). Maintaining the vacuum, the Thunberg flask was placed with its "beak" in liquid nitrogen contained in a wide-necked vacuum flask (Figs.4 and 5). Sublimation was complete in about 20 minutes after which the vacuum was released by allowing the entry of air dried by a calcium chloride column. After thawing, the distillate was stored at 4°C until

assayed. A diluted sample of the injection solution was similarly treated.

Vacuum distillation was also used in the preparation of the intravenous injection solution to remove pyrogens from the unsterile stock solution of tritium . (II. 8. a.p.27).

(f) Tritium Counting Apparatus and Technique: A Nuclear Enterprises (G.B.) Ltd. Tritium Counter (NE 8301) (Fig.6) was used with a dioxane-based scintillator fluid (NE 220). An aliquot of tritiated plasma distillate, dispensed gravimetrically, was added to the liquid scintillator in quartz ampoules. The activity of the tritiated plasma distillate was compared with those of the similarly prepared diluted samples of the injection solution of tritiated water. Each plasma sample was counted in duplicate in separate quartz ampoules and the mean result from all plasma samples was taken as the total body water.

One difficulty found was that the observed response to a given activity of tritiated water depended on the particular quartz ampoule used. The ampoules were therefore cross-calibrated to obtain correction factors.

A detailed description of the apparatus, counting technique and calibration of ampoules is given in Appendix 1. This was provided by Mr.A.H.Smith, Medical Physics Department, University of Leeds, who established the tritium counting procedure, in his research work, for the Nuclear Enterprises Ltd. apparatus.

III. EXPERIMENTAL RESULTS WITH TRITIATED WATER

III. EXPERIMENTAL RESULTS WITH TRITIATED WATER

III. I. Removal of Pyrogens by Vacuum Distillation:

A sample of the Proposed International Reference Preparation for Pyrogen (material from S.Marcenscens) was used in an experiment to show that pyrogens were removed by vacuum distillation. The pyrogenic material was dissolved in 3 ml sterile saline. One aliquot of 1 ml was vacuum distilled, diluted in 300 ml 0.9 per cent sterile, pyrogen-free saline and autoclaved as for the intravenous injection of tritiated water while another aliquot of 1 ml was similarly diluted and autoclaved but not vacuum distilled. Animals injected with the distilled solution showed no evidence of a pyrogen reaction while those injected with non-distilled solution developed an immediate pyrogen reaction and died[x].

It is concluded that vacuum distillation of unsterile tritiated water followed by autoclaving in sealed Pyrex glass ampoules provides a sterile pyrogen-free solution suitable for intravenous injection. No reaction has been noticed in over one hundred intravenous injections into patients.

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[x] The animals were injected by Dr.Shone and his staff at the Regional Transfusion Laboratory, Leeds 14, whose co-operation was appreciated.

III. 2. "In Vitro" Dilution Experiment:

In this experiment tritiated water was added to a known volume of tap water. After distribution of the tritiated water, samples were taken, assayed and the volume of distribution measured and compared with the known volume of tap water.

(a) Procedure: 264 μC of tritiated water were added to a carboy containing exactly 40 litres of tap water which was then stirred for a few minutes. After 24 hours a sample was taken from the fluid at the top of the carboy and another from the fluid at the bottom. One aliquot from each sample was vacuum distilled and prepared for assay while another aliquot was prepared without prior vacuum distillation. Duplicate or triplicate assays were made for each aliquot and the mean result calculated (Table 2). In the case of one particular aliquot, only one measurement was obtained. Further aliquots were prepared and a second measurement made two days later.

(b) Discussion: Mean results were similar to the 40 litres measured into the carboy and the errors ranged from an underestimate of 1.7 per cent to an overestimate of 2.7 per cent. There was no significant difference in samples taken from the top and from the bottom of the carboy and there was no evidence that vacuum distillation of the samples affected their tritium content. Although all the errors were positive in the second series of measurements, made 48 hours after the first series, the errors themselves were small and within the range of experimental error.

Table 2. Repeated measurement of Volume of Distribution (litres) of Tritiated Water in a Carboy containing 40 litres tap Water.

	Site of Sample	Sample Distilled	Measurements (l)	Mean (l)	Per cent Error
First Measurement	Top	Yes	39.7, 37.9, 40.3	39.3	-1.7
	Top	No	40.4	40.4	+1.0
	Bottom	Yes	38.9, 38.6, 41.0	39.5	-1.3
	Bottom	No	41.6, 40.5	41.1	+2.7
Second Measurement	Top	Yes	40.4, 39.7	40.1	+0.25
	Top	No	40.8 40.2	40.5	+1.3
	Bottom	Yes	40.5, 40.3	40.4	+1.0
	Bottom	No	40.5, 40.1	40.3	+0.8

There was an interval of 48 hours between the first and second measurements.

It is concluded that with an "in vitro" experiment, tritiated water could accurately measure a volume of fluid of 40 litres with a maximum error of 2.7 per cent. Vacuum distillation of the samples had no significant effect. The accuracy was reproducible with a second series of measurements.

III. 3. Precision of Measurement of Body Water by Dilution of Tritiated Water.

The accuracy of measurement of body water by tritiated water may be assessed in three ways. The technical accuracy of the apparatus used can be calculated, the activity of a series of tritiated plasma samples from one subject assayed on two separate occasions can be compared (duplicate assays) and the body water of a subject can be measured on two occasions separated by a time interval during which there has been no reason to expect a major change in body water content (repeated measurements).

(a) Technical Accuracy: Since radioactivity is a random process the accuracy of the assay depends in part upon the number of counts that have been accumulated. Allowing for this and for the error involved by the necessity of applying a correction factor to the quartz ampoules to establish them on a comparable basis, Smith (1963) calculated that our apparatus had a technical accuracy equivalent to a standard deviation of $\pm$ 2 per cent.

(b) Duplicated Assay: Two separate sets of counting samples were prepared from the same series of plasma samples for each of two

dogs and three adult males. Aliquots of the tritiated plasma samples were vacuum distilled, dispensed and counted on separate occasions (Table 3). The total body water was calculated as the mean value of two or three plasma samples, each sample having been measured in duplicate. The greatest discrepancy was 1.9 litres in a body water of 42.8 litres (subject J.A.) an error of 4.4 per cent. On this occasion there was reason to believe that a technical fault in the apparatus was present during the first measurement; this was remedied after only two plasma samples had been counted. Apart from this result duplicate measurements were reproducible within 0.7 per cent, which is within the technical accuracy previously described.

(c) Repeated Measurement of Total Body Water: In six subjects, five males and one female, the total body water was measured by the dilution of tritiated water on two or more occasions separated by an interval varying from two days to seven months. Subjects were chosen in whom there was no reason to believe that there had been any significant alteration in body composition or body water content. The results are shown in Table 4. Only small changes were found in body weight and total body water, and for the latter they ranged from a fall of 7.9 per cent to a gain of 5.7 per cent of the previous measured volume. The factors influencing this range of results are the technical accuracy of the apparatus, errors introduced in the dispensing and injecting of tritiated water, and true variation in the total body water content of the body.

From the results which have been described and from values obtained with 82 measurements of the total body water by the dilution of tritiated water, Smith (1963) calculated that our method had an overall experimental error equivalent to a standard deviation of ± 3 per cent. Repeated measurements of total body water in the same individual showed an average deviation about 4 per cent of total body water with antipyrine (Steele, Berger, Dunning and Brodie, 1950), about 4.6 per cent with N.A.A.P. (Brodie, 1951) and about 2.0 per cent with deuterium (Schloerb et al. 1950). The results of our experiments show that the dilution of tritiated water using the technique described gives reproducible measurements of the total body water within the experimental accuracy of the method and the true variation in water content of the body. The accuracy of the method compares favourably with the results reported for other test substances.

Table 3. Duplicate Assay of Total Body Water (litres) on Two Dogs and Three Adult Males, from Tritiated Plasma Samples Prepared and Counted Separately.

Subject	First Measurement of Three Plasma Samples			Mean Body Water (litres)	Second Measurement of Three Plasma Samples			Mean Body Water (litres)	Per cent Difference of Lesser Volume
	1	2	3		1	2	3		
Dog 63	18.2,	18.6,	19.0	18.6	18.0,	18.9,	-	18.5	0.5
Dog 64	19.0,	18.5,	18.3	18.6	-	18.8,	18.5	18.7	0.5
A.F.	39.8,	41.3,	43.0	41.4	41.5	41.7,	41.8	41.7	0.7
J.A.	42.7,	46.8	-	44.7	42.9,	43.0,	42.5	42.8	4.4
H.M.	30.2,	31.6,	30.9	30.9	30.5,	30.7,	31.4	30.9	0

The result for each plasma sample is the mean of a duplicate measurement.

Table 4. Repeated Measurement of Total Body Water in Humans[x] by Dilution of Tritiated Water.

Patient	Sex	Time Interval	Body Weight kg	Body Water l	Difference Body Weight kg(%)	Difference Body Water l(%)
A.T.	M	4 months	70.0	39.1		
			66.8	39.2	-3.2(4.6)	+0.1(0.3)
A.R.	M	7 months	54.6	35.6		
			51.8	36.9	-2.8(5.1)	+1.3(3.7)
J.L.	M	2 days	69.1	40.1		
			68.0	39.8	-1.1(1.6)	-0.3(0.8)
A.B.	M	6 days	56.0	33.1		
			54.0	34.2	-2.0(3.6)	+1.1(3.3)
H.B.	F	17 days	57.2	29.1		
		35 days	57.3	26.8	+0.1(0.2)	-2.3(7.9)
			56.2	26.3	-1.1(1.9)	-0.5(1.9)
J.C.	M	5 months	40.1	29.2		
			39.2	28.8	-0.9(2.2)	-0.4(1.4)

[x]Subjects were chosen in whom there was no reason to believe that there had been any significant change in body composition or body water content.

III. 4. Comparison of the Results of Total Body Water Calculated from Plasma and Urine Samples:

As many of the patients studied suffered from various renal diseases, including renal failure with oliguria, the volume of the body water was routinely calculated from the specific activity of tritiated water in several plasma samples and the mean result taken. In four subjects, three females and one male, without renal disease, "spot" urine samples were obtained at the same time as the plasma samples, after the bladder had been emptied one and a half hours following administration of tritiated water. The urine samples were prepared by vacuum distillation technique (II. 8. e.p.29) and the tritium content assayed. The mean volumes of total body water calculated from these urine samples were compared with those from plasma samples (Table 5). Although the urine samples gave a slightly larger total body water volume in three subjects, the difference is not significant. Siri and Evans (1962) reported no significant differences in the specific activity of tritium assayed in blood and urine in some 300 humans and many animal studies.

It was concluded that in patients without renal disease, "spot" urine samples were suitable for the measurement of total body water by the dilution of tritiated water, provided the bladder had been emptied after administration of the tritiated water and before the first sample was taken.

Table 5. Comparison of Results of Total Body Water, Measured by the Dilution of Tritiated Water, Calculated from the Mean Concentrations in Plasma and Urine Samples.

Patient	Sex	Diagnosis	Weight (kg)	Total Body Water (litres) Calculated from Plasma	Total Body Water (litres) Calculated from Urine	Plasma / Urine
C.H.	F	Normal	56.4	26.6	26.6	1.00
D.S.	F	Normal	52.7	27.8	28.0	0.99
J.C.	M	Crohn's disease	39.6	28.8	29.1	0.99
S.A.	F	Normal	49.6	26.2	27.3	0.96

III. 5. Summary:

(a) The physical properties of tritium and their relationship affecting its use as a test substance for measurement of total body water have been described and discussed. The principles of tritium counting have been outlined and the apparatus has been described.

(b) Methods have been described, and proved experimentally, for the preparation of pyrogen-free, sterile solution of tritiated water for intravenous injection.

(c) Evidence has been presented to show that tritiated water gives accurate and reproducible results in a dilution experiment.

(d) Experiments have been performed to show that measurement of the total body water by the dilution of tritiated water gives reproducible results on duplicate assays and repeated measurements on the same individual.

(e) Following the technique described, urine samples have been shown to give similar results to those of plasma samples for measurement of the total body water.

IV. EXPERIMENTAL COMPARISON OF THE VOLUMES OF DISTRIBUTION OF UREA, CREATININE, N-ACETYL 4-AMINO PHENAZONE (N.A.A.P.) AND TRITIATED WATER.

IV. EXPERIMENTAL COMPARISON OF THE VOLUMES OF DISTRIBUTION OF UREA, CREATININE, N-ACETYL 4-AMINO PHENAZONE (N.A.A.P.) AND TRITIATED WATER

IV. 1. Introduction:

been discussed (I.1.p.2) but there is considerable evidence that it may not be freely diffusible throughout the body. Walser and Bodenlos (1959) showed that 25 per cent of endogenous urea was hydrolysed by bacteria in the intestine and suggested that this might lead to an error in the estimation of body water by urea infusion. Also, there is biochemical evidence that some urea may be bound to protein in the body. Ralls (1943) found that the ratio of urea concentration in water of red blood cells to plasma water was 1.14/1.00. Pasynskii and Chernyak (1950) reported that albumin and globulin adsorbed urea in quantities not related to their respective water contents. Murdaugh and Doyle (1961) showed that urea is bound by haemoglobin and albumin, that the quantity bound is related to the plasma urea concentration and that the binding does not become saturated in urea concentrations up to 400 mg/100 ml.

Blackmore and Elder (1961) compared the amount of urea removed by haemodialysis with the expected removal calculated from the fall in plasma concentration, assuming a body water of 57 per cent of body weight. In most patients the two values did not agree and they concluded that in some circumstances urea might exist in both diffusible and non-diffusible forms, although they made no allowance

for endogenous urea production during haemodialysis, for redistribution of urea immediately after dialysis, nor for variation in body water. Shackman et al. (1962) estimated that urea required fifteen hours to reach equilibrium of distribution following the haemodialysis of some patients when the pre-dialysis and post-dialysis hourly increments of endogenous urea production were compared. They suggested that this long delay could not be due solely to redistribution but probably reflected a slow release of bound urea.

Reports on the volume of distribution of creatinine have varied considerably. Dominguez et al. (1937) regarded the body water as having two fluid compartments and calculated a creatinine space of 63 per cent of body weight for normal dogs. Greenberg et al (1952) estimated the creatinine space to be 48 per cent of body weight for intact dogs from experiments in which equilibrating infusions of creatinine were given and allowance made for the urinary excretion. Sapirstein et al. (1955) found the volume of distribution of creatinine to be 37 per cent of body weight in nephrectomized dogs and based their calculations on the creatinine arterial disappearance curve. Both Dominguez et al. (1937) and Sapirstein et al. (1955) based their calculations on the assumption that the body water could be divided into two compartments, the extracellular and the intracellular fluid compartments, and that a substance must penetrate the extracellular fluid to reach the intracellular fluid. While a two compartmental body fluid system exists anatomically, attention has been drawn to

the fact that the final volume of distribution of a substance represents the average of an infinite number of body fluid units of varying concentrations, (I.2. p.7) and cannot be resolved mathematically on a two compartmental system.

Although there is considerable evidence that urea may not be freely diffusible in the body and reports on the distribution of creatinine have varied widely, few attempts have been made to compare the volumes of distribution of these metabolites with the total body water as measured by the generally accepted tracers. (I.3.e.p.12). Edwards (1959) reported that in the ureter-ligated rabbit the volume of distribution of creatinine was similar to the body water measured by antipyrine dilution and by desiccation and Schloerb (1960) found similar volumes of distribution for urea, creatinine and tritiated water in nephrectomized dogs. However, Bradbury (1961), using urea and deuterium dilution to measure body water, found urea volumes smaller than those of deuterium, the mean difference being -5.3 per cent.

IV. 2. Object of Experimental Study:

The volumes of distribution of urea and creatinine are fundamental to the mathematical problems associated with their extraction from the body by haemodialysis and this study sought to compare their volumes of distribution with those of modern tracers. The present investigation measured and compared the volumes of distribution and rates of equilibration of urea, creatinine,

N-acetyl 4-amino phenazone (N.A.A.P.) and tritiated water following intravenous administration in 23 bilaterally nephrectomized dogs. For the purposes of this study the volumes of distribution of urea and creatinine, as well as those of N.A.A.P. and tritiated water, may be defined as that volume of water in which the total body quantity is assumed to be homogeneously dispersed at a concentration equal to that in the plasma water. The validity of the results was tested in seven of the dogs by expanding the volume of distribution with a known volume of intravenous fluid; after re-equilibration the measured volume of redistribution was compared with the expanded volume of distribution.

IV. 3. Methods:

(a) Preparation: Adult mongrel dogs of both sexes, weight 9.9 - 29.5 kg. were used. They were starved for eighteen hours but were allowed water. Anaesthesia was induced and maintained by Pentobarbitone Sodium (Abbott) and bilateral nephrectomy was performed through a mid-line abdominal incision. Intravenous injections were given through a polythene cannula introduced into the inferior vena cava through a tributary of the femoral vein. Blood samples were obtained from a cannula in the carotid artery and, before sampling, the blood occupying the dead space was collected and returned to the animal. The blood pressure was monitored from the other carotid artery. Clotting in the cannulae was prevented by local heparin solution. The animals were sacrificed at the end of the experiment.

(b) Injection Solutions: A solution of urea 30 g., creatinine 1.5 g. and N.A.A.P. 750 mg in 200 ml 0.9 per cent saline was given intravenously at a constant rate over 30 minutes; this is referred to subsequently as the infusion solution. These quantities of urea and creatinine were chosen to produce final plasma concentrations similar to those in uraemia, giving a plasma urea nitrogen concentration of 150-200 mg/100 ml and a plasma creatine concentration of 15-20 mg/100 ml.

Tritiated water, total activity 300-500 uc, was diluted in 50 ml 0.9 per cent saline and given separately by intravenous injection.

(c) Analytical Methods: Blood samples were collected in heparinized tubes at half-hourly or hourly intervals, the plasma being separated immediately and stored at 4°C until analysed.

Urea was measured by a modification of the carbamidodiacetyl reaction using the Technicon Autoanalyser. Plasma samples and the infusion solution were measured in triplicate and compared with a series of standard solutions. Creatinine was measured by the adsorption method of Edwards and Whyte (1958) and N.A.A.P. by the method of Brodie et al. (1951), all estimations being performed in duplicate. The technique of preparing and measuring tritiated water samples is described in Section II and Appendix 1.

(d) Experimental Procedure: (i) Group A (3 dogs). Bilateral nephrectomy was performed and hourly blood samples taken for eleven hours to observe the endogenous increment of urea and creatinine in the plasma.

(ii) Group B (16 dogs). The same technique as used in Group A was followed for three hours, after which the injection solutions were given. Further blood samples were taken for eight hours.

(iii) Group C (7 dogs). After bilateral nephrectomy the initial blood sample was collected, the injection solutions given and further blood samples taken for seven hours. A measured volume of fluid, approximately 10 per cent of the expected body water, was then given intravenously over one hour, four dogs receiving 0.9 per cent saline, one distilled water, one isotonic urea solution and the last 5 per cent dextrose in water. Further blood samples were taken for six hours.

Various combinations of the injected substances were used (Table 7), N.A.A.P. being given to twenty-two dogs, urea to eighteen, creatinine to eleven and tritiated water to fourteen.

(e) Calculations: Plasma concentrations of urea nitrogen and creatinine were plotted against time (Figs. 7 and 8) and endogenous production gave a steady rise before injection and after equilibrium of distribution. By extrapolation to the mid-point of injection the difference in plasma concentration produced by the quantity infused, was obtained. This was corrected to plasma water concentration using the factor 0.93, and from this the final volume of distribution was calculated. Plasma concentrations of N.A.A.P. were also extrapolated and the volume of distribution calculated from the plasma water concentration at the mid-point of injection. The volume of distribution of tritiated water was calculated from the mean of three

or four samples, which were taken between two and five hours after injection (Prentice et al. 1952).

A correction was applied to the calculated volumes of distribution to allow for fluid injected or removed during the experiment. No allowance was made for insensible loss.

In Group C, similar calculations were applied extrapolating to the mid-point of administration of additional fluid.

IV. 4. Results:

(a) Group A Endogenous Production of Urea and Creatinine: There was a constant increment, with insignificant variation, in the urea and creatinine concentrations in the plasma during eleven hours. The mean hourly increment of urea nitrogen was 2.42 mg/100 ml and of creatinine was 0.17 mg/100 ml.

(b) Group B (i) Endogenous Production of Urea and Creatinine: Before administration of the infusion solution the hourly increment in urea and creatinine concentrations in the plasma was constant with insignificant variation. After infusion, the hourly increment was again constant but at a different value.

(ii) Effect of the Infusion Solution on Endogenous Production of Urea and Creatinine (11 dogs): The mean hourly increment of plasma urea nitrogen was 2.24 mg/100 ml before infusion and 2.53 mg/100 ml after infusion (Table 6). Although the mean value was greater after infusion, there was considerable individual variation, one animal increasing from 1.9 mg/100 ml before infusion, to 4.5 mg/100 ml after infusion, while another decreased from 1.1 mg/100 ml to 0.8 mg/100 ml.

The mean hourly increment of plasma creatinine was 0.18 mg/100 ml before infusion and 0.12 mg/100 ml after infusion (Table 6). In comparison with the variation in urea nitrogen increments before and after infusion, the post-infusion increment of plasma creatinine was less than the pre-infusion increment in every experiment except one in which they were equal. The greatest fall was from 0.17 mg/100 ml before infusion to 0.03 mg/100 ml after infusion.

(iii) Time Required for Equilibration: Tritiated water equilibrated rapidly and samples taken one to five hours after administration gave volumes of distribution identical within the experimental error of the method. In the results only mean values obtained from samples taken from two hours onwards were used to avoid any uncertainty as to whether or not equilibration had occurred at one hour in individual experiments. Urea equilibrated in one and a half to two and a half hours (Fig.7), N.A.A.P. in three hours and creatinine in four and a half to six hours (Fig.8). The rates and volumes of distribution were not affected by omitting any one substance from the infusion solution.

(c) Groups B and C - Volumes of Distribution (Table 7): Urea and N.A.A.P. had mean volumes of distribution which were similar at 59 per cent and 57 per cent of body weight respectively, while creatinine and tritiated water were slightly larger at 62 per cent and 61 per cent. When paired results were taken, the difference in the mean volumes of distribution was significant between tritiated water and N.A.A.P. ($p < 0.01$) and between creatinine and N.A.A.P. ($p < 0.001$). The slight difference between urea and N.A.A.P. was not significant.

Table 6. Endogenous Production of Urea and Creatinine in Bilaterally Nephrectomized Dogs Before and After Intravenous Injection of a Solution of Urea, Creatinine and N.A.A.P.

No.	Plasma Urea Nitrogen mg/100 ml/hour			Plasma Creatinine mg/100 ml/hour		
	Before Injection	After Injection	Difference	Before Injection	After Injection	Difference
4	2.7	2.7	0.00	0.27	0.10	-0.17
5	1.9	2.5	+0.6	0.18	0.18	0.00
6	1.9	4.5	+2.6	-	-	-
7	-	-	-	0.14	0.10	-0.04
8	3.0	4.1	+1.1	0.20	0.14	-0.06
9	2.5	2.4	-0.1	0.11	0.10	-0.01
10	1.9	2.1	+0.2	0.23	0.12	-0.11
11	2.4	1.7	-0.7	0.17	0.03	-0.14
12	2.7	3.3	+0.6	0.17	0.14	-0.03
13	1.9	2.6	+0.7	0.25	0.23	-0.02
14	2.1	0.8	-1.3	0.16	0.08	-0.08
15	1.6	1.1	-0.5	0.15	0.06	-0.09
Mean	2.24	2.53	+0.29	0.18	0.12	-0.06

Table 7. Volumes of Distribution of Tritiated Water, N.A.A.P., Urea and Creatinine following Intravenous Administration in Nephrectomized Dogs.

			H T O		N.A.A.P.		UREA		CREATININE		H T O / N.A.A.P.	UREA / N.A.A.P.	CREATININE / N.A.A.P.
No.	Sex	Weight (Kg)	Vol. (l)	%wt.	Vol. (l)	%wt.	Vol. (l)	%wt.	Vol. (l)				
4	M	12.74	-	-	7.5	58.9	7.7	60.4	8.0	62.8	-	1.03	1.07
5	M	19.32	-	-	-	-	12.1	62.6	12.2	63.1	-	-	-
6	M	18.20	-	-	11.1	61.0	11.8	64.8	-	-	-	1.06	-
7	M	11.95	-	-	7.5	62.8	-	-	7.7	64.4	-	-	1.03
8	F	12.34	-	-	7.3	59.2	7.0	56.7	7.8	63.2	-	0.96	1.07
9	F	11.08	-	-	6.7	60.5	6.2	56.0	7.4	66.8	-	0.93	1.10
10	M	17.31	-	-	9.1	52.6	9.2	53.1	9.5	55.5	-	1.01	1.05
11	M	18.10	-	-	10.8	59.7	11.3	62.4	11.6	64.1	-	1.05	1.07
12	F	13.29	-	-	7.0	52.7	7.6	57.2	8.0	60.2	-	1.09	1.14
13	F	25.97	-	-	14.3	55.1	13.8	53.1	14.7	56.6	-	0.97	1.03
14	M	13.89	-	-	8.4	60.5	8.3	59.8	8.7	62.6	-	0.99	1.04
15	M	12.66	8.7	68.7	7.8	61.6	7.3	57.7	8.2	64.8	1.12	0.94	1.05
16	F	19.37	12.1	62.5	10.6	55.8	-	-	-	-	1.12	-	-
17	F	13.84	9.9	62.5	9.0	56.8	9.1	57.4	-	-	1.10	1.01	-
18	M	9.93	6.2	62.4	5.9	59.4	-	-	-	-	1.05	-	-
19	M	29.51	17.8	60.3	14.7	49.8	-	-	-	-	1.21	-	-
20	M	21.56	-	-	11.8	54.7	12.2	56.6	-	-	-	1.03	-
21	M	20.31	11.4	56.1	10.3	50.7	-	-	-	-	1.11	-	-
22	F	15.61	8.9	57.0	8.1	51.9	8.4	53.8	-	-	1.10	1.04	-
23	M	20.82	12.4	59.6	11.1	53.3	11.3	54.3	-	-	1.12	1.02	-
24	F	18.07	11.4	63.1	10.7	59.2	11.1	61.4	-	-	1.07	1.04	-
25	M	15.94	8.3	52.1	7.7	48.3	8.0	50.2	-	-	1.08	1.04	-
26	M	15.10	10.2	67.5	9.5	62.9	9.9	65.6	-	-	1.07	1.04	-
Mean				61.1		56.7		58.0		62.2	1.10	1.01	1.07
S.D.				4.8		4.4		4.4		3.4	0.47	0.44	0.34

(d) Group C - Expansion of Volumes of Distribution: Table 8 summarizes the results of comparison of body water measurement, using urea, N.A.A.P. and tritiated water, before and after the injection of known incremental volumes of fluid. There significance is referred to in the Discussion.

IV. 5. Discussion:

The presence of normal renal function complicates the measurement of the volume of distribution of endogenous metabolites. More accurate observations can be made on the nephrectomized animal or the oliguric patient, providing there is a constant rate of production of the metabolites. In the nephrectomized dog the endogenous production of urea and creatinine was shown to be constant over eleven hours (Group A). The mean hourly increments in plasma of 2.42 mg/100 ml for urea nitrogen and 0.17 mg/100 ml for creatinine were similar to the values of 2.1 mg/100 ml and 0.15 mg/100 ml respectively, reported by Schloerb (1961) who also showed that they remained constant over four days in bilaterally nephrectomized dogs. After infusion (Group B) the endogenous accumulation of urea varied, some animals having an increased and some a decreased production. This alteration was not related to the rate of production before infusion nor to the size of the animal. However, the endogenous production of creatinine was reduced in every experiment, except one in which it remained constant after administration of the infusion solution. No explanation was found for these alterations. Although the rate of production

altered, the constancy of accumulation of waste metabolites, before and after infusion, showed that the nephrectomized dog was a suitable preparation.

These results were in agreement with the findings of Schloerb (1960) that tritiated water equilibrated in one hour and urea in one and a half to two and a half hours. The administration of exogenous urea showed no evidence of a delayed equilibration as was found by Schackman et al. (1962) in the redistribution of urea in the human body after haemodialysis. The equilibration of creatinine varied from four and a half to six hours; this compared with four hours accepted by Schloerb (1960) in the dog and six hours by Edwards (1959) in the rabbit. Serial estimations confirmed the observations of Brodie et al. (1951) that N.A.A.P. equilibrated in three hours.

The mean volume of distribution of urea was 58 per cent of body weight, of creatinine 62 per cent, of N.A.A.P. 57 per cent and of tritiated water 61 per cent (Table 7). The values for urea, creatinine and tritiated water are of the same order as those of Schloerb (1960) in dogs and of those of Edwards (1959), for creatinine distribution in rabbits.

There was a significant difference in the mean volumes of distribution of tritiated water and N.A.A.P. in our experiments. This is in agreement with the results of other investigators and its relationship to the slow penetration of gastro-intestinal water by N.A.A.P. and to the exchange of tritium with hydrogen

ions in the body other than those in the water molecule has been discussed (I. 3. c. p.10 and I. 3. e. p.12). Although paired results are not available for tritiated water and creatinine, the significant difference in the volume of distribution of either when compared with that of N.A.A.P. implies that they both would have similar volumes of distribution. There was no significant difference in the volumes of distribution of urea and N.A.A.P.

If the volume of distribution of an indicator is accepted as a measure of the body water, then rapid expansion of that body water should produce a comparable fall in the plasma water concentration of the indicator, irrespective of the composition of the expanding solution. In Group O experiments this theory was tested for urea, N.A.A.P. and tritiated water using various expanding solutions. In all experiments the volume of redistribution of the indicator was similar to the expanded body water calculated from the volume of distribution increased by the additional fluid volume (Table 8). While it is appreciated that the volume of additional fluid given is small in relation to the errors in the analytical methods used, the experimental preparation would not accept a larger volume during a short period of administration. Although little statistical significance can therefore be given to the results, at least it may be noted that in every experiment the change in volume of redistribution was in the right direction and reasonably close to the expanded value.

These experiments thus show that urea and creatinine, which have volumes of distribution similar to those of N.A.A.P. and tritiated water, are suitable indicators for measurement of body water. Rapid expansion of the body water can be measured by alteration in the plasma concentration of these substances.

IV. 6. Summary:

(a) Following intravenous injection in the dog, tritiated water reached equilibrium of distribution in one hour, urea in one and a half to two hours, N.A.A.P. in three hours and creatinine in four and a half to six hours.

(b) Urea and creatinine had volumes of distribution in the dog similar to those of N.A.A.P. and tritiated water.

(c) Small but significant differences were present between the mean volumes of distribution of creatinine and N.A.A.P. and between those of tritiated water and N.A.A.P.

(d) Rapid expansion of the body water could be measured by alteration in the volumes of distribution of urea, N.A.A.P. and tritiated water.

Table 8. Comparison of the Measured Volumes of Distribution of Urea, N.A.A.P. and Tritiated Water Before and After the Addition of a Known Incremental Volume.

No.	Expanding Fluid	Added Vol.(1)	UREA		N.A.A.P.		TRITIATED WATER	
			Vol.A	Vol.B	Vol.A	Vol.B	Vol.A	Vol.B
20	0.9% Saline	1.3	13.5	13.4	13.1	13.2	-	-
21	0.9% Saline	1.2	-	-	11.5	11.4	12.6	12.5
22	0.9% Saline	1.0	9.4	9.5	-	x	9.9	10.0
23	0.9% Saline	1.0	12.3	12.2	-	x	13.4	14.0
24	5% Dextrose in water	1.0	12.1	12.3	11.7	11.6	12.4	12.6
25	Distilled Water	1.0	9.0	9.0	8.7	8.6	9.3	9.5
26	Isonomic Urea Solution	1.0	10.9	11.2	-	x	11.2	11.2

xExpanded N.A.A.P. space not measured due to the presence of sodium thiocyanate in Experiment Nos. 22 and 23 and to haemolysis in Experiment No.26.

Vol.A Initial Measured Volume of Distribution + Added Volume
Vol.B Final Measured Volume of Distribution

V. THE MEASUREMENT OF TOTAL BODY WATER AT HAEMODIALYSIS

V. THE MEASUREMENT OF TOTAL BODY WATER AT HAEMODIALYSIS

V. 1. Introduction:

The relationship of the volumes of distribution of urea and creatinine to the treatment of renal failure was discussed in Section I. 5. p. 17. During haemodialysis reduction in plasma concentration of urea and creatinine largely depends on the total load of these metabolites present in the body. The total load is the product of the plasma concentration and the volume of distribution of the metabolite and this latter value varies widely among patients. Infusion experiments have shown that in the nephrectomized dog urea and creatinine are distributed in the body water phase (Section IV). If the same is true for the patient in renal failure, it should be possible to estimate the total body water from the amount of urea or creatinine removed by haemodialysis and the change in plasma concentration effected, as was suggested by Black (1959). Measurement of the body water by this method at the first dialysis would allow more accurate prediction of the expected fall in plasma concentration of urea and creatinine at subsequent dialyses. Serial measurements might show alteration in the state of hydration of the patient and would aid in the calculation of changes in body composition, for although serial measured body weights will usually provide an adequate precaution against overhydration, the data may be difficult to evaluate if

there is weight loss.

In men, a few single studies of body fluid compartments during acute renal failure suggested an increase in total body water and extracellular fluid volume (Schwartz, Tomovic and Schwartz, 1951; Sirota and Kroop, 1951; and Smith, Madisso and Pitts, 1954). Swan and Merrill (1953) thought that the polyuria of the diuretic phase of acute renal failure did not entirely represent inability of the kidneys to conserve electrolytes and water but was a mechanism for their excretion when they had accumulated to excess during the disease. Remenchik, Schoenberger and Dyniewicz (1958) studied eight patients with acute renal insufficiency and reported an increase in total body water of five patients and in extracellular fluid volume in all patients. Their results are difficult to assess because of the wide range of normal values for these fluid compartments, but support is given by the observed decrease of these volumes in three patients who recovered. Other attempts at direct measurement of body water in oliguric patients have used deuterium (Haley, Edelman and Moore, 1951) and tritiated water (Watten, Doolan, Hutchin, Canada and Harper, 1956).

An appreciation of the factors governing fluid balance in renal failure has increased, it has become apparent that the degree of hydration of the patient in the early days of the disease is affected by the lesion which precipitated renal failure, by the wit of the physician to make an early diagnosis and by his ability to control fluid balance. Dehydration is found when acute renal failure is precipitated by such conditions as severe gastro-enteritis or improper

management of a proximal small bowel fistula. At the other extreme gross overhydration is seen when it follows a surgical operation and the oliguria, mistakenly diagnosed as due to pre-operative fluid deprivation, is treated with copious intravenous fluid.

Attempts to calculate the volumes of distribution of creatinine and urea from their extraction during haemodialysis for renal failure have had only limited success, though the results would have considerable value in the management of this condition. Edwards (1959) made 10 comparisons on eight human subjects and found that creatinine underestimated the predetermined antipyrine space by an average of 2 litres. Vaughan, Doolan, Theil and Alpen (1961) measured the body water by dilution of tritiated water and compared the result with the body water measured by the extraction of both tritiated water and urea during haemodialysis. They considered that the urea extraction technique was potentially unreliable. Blackmore and Elder (1961) found considerable discrepancy between the calculated and actual plasma urea concentrations at the end of dialysis, and concluded that urea was not a freely diffusible, rapidly equilibrating substance in all cases.

V. 2. Object of Experimental Study:

In this investigation, the volumes of distribution of urea and creatinine have been calculated from alterations in their plasma concentration effected by haemodialysis and the total amounts removed. The results are compared with the body water measured in the immediate post-dialysis period by the dilution of tritiated water and N.A.A.P.

V. 3. Methods:

(a) Subjects Studied: Twenty-five series of observations were made on 20 patients, 11 male and 9 female, with traumatic, obstetric and pyelo- and glomerulonephritic renal failure; a miscellaneous group comprised patients with renal failure secondary to congenital polycystic kidneys, myelomatosis and dehydration. A summary of the clinical histories is given in Appendix 2.

(b) Apparatus: Haemodialyses were performed with a modified Kolff artificial kidney (Fig.9) described by Parsons and McCracken (1958). The duration of dialysis was usually 6 hours and the bath fluid, of 100 litres volume, was changed every 2 hours. Blood flow was constant within the range 250 ml to 450 ml/minute.

(c) Procedure: A venous blood sample was taken at least 3 hours prior to dialysis; when possible this interval was extended to 24 hours. Samples from the arterial cannula were taken at the start, at hourly intervals during dialysis and at its termination. Solutions of N.A.A.P., 1.2 g, and tritiated water, 250 μC, were prepared separately each in 50 ml 0.9 per cent saline and given intravenously immediately following dialysis. Frequent venous blood samples were taken at varied intervals in the 24 hours following dialysis. The patient was weighed at the beginning and end of dialysis.

(d) Analytical Methods: Methods for the estimation of urea, creatinine, N.A.A.P. and tritiated water have been described (IV. 3. o.p.48 and Appendix 1).

(c) Calculations: Allowance was made for the endogenous production of urea during dialysis and for equilibration of urea in the body after dialysis, by plotting the plasma concentrations against time and extrapolating both to the time of completion of the dialysis (Fig.10). The corrected values obtained were converted to plasma water concentrations by the factor 0.93 and the total body water calculated from the formula:

$$TBW = \frac{U}{I-F} \qquad (7)$$

where TBW is the total body water in litres, U is the total amount of urea in grams present in the dialysing fluid and I and F respectively the corrected initial and final plasma water concentrations in grams/litre. If alteration in body weight occurred during dialysis, reflecting a change in total body water, then correction was made for the redistribution of total body urea following dialysis into a larger or smaller volume of body water:

$$TBW = \frac{U \pm WI}{I - F} \qquad (8)$$

where W is the weight change in kilograms during dialysis. The factor WI was subtracted for weight loss and added for weight gain. If body weight changed due to a gain or loss of water other than intravenously administered fluid, then the volume of fluid in the dialysing bath would also alter. The small volumes concerned were considered insignificant in relation to the total volume of the dialysing fluid.

V. 4 Results:

(a) Redistribution of Urea and Creatinine after Dialysis: Blood samples were taken at frequent intervals after dialysis over a period of 18 hours and analysed for urea content on 21 occasions and for creatinine content on 9 occasions. The mean time for urea distribution to equilibrium was 5 hours with a range from 2 hours to 6 hours and the mean time for creatinine redistribution was 6 hours with a range from 4 to 8 hours. In no patient was there evidence of delayed distribution beyond these times.

(b) Volumes of Distribution: (Table 9): For most patients the volumes of distribution of urea and creatinine were similar to that of N.A.A.P. Comparison of paired results showed the ratio of the volumes of distribution of creatinine to N.A.A.P. to be 1.03 (S.D.$\pm$ 0.06) while for urea the ratio was 1.07 (S.D.$\pm$ 0.12). If two patients (I.L. and F.L.2) in whom there was a marked discrepancy between the volumes of distribution of urea and N.A.A.P. are excluded, the ratio falls to 1.03 (S.D.$\pm$ 0.08). The ratio of the volumes of tritiated water to N.A.A.P. was high (1.29 S.D.$\pm$ 0.12) and, as would be expected, comparison of paired values between those for urea and those for tritiated water gave a ratio of 0.05 (S.D.$\pm$ 0.08).

Table 9. Total Body Water Measured by Volume of Distribution of Tritiated Water and N.A.A.P. and by Extraction of Urea and Creatinine during Haemodialysis.

Name	No. of Dialysis	Duration (hours)	Body Wt. (kg)	Volumes of Distribution (litres) THO	NAAP (%wt)	Urea	Creat-inine	THO / NAAP	Urea / NAAP	Creat: NAAP	Urea / THO
F.H.	1	6	72.0+	-	39.5 (54.9)	39.4	42.4	-	1.00	1.07	-
M.P.	1	4	57.3+	-	23.2 (40.5)	22.3	22.8	-	0.96	0.98	-
J.D.	1	6	82.0	-	36.0 (43.9)	36.5	35.8	-	1.01	0.99	-
E.W.	1	6	47.6	-	24.9 (52.3)	26.9	25.4	-	1.08	1.02	-
W.G.	3	4	61.0+	-	26.4 (43.3)	26.2	25.4	-	0.99	0.96	-
P.O.	2	6	76.0+	-	34.3 (45.1)	35.0	36.0	-	1.02	1.05	-
R.B.	2	6	70.1+	-	32.6 (46.5)	35.1	35.9	-	1.08	1.10	-
S.S.	3	6	62.0+	-	29.8 (48.1)	30.7	30.3	-	1.03	1.02	-
P.H.	1	6	107.3	-	39.9 (37.2)	47.0	37.5	-	1.18	0.94	-
I.L.	1	6	73.5	-	38.4 (52.3)	51.6	44.3	-	1.34	1.15	-
J.C.	3	6	56.7	-	28.8 (50.8)	29.7	30.3	-	1.03	1.05	-
G.A.	1	6	57.3	-	38.3 (66.8)	40.2	40.8	-	1.05	1.07	-
R.A.	3	6	62.4+	-	34.3 (55.0)	31.9	33.9	-	0.93	0.99	-
J.L.	1	4	67.6	40.1	31.0 (45.9)	36.8	-	1.33	1.19	-	0.92
	2	6	66.5	39.8	- -	34.4	-	-	-	-	0.86
	3	6	69.7	43.4	- -	37.7	-	-	-	-	0.87
P.L.	1	6	62.6	40.4	29.8 (47.6)	-	-	1.36	-	-	-
	2	6	59.0	33.3	23.5 (39.8)	31.8	-	1.42	1.35	-	0.95
B.J.	2	6	75.0	36.2	34.3 (45.7)	34.0	-	1.06	0.99	-	0.94
A.B.	1	6	56.0	33.1	26.3 (47.0)	27.1	-	1.26	1.03	-	0.82
E.B.	1	6	57.2	29.1	21.5 (37.6)	22.7	-	1.35	1.06	-	0.78
	2	6	57.3	26.8	- -	20.4	-	-	-	-	0.76
	3	6	56.2	26.3	21.6 (38.4)	21.8	-	1.22	1.01	-	0.83
J.H.	1	6	64.0	46.8	- -	43.9	-	-	-	-	0.94
M.H.	1	6	55.2	35.3	- -	25.1	-	-	-	-	0.71
						Mean		1.29	1.07	1.03	0.85
						S.D.		0.12	0.12	0.06	0.08

+Body weight is approximate

(c) Repeated Measurements at Successive Dialyses: (Table 10):

With a few exceptions, repeated measurements at successive dialyses gave results consistent with the expected change in body water as observed by alteration in patient's body weight. In one patient, however, (I.L.) the volume of distribution of urea measured at the first dialysis showed a considerable discrepancy from the corresponding value for N.A.A.P., yet at the second dialysis 13 days later, the value for urea distribution was the same as that found for N.A.A.P. at the first dialysis. This is discussed below. In another patient (R.A.) there was wide variation in five successive dialyses. This patient had a high rate of protein breakdown due to his multiple injuries and the first dialysis was limited to a period of 2 hours.

V. 5. Discussion:

The biochemical evidence that urea may be bound in the body has been discussed (IV. 1. p.44). Schackman et al. (1961) found that in some patients there was a delay as long as 15 hours in the redistribution of urea to equilibrium in the body following haemodialysis. Though frequent serial observations were not made in all our patients a considerable number were examined and no evidence of delayed redistribution was found, though the results are difficult to interpret in some patients in whom there was alteration in the rate of endogenous production of urea following dialysis. While the mean time for redistribution of urea following dialysis was 6 hours, in our experiments on nephrectomized

dogs exogenous urea distributed in 1½ to 2½ hours (IV. 4 b.iii p.51) suggesting that other factors may be affecting the release of intracellular urea following dialysis. The redistribution of creatinine in 6 hours is similar to the value found in the animal experiments (IV. 4. b.iii.p.51).

Measurement of the total body water by extraction of urea and creatinine at haemodialysis proved to be a satisfactory method when compared with the results obtained by the distribution of N.A.A.P. (Table 9). There were, however, exceptions. One of these (I.L.) was a patient who was oedematous and suffered from long-standing chronic uraemia due to glomerulonephritis. In this case the volume of distribution of urea was 13 litres different from that of N.A.A.P. yet at his second dialysis 13 days later there was close correspondence between the volume of distribution of urea and the value for N.A.A.P. previously observed. Blackmore, Elder and Bowden (1963) found that in some patients with chronic uraemia the red cell urea concentration was considerably greater than that of the plasma, and that this was present prior to the first dialysis but not thereafter. Red cell urea concentrations were not examined in the present investigation but this may have been a factor producing the discrepancy observed. For the other exception, patient (P.L.), it would appear from comparison with other results obtained that an error had occurred in the measurement of the volume of distribution of N.A.A.P.

The total body water as measured by N.A.A.P. distribution and expressed as a percentage body weight, ranged from 37.2 per cent to 66.8 per cent. These values represent varying degrees of obesity and various states of over and under hydration. Blackmore and Elder (1961) calculated a theoretical efficiency for haemodialysis from the known urea clearance of their artificial kidney and the initial plasma urea but they assumed a total body water for the patient of 57 per cent of body weight, this being the average figure quoted in the world literature. Comparison of this with our values shows that a large error would be introduced into their calculations by this assumption. In addition they made no allowance for endogenous production of urea during dialysis, nor for redistribution of urea to equilibrium after dialysis. Only in patients with renal failure due to obstetric causes did they obtain a satisfactory correlation. These patients are the most stable for metabolic studies in renal failure having usually a low and steady rate of endogenous urea production. The difficulty of accurate measurements in a patient with a high rate of protein breakdown and in whom the clinical state was varying considerably from day to day is shown in our patient R.A. in whom wide variations in the volume of distribution of urea were found.

The effect of redistribution of urea following dialysis is shown in Fig.11. In this patient the plasma urea nitrogen concentration was measured at the start of dialysis, at the two-hourly

change in both fluids, at termination of dialysis and at half-hourly intervals in the post-dialysis period. Calculated from the total six-hour dialysis and allowing for redistribution of urea after dialysis, the urea space was 40 litres. After 6 hours of dialysis, redistribution produced a rise in the plasma urea nitrogen concentration which was 5 per cent of the total fall. Taking volume of distribution of urea as 40 litres the true plasma concentration after theoretical redistribution was calculated for the two-hour and four-hour periods of dialysis, the quantities of urea removed at that time being known. When these calculated values were compared with the actual values measured at those times, it was found that after 4 hours of dialysis redistribution would have produced an elevation in the plasma urea nitrogen concentration of 10 per cent and at 2 hours an elevation of 19 per cent of the total fall. The artificial kidney removes urea from the extracellular fluid compartment more rapidly than urea redistributes between the intracellular and extracellular water. This effect is more readily observed in a dialysis of short duration and is one explanation why unreliable results may be obtained for measurement of the total body water by urea extraction in short dialyses. In addition, the errors of extrapolation are proportionally greater in a dialysis of short duration.

Table 10. Repeated Observations on the Total Body Water Measured at Haemodialysis.

Name	No. of Dialysis	Body Weight (kg)	Volumes of Distribution (litres)			
			H.T.O.	N.A.A.P.	Urea	Creatinine
R.B.	2	70.1	-	32.6	35.1	35.9
	3	71.0	-	-	37.2	35.8
F.S.+	2	61.5	-	-	32.3	36.0
	3	62.0	-	29.8	30.7	30.3
I.L.	1	73.5	-	38.4	51.6	44.3
	2	72.0	-	-	38.4	-
F.O.	2	76.0	-	34.3	35.0	-
	3	76.0	-	-	34.7	-
P.L.	1	62.6	40.4	39.8	-	-
	2	59.0	33.3	25.5	31.8	-
J.L.	1	67.6	40.1	-	36.8	-
	2	66.5	39.8	-	34.4	-
	3	69.7	43.4	-	37.7	-
K.B.	1	57.2	29.1	-	22.7	-
	2	57.3	26.8	-	20.4	-
	3	56.2	26.3	-	21.8	-
R.A.+	1	66.0	-	-	41.2	-
	2	67.9	-	-	26.0	-
	3	62.4	-	34.3	31.9	33.9
	4	62.5	-	-	40.0	-
	5	62.0	-	-	26.0	-

+Absolute values of body weights not certain.

V. 6. Summary:

(a) The total body water of a patient in renal failure can be measured by the extraction of urea and creatinine during haemodialysis.

(b) With exceptions, the total body water measured by this technique compares closely with that measured by the dilution of N.A.A.P. and tritiated water.

(c) Exceptions include patients with a high rate of protein breakdown and possibly those in chronic renal failure who may have a volume of distribution for urea greater than the total body water.

VI. THE MEASUREMENT OF WHOLE BODY POTASSIUM

VI. THE MEASUREMENT OF WHOLE BODY POTASSIUM

VI. 1. Historical Introduction:

The potassium content of the human body has usually been measured as total exchangeable potassium using tracer doses of the radioactive isotope, ^{42}K. Corsa et al.(1950) described the technique in detail and numerous reports of the value for exchangeable potassium in both health and diseased states have appeared in the literature. Comparison of isotope dilution studies in rats with chemical analyses of the hair-free carcasses showed that 98 per cent of the carcass potassium was exchangeable (Talso et al. 1960).

Interest in the natural radioactivity of the human body has led, during the last decade, to the development of techniques for the measurement of whole body potassium. These are based on the detection of gamma radiation from the potassium isotope ^{40}K, which is present in all naturally occurring potassium. The original interest in body radioactivity was in the measurement of acquired radium burdens and was stimulated by the use of radium salts medicinally and by the tragic results of the ingestion of radium by the early luminizers. The first measurements were made with ionization chambers (Schlundt, Barker and Flinn, 1929). The modern development of accurate quantitative measurement was initiated by Evans (1937) who took account of body geometry, internal radiation absorption and the effect of the absorption of background radiation by the subject.

The first apparatuses were crude but from 1950 onwards more satisfactory methods were developed which allowed the detection of not only these acquired radium burdens but the natural gamma activity of the body which is predominantly due to ^{40}K. Sievert (1951) developed the first apparatus with a sensitivity sufficient for measurement of the natural gamma ray activity of the body. In a surface laboratory with water tank shielding against background radiation, he used cylindrical ionization chambers arranged around the subject. Later Sievert (1955) obtained more accurate results with similar equipment in an underground laboratory beneath about 55 metres of rock which reduced the cosmic ray background. In Leeds, Burch and Spiers (1953) used high pressure ionization chambers with water shielding supplemented by steel. This apparatus determined the natural gamma ray activity of the subject with a standard error of about ± 20 per cent with a single two-hour measurement. This meant that the total body potassium could be measured with the same degree of accuracy since the gamma radiation arose almost entirely from the ^{40}K content of the body.

The advent of large-scale scintillation apparatus superseded both the ion chamber and the Geiger counter apparatus and led to the accurate measurement of the natural gamma ray activity of the body. The large liquid scintillators developed by Anderson (1956), the sodium iodide apparatus pioneered by Marinelli (1956) and the three-unit plastic scintillator apparatus evolved by Burch, Hughes, Iinuma, Overton and Appleby (1962) were all capable of measuring

the total body potassium with a statistical error of a few per cent in an observation time of about 15 minutes. These apparatuses were also capable of analysing the gamma ray spectrum with varying degrees of resolution. From the late 1950s this has become necessary because the measurement of whole body potassium has been complicated by the universal acquisition by the population of a body burden of caesium-137 (^{137}Cs) from the ingestion of fallout products following the testing of nuclear devices in the atmosphere.

Whole body counters can be used for radiological protection monitoring, natural potassium measurements and low level tracer work and during the present decade have been developed primarily to deal with radiological protection problems. They have been used to measure radioactive body burdens acquired accidentally or from occupational exposure. Whole body potassium measurements have formed the basis of studies on the proportions of fat and lean mass in the body (Allen, Anderson and Langham, 1960; Forbes, Gallup and Hurst, 1961; Remenchik and Miller, 1962). This technique developed for humans has been applied by a few agriculturists (Zobrisky, Naumann, Dyer and Anderson, 1959; Kirton, Pearson, Nelson, Anderson and Schuch, 1961) to study body composition of livestock on the basis of the relationship of potassium to lean body mass advanced by Woodward, Trujillo, Schuch and Anderson, 1956). Gamma ray spectrometry has also been used to study the potassium and lean meat content of hams (Pringle and Kulwich, 1961). Whole body counting provides a new approach to a number of tracer investigations which can be carried out at levels far below those hitherto used

when excreta were assayed. Examples are uptake studies with ^{47}Ca, and ^{59}Fe and ^{58}Co labelled vitamin B 12. As the investigations can be continued over a longer period of time the slower metabolic compartments of the body have been brought within range of tracer methods.

Although there have been several studies of whole body potassium measurement by gamma ray spectrometry in normal males and females, one of the largest being that of Anderson and Langham (1959), there have been few clinical studies in diseased states. The expensive apparatus required has been associated with radiation protection schemes and with the nuclear energy laboratories, rather than with general hospitals. The poor clinical state of some patients with potassium depletion precludes transporting them to a whole body counter away from the hospital and even if one is available within the hospital, most apparatuses have not been designed primarily for the comfort of an ill patient. Clinical studies which have been performed include those of Blahd, Cassan and Lederer (1962). They measured whole body potassium in relation to muscular dystrophy and myotonia atrophica and showed moderate to severe depletion of body potassium the extent of which appeared to be related to the severity of the disease, especially in the former condition. Remenchik and Miller (1962) studied whole body potassium in six patients in whom it was depleted by antihypertensive therapy, and in four patients suffering from gross obesity.

VI. 2. Principles of Whole Body Counting:

Natural potassium consists of three isotopes, ^{39}K, ^{40}K and ^{41}K in constant abundances of 93.08, 0.0118 and 6.91 per cent respectively (Strominger, Hollander and Seaborg, 1958). Of these the ^{40}K isotope is naturally radioactive. It has an extremely long half-life of 1.3×10^9 years and decays by two alternative branches to calcium^{40} with the emission of beta particles (89 per cent) or to argon^{40} with the emission of gamma rays (11 per cent). The beta particles are in effect completely absorbed in the tissue of the body but the gamma rays, of energy 1.46 MeV, although undergoing some absorption in the body, can be detected by a suitable counter placed externally. Because the number of gamma photons emitted per second from the potassium in the body is small (less than approximately 3.4 per gram of natural potassium), a detector of high sensitivity is required. A shield must be provided to screen this detector from the natural background radiation which would otherwise produce a far higher response in the detector than the body's ^{40}K radiation.

Scintillation detectors are generally classified as organic scintillators, both liquid and plastic, and heavy element crystals such as sodium iodide or caesium iodide. Spectrometric properties arise from the nearly proportionate conversion of secondary electron energy in the scintillator into light and the conversion of the light flashes into electrical pulses by a photomultiplier tube. These pulses can then be analysed on an amplitude basis and counted to give a pulse-

height distribution which is related to the original gamma ray photon energy spectrum. The spectral response of the scintillator apparatus is primarily dependent on the nature of the physical interaction of the photon with the scintillator. In the sodium iodide crystal, a considerable fraction of incident photons are absorbed by the photo-electric process giving a comparatively sharp photo-peak, with a small spread of amplitude in the pulse-height distribution. In organic scintillators of moderate size, photons are absorbed mainly by the Compton process and for any single photon energy all pulse sizes appear up to the maximum possible Compton transfer of energy at a first collision. Asymmetrical and rather broad peaks then characterize the pulse-height distribution but nevertheless useful spectrometric resolution can be obtained. Thus, by using a scintillation detector pulse-height techniques can be employed to assist in the resolution of gamma rays of ^{40}K from other gamma radiation present, in particular, that from ^{137}Cs acquired from fallout.

The problem of calibration is inherent in the measurement of radioactivity distributed in the human body. If the spatial distribution of radioactivity remains sensibly unaltered relative measurements show the variation of radioactivity or amount of radio-isotope present with time in any one subject, and avoid systematic and calibration errors. However, when absolute measurements of radioactivity are required the relationship between instrument

response and known amounts of the radio-isotope in the spatial distribution of the body, must be determined. The equipment is calibrated by comparison of the net response (i.e. observed count-rate minus background) to the body's ^{40}K radiation with the net response to potassium uniformly distributed as potassium chloride in aqueous solution throughout a phantom consisting of polythene containers approximating to the size and shape of the subject. Correction is made for the different geometrical distributions of potassium and different magnitudes of self-absorption of the ^{40}K gamma radiation in the subject and in the phantom. This is done by administering small quantities of the artificial radioactive isotope ^{42}K to the subject and to the phantom (Burch and Spiers, 1953). This isotope, of half-life 12.45 hours, emits beta particles and also gamma rays of energy 1.52 MeV, the latter having almost identical absorption and scattering characteristics to those of the gamma rays from ^{40}K. The correction factor is obtained by comparing the ^{42}K count-rate for the subject after equilibrium distribution of the ^{42}K with the count-rate of the phantom. The appropriate value for the background count-rate is obtained with a water-filled phantom in the counting position to simulate the absorption of the background radiation by the subject or phantom. The details of calibration for the present study are described in Section VII.

VI. 3. Contaminants:

The possibility must not be overlooked that a subject may contain an unsuspected radio-isotope, acquired incidentally or administered for diagnostic or therapeutic purposes, and emitting gamma rays of such an energy that they are not readily distinguishable from the ^{40}K gamma radiation. In the Leeds plastic scintillator apparatus, two single-channel analysers select the pulses originating from the ^{40}K gamma radiation, one analyser being set to select the upper portion and the other the lower portion of the potassium peak in the pulse-height spectrum. Examination of the ratio of count-rates obtained in the two analysers would, in the presence of an interfering isotope, show an alteration from the expected ratio. A further check can be made by monitoring the high energy part of the spectrum with an analyser set above the ^{40}K energy; this would detect, for example, the presence of thorotrast in a patient. Administered ^{131}I if present in small quantities, and the body burden of ^{137}Cs are both registered by the apparatus but can be discriminated against by the single-channel analysers. ^{47}Ca, ^{59}Fe and ^{82}Br will all interfere with counting of ^{40}K. Detection of contaminants is easier with the higher resolution of the sodium iodide crystal than with the liquid or plastic scintillators. In practice, the commonest "contaminant" likely to interfere with ^{40}K measurement is a wrist-watch with luminous paint containing radium.

VI. 4. Types of Whole Body Counters:

Scintillation detectors are sensitive to the geometry or spatial distribution of the radiation source which they are measuring and also to the degree of self-absorption of the radiation in the source. Various types of apparatus have been developed to overcome these problems.

(a) 4 Pi Geometry: Fig.12. In the Los Alamos whole body counter (Anderson, 1956) a hexagonal-sided hollow cylinder of liquid scintillator is used. This is viewed by a group of 24 photomultiplier tubes. The subject is placed on a cradle outside the steel shield and is then propelled to the hollow centre of the cylinder. As the subject is, in effect, totally enclosed a 4 pi geometry is obtained, and the response of the counter is independent of the position of the source. Although the spatial distribution of the ^{40}K is not important the gamma radiation is still attenuated by self-absorption.

(b) Chair Geometry: The Miller-Marinelli geometry or chair geometry (Marinelli, 1956) uses a thallium activated sodium iodide crystal, NaI (Tl), and the source-detector distance is kept constant by placing the subject in a chair so that he forms an arc in relation to the detector. (Fig.13). If the source-detector distance is constant the sensitivity of the apparatus should also be constant i.e. counts/second per unit activity. The apparatus has a low sensitivity as the arc of the body is so wide in radius and there is still the problem of self-absorption.

(c) Leeds Plastic Scintillator: Fig.14. Three plastic scintillators, 20 x 10 x 6½ inches are used, one in front of the subject and two behind (Burch, et al.1962). The subject is wrapped around the front unit and the top back unit extends above the head. Each scintillator unit is viewed by two photomultiplier tubes. During measurement the patient can be observed through the water window and communication is maintained with a two-way loud speaker system. The shield is 5 inches thick steel, lined with 0.5 inches lead. The apparatus is accommodated underground in the basement of the Wellcome Wing, The General Infirmary at Leeds. This basement laboratory has a concrete ceiling 2 feet 6 inches thick to give further shielding and is remote from intense radiation sources elsewhere in the hospital. Only recently has this apparatus been sited with direct access from the wards so that ill patients can be measured and the studies presented here are among its first large-scale clinical uses.

VI. 5. Precision of Whole Body Counting:

The precision of whole body counting can be assessed in terms of the accuracy of counting, the variation in repeated measurements on a single subject and the absolute accuracy of measurement.

(a) Accuracy of Counting: This depends on the random statistical nature of the radioactive decay process, instability in the electronics and alteration in background radiation due to variations in cosmic ray activity and in airborne radioactivity

which is mainly radon and its daughter products. In the Leeds apparatus a counting time of 1,000 seconds is used for the subject with a total of 2,000 seconds counting time for background. For a subject containing 150 g potassium the standard deviation of the counting statistics is ± 1.1 per cent (Hughes, 1963 a) and this is increased to approximately ± 2 per cent when the electronic and background instabilities are included (Hughes, 1963 b).

(b) Repeated Measurements on a Single Subject: Repeated observations on the same subject have a standard deviation from the mean of ± 3 per cent (Hughes, 1963 a). Part of this is due to the accuracy of counting, part to minor alterations in the position of the subject in relation to the detectors and part may be due to natural variation in the true total potassium content of the body. It has been suggested that there may be natural variation in the total potassium content of the body by as much as 7 per cent (Evans, 1963).

(c) Absolute Accuracy: From one observation of the ^{40}K response the absolute value of the whole body potassium can be calculated by formula with a standard deviation of ± 3.5 per cent (VII.3.p.92). If calibration with ^{42}K is used, the standard deviation is reduced to about ± 2.9 per cent (VII. 1.p.89). This value includes the statistics of counting, errors in dispensing and apparatus instability (Hughes, 1963 b). It assumes that calibration by ^{42}K is correct in principle.

Blainey, Cooke, Quinton and Scott (1954) discussed the errors

involved in exchangeable potassium measurements. They found that two determinations would have to differ by 120 mEq per 1,000 mEq (12 per cent) of exchangeable potassium, for the difference to be significant and considered that this was consistent with the results published by other workers. Comparison of these figures with the analysis of errors in whole body counting given above, shows the latter to be a more accurate method of body potassium measurement.

VI. 6. Advantages and Disadvantages of Potassium Measurement by Whole Body Counting:

Whole body potassium counting provides a rapid method for potassium measurements which can be repeated an indefinite number of times and if necessary, at short time intervals. As very little or no radioactive substances need be administered, there is minimal or no radiation hazard and the method is suitable for potassium measurement during pregnancy and in children. The measurement itself is rapid, taking only 16 minutes of the patient's time and the result is almost immediately available. Exchangeable potassium studies, on the other hand, require the administration of a relatively large dose of ^{42}K and frequent measurements are precluded by the time necessary for decay of a previous dose. In addition, the long equilibration time of ^{42}K and the need for chemical as well as radioactive assays delay calculation of the result. One disadvantage of the whole body counter is that the subject must be

fit enough to walk a few steps with assistance from the door of the steel shield to the chair. In an ill patient, and in particular one with severe potassium deficiency, this may not always be possible. A more suitable apparatus for these patients would be one in which the patient could lie on a stretcher throughout the measurement and such an apparatus will shortly be available in Leeds. A few patients of nervous disposition have experienced claustrophobia and have refused to complete the measurement. Patients can be observed through the water window and conversation maintained if necessary with the two-way loudspeaker system. For general installation the apparatus has the disadvantage that it is extremely expensive and requires highly trained staff for its use and maintenance.

VII. ABSOLUTE CALIBRATION OF WHOLE BODY COUNTER

VII. ABSOLUTE CALIBRATION OF WHOLE BODY COUNTER

VII. 1. Absolute Calibration by ^{42}K Technique:

It is difficult in whole body counting to obtain an accurate calibration factor to relate the quantity of radio-isotope in a specific subject to the observed count-rate. When serial measurements of body potassium in the same subject were required, the absolute value of body potassium has been less important than the relative value. Such serial estimations have carried a standard deviation of $\pm$ 3 per cent. (VI. 5.b. p.84). However, in clinical studies with patients suffering from potassium deficiency and with patients whose body size and shape varied greatly with time, e.g. pregnant women and oedematous patients, thus altering significantly the geometry, it became necessary to estimate absolute values for whole body potassium. The technique of administering oral ^{42}K to both subject and to a phantom was used to establish the calibration factor appropriate to the individual subject. The method was described by Burch and Spiers (1953)

In this procedure the subject's ^{40}K response is first measured. About 0.75 μC ^{42}K is administered to both subject and to a simple polythene phantom. The ^{42}K is allowed time to distribute in the body until its spatial distribution is broadly the same as the natural potassium. Complete metabolic equilibrium is not necessary as in the case of exchangeable potassium estimations.

The time required for satisfactory mixing and distribution will be discussed later. The subject is counted again at the end of the mixing period, thus giving a measure of the ^{40}K + ^{42}K burden. The quantity of ^{42}K administered was chosen so that the ^{42}K + ^{40}K count-rate at this time was approximately ten times greater than the ^{40}K count-rate. Thus the ^{42}K count-rate could be obtained by subtraction without introducing significant error due to alteration in the basic ^{40}K content of the subject between the two measurements and the experimental error in determining ^{40}K.

The equivalence between the ^{42}K and natural potassium was established in vitro by measuring the ^{42}K phantom and an identically shaped and positioned phantom containing potassium chloride in aqueous solution. This relationship was then applied to the ^{42}K in the subject. Thus the quantity of ^{42}K left in the subject at the time of the second measurement and its equivalent in grams of natural potassium could be calculated. This information was then used to convert the initial ^{40}K count-rate into grams of natural potassium. It was found that urinary excretion up to 24 hours was not usually greater than about 5 per cent of the administered ^{42}K and the faecal excretion was about 1 per cent. During the mixing period, urine and faeces were collected and the ^{42}K content assayed, allowance being made for radioactive decay.

This method of calibration with ^{42}K proved satisfactory and the absolute value for whole body potassium carried a standard deviation of ± 2.9 per cent (Hughes, 1963 b).

VII. 2. Distribution Time for ^{42}K.

The time necessary for adequate spatial distribution of ^{42}K in the body was investigated by following the ^{42}K count-rate against time after oral and intravenous administration of a dose of ^{42}K. This experiment was performed on five of us who were involved in the present study and who were all classed as healthy, normal males. A typical result is shown in Fig.17 which is a logarithmic plot of the ^{42}K count-rate, corrected for excretion, against time for both oral and intravenous administration. After Point A in the top curve which represents oral administration, and Point B in the bottom curve for intravenous administration, the observed results follow the physical half-life of ^{42}K, namely 12.45 hours. It was concluded that adequate distribution of ^{42}K took place in about 12 hours following an oral dose and about 5 hours following intravenous injection. Using oral ^{42}K and a NaI (Tl) crystal, Delwaide, Verly, Colard and Boulenger (1962) reported a mixing time of 10 hours.

Oral administration of ^{42}K for calibration avoided the problem of preparing a sterile solution for intravenous injection but had the disadvantage that the second measurement in the whole body counter could not be made on the same day as the ^{40}K measurement. In addition urine and possibly faecal collections were required for measurement of their ^{42}K content. However, intravenous injection of ^{42}K involved considerably greater labour to prepare and administer. It was still necessary to collect urine and sometimes faeces and

although the mixing time was shorter it was not usually possible to carry out the whole procedure within normal working hours. It was concluded that in the present studies intravenous administration did not justify the extra effort involved.

VII. 3. Calibration Based on Body Geometry:

In order to eliminate the ^{42}K procedure and to provide a result with minimal delay, an attempt was made to obtain some correction factor to apply to the ^{40}K count-rate depending on the size and shape of the individual subject. Having established the mixing time for ^{42}K and that oral administration was satisfactory for our purposes, 45 observations using the ^{42}K technique were made on 42 subjects, 29 males and 13 females, of whom 25 were normal and 17 suffered from various diseases; these latter subjects included those with potassium deficiency or other metabolic derangements. Inspection of the results showed that they fitted a simple formula, based on self-absorption, and which was an analysis similar to one of those given by Meneely, Ball, Ferguson, Payne, Lorimer, Weiland, Rolf and Heyssel (1962). All results presented in the tables were then calculated on the basis of this formula:

$$WBK = (c/r)\ a \exp\left(b\left(\frac{W}{H}\right)^{\frac{1}{2}}\right) \qquad (9)$$

where WBK was the whole body potassium in grams, c/r is the ^{40}K count-rate, a and b are constants with values in our work of a = 3.324 and b = 1.289. W is the subject's weight in kg and H is height in cm. In this formula $\frac{W}{H}$ is a measure of the cross-sectional area of a

cylindrical subject and the square root of this is a measure of the radial thickness. The exponential term represents absorption. This formula is based on the model of the human body as a cylinder with a central core of activity surrounded by an absorbing sleeve. Thus an approximate correction for the absorption of the gamma rays on traversing the body is made by the exponential term. It was found that by taking the measured ^{40}K count-rate (in counts/second normalized in terms of the sensitivity of the apparatus to the reference potassium chloride source) and applying this exponential formula, that the calculated values for whole body potassium obtained were in good agreement with those obtained by ^{42}K calibration. For the 45 observations, which included normal and diseased subjects, the difference in the values for the potassium content given by the formula and given by ^{42}K calibration carried a standard deviation of $\pm$ 3.5 per cent which is almost entirely compatible with the experimental accuracy (Table 11).

Table 11. Comparison of Absolute Calibration of Whole Body Potassium Measurements by Formula Based on Body Geometry and by ^{42}K Technique.

No.	Age	Weight	Height	$^{K}_{calc.}$	$\frac{(^{K}_{calc.} - ^{K}_{42}) \times 100}{^{K}_{42}}$
	yrs.	kg	cm	g	%
Males - Normals.					
1	33	50.9	168	102.2	-7.8
2	34	52.9	168	97.9	-11.4
3	36	55.7	153	110.3	-0.1
4	27	73.7	165	131.8	-3.4
5	22	79.6	178	154.1	-6.6
6	63	85.0	161	128.0	+1.4
7	62	68.5	174	118.1	+3.9
8	62	66.8	174	108.8	-1.1
9	54	60.9	171	121.0	+1.7
10	51	61.0	163	108.1	+2.7
11	51	72.7	173	144.2	+3.7
12	57	89.5	176	137.4	0
13	31	56.9	166	117.6	-0.7
14	75	64.5	164	86.3	+6.9
15	63	46.9	167	89.1	+5.3
16	55	54.7	151	111.3	+5.2
17	42	62.7	168	133.6	+1.1
18	54	66.3	170	124.5	-5.9
19	49	82.3	179	117.8	-5.8
20	65	56.6	155	103.6	+1.4
21	41	81.9	172	158.1	+7.4
22	51	89.6	175	130.2	-0.6
23	34	94.7	181	155.7	-2.8
24	51	75.3	173	123.0	-1.0
25	42	72.1	166	131.0	+2.0
Males - Pathological					
26	22	59.9	181	121.2	+2.0
27	60	69.1	173	110.4	-6.1
28	49	66.0	173	117.8	+0.7
29	57	65.5	177	126.0	+0.4
30	23	48.0	170	98.0	-0.3
31	47	42.1	163	65.6	-8.5
32	47	41.7	163	63.7	-0.3

No	Age	Weight	Height	$K_{calc.}$	$\frac{(K_{calc.} - K_{42}) \times 100}{K_{42}\%}$
	yrs.	kg	cm	g	
Females - Normals					
33	20	42.8	158	69.4	-3.7
34	50	55.2	154	83.1	-3.2
35	60	60.0	151	81.6	+3.3
Females - Pathological					
36	25	60.5	173	108.7	-5.2
37	21	61.7	163	76.0	+5.6
38	50	54.1	164	68.1	-0.1
39	43	57.7	157	68.4	+3.1
40	40	56.4	157	81.6	+4.9
41	41	52.1	159	74.5	-0.8
42	76	55.4	155	70.9	-4.2
43	49	36.4	165	63.2	-4.2
44	54	60.2	154	75.7	-1.6
45	30	48.0	161	78.7	-5.9

38 out of 45 (84 per cent) $\leq \pm$ 6 per cent.

43 out of 45 (95 per cent) $\leq \pm$ 7.8 per cent (equivalent to S.D. of 3.9 per cent).

VIII. MEASUREMENT OF WHOLE BODY POTASSIUM IN NORMAL MALES

VIII. THE MEASUREMENT OF WHOLE BODY POTASSIUM IN NORMAL MALES

VIII. 1. Introduction:

Before using the whole body counter for clinical studies of potassium depletion, a survey was made of whole body potassium content in normal male subjects. It was originally intended to investigate potassium depletion associated with hyperchloraemic acidosis following total cystectomy and uretero-colic anastomosis. As this operation is performed more commonly in the male it was decided that the first group of control subjects should also be males. The scope of these investigations has now been widened and one object of future study will be a survey of body potassium content in normal female subjects.

Previous investigators examined exchangeable potassium as a parameter of body composition and showed that the highest correlation was obtained between exchangeable potassium and total body water (I. 4. d. p.16). The present study examines the correlation between whole body potassium measured by gamma ray spectrometry and total body water measured by the dilution of tritiated water in normal male subjects.

VIII. 2. Methods:

The subjects for this study included 22 male patients who had been hospitalized for minor surgical procedures or for diagnostic studies. None had disease entities which are known to influence water or electrolyte metabolism. During the studies patients continued on normal fluid intake and hospital diet. All subjects

were weighed in pyjamas and dressing gowns for which allowance was made, and their height was measured without shoes in the standing position.

Whole body potassium was measured in the Leeds plastic scintillator counter described by Burch et al. (1962).(VI.4. c.p.83). The subjects and initial and final backgrounds were counted for a minimum of 1,000 seconds each (Hughes, 1963 a). Absolute calibration by the ^{42}K technique was used on most of these normal males but for uniformity the results based on the formula associated with body geometry have been used in all cases (Section VII).

Total body water was measured by the dilution of tritiated water following the technique described in Section II.

VIII. 3. Results: The values obtained for serum concentration of potassium, whole body potassium and total body water by dilution of tritiated water are given in Table A together with the weight, height and age of the subjects. In 3 subjects these measurements were repeated after an interval which was 1 month for 2 of the subjects and 4 months for the third subject.

The following regression equations for these data, with the probable errors for the constants, were established by the Electronic Computing Laboratory, University of Leeds, using a least squares fitting programme:

$$WBK = (3.16 \pm 0.20)\ (TBW) \pm (7.58 - 8.16) \qquad (10)$$

S.D. from regression = 7.43 gK.

$$WBK = (1.05 \pm 0.14)\ (\text{Body Weight}) + (48.74 \pm 10.03) \qquad (11)$$

S.D. from regression = 12.67 gK.

where WBK is whole body potassium in grams, TBW is the total body water in litres and body weight is measured in kilograms.

VIII. 4. Discussion:

Anderson (1963) showed that the ratio of whole body potassium to body weight decreased with advancing years. In the age range 18 years to 74 years the value for males decreased from 2.2 to 1.6 grams potassium per kilogram (gK/kg). The average value for the present study was 1.74 gK/kg for males ranging from 34 years to 75 years.

Talso et al. (1960) measured exchangeable potassium in 37 males and 13 females and obtained the following regression equations with total body water measured by the dilution of N.A.A.P. and with body weight.

$$EBK = 3.27\ (TBW) + 0.38 \qquad (12)$$

S.D. from regression = 11.5 gK

$$EBK = 1.72\ (\text{Body Weight}) - 3.29 \qquad (13)$$

S.D. from regression = 21.7 gK

in which EBK is the exchangeable body potassium in grams, TBW the total body water in litres measured by the dilution of N.A.A.P. and body weight is measured in kilograms. The regression obtained against total body water is similar to equation (10) in the present study even though different test substances were used. For regression against body weight, the constant in equation (11) is smaller than that in equation (13) but this is to be expected in a group containing females who have a lower potassium content in relation to gross body

weight than males (Anderson, 1963).

The potassium concentration is different for the various tissues in the body. Approximate values have been summarized by von Döblen (1962) who separated the lean body mass into muscle and the lean muscle-free body mass. He gave the concentration of potassium in muscle as 3.2 g/kg compared with 1.6 g/kg for the lean muscle-free body mass, the latter comprising skeleton, skin, blood, C.N.S., liver and other viscera. Therefore the division of the body into the two components of lean body mass and body fat as it is usually considered for body water studies, is not sufficient for potassium measurement which must be considered in the light of the different potassium concentrations in the two subdivisions of the lean body mass. When these concentrations apply in health, simultaneous equations can be constructed which will give values for muscle, non-muscle lean and body fat. However, in disease, where these concentrations probably do not apply, the constants in the simultaneous equations would be uncertain and this analysis would be inapplicable. Therefore the present investigation has examined the gross potassium content in relationship to total body water which is representative of the lean body mass. It was appreciated that this introduced an uncertainty in the relationship of whole body potassium to total body water because of the variation in the mass of muscle to non-muscle lean tissue in the patient. However, it was considered justifiable to investigate the whole body potassium and total body water relationship on the grounds that this provided a convenient measurement for clinical studies.

It was appreciated that errors may be more pronounced at the extremes of body composition. Thus an extremely muscular subject might have considerable potassium loss before the relationship of whole body potassium to total body water falls below normal. At the other extreme, a non-muscular subject might be regarded as potassium deficient though in actual fact he could be normal.

A closer relationship might be found between whole body potassium and intra-cellular water. There is no direct technique, however, for measuring the latter and the inaccuracies introduced by the additional experimental determination of extra-cellular fluid and its interpretation probably outweigh the improvement in the theoretical model.

IX. WHOLE BODY POTASSIUM MEASUREMENTS IN DISEASE

IX. WHOLE BODY POTASSIUM MEASUREMENTS IN DISEASE

IX. 1. Introduction:

Although there are many reports on exchangeable potassium measurements in disease, there have been few reports in the literature on whole body potassium measurement. This is due, at least in part, to the absence of whole body counting facilities of sufficient sensitivity in hospitals. (VI. 6. p.85).

In the present work, studies were made on potassium deficiency associated with uretero-colic anastomosis, and in a group of miscellaneous conditions, including Crhon's disease, renal failure and hypertension. Also the potassium-water relationship was studied in a few oedematous patients.

IX. 2. Whole Body Potassium Deficiency Associated with Uretero-Colic Anastomosis.

(a) History: The operation of uretero-colic anastomosis had been performed for many years and although acidosis was occasionally reported as a complication, it was only with the report of Ferris and Odel (1950) that the high incidence and pattern of body fluid and electrolyte disturbances were appreciated. They found hyperchloraemic acidosis in 79 per cent of 141 patients with bilateral uretero-colic anastomosis. It has been suggested that the hyperchloraemic acidosis is due either to absorption of urinary chloride from the gut or that it is the result of impaired renal function, particularly of the

tubular base-sparing mechanisms. Wilkinson (1952) considered that the acidosis followed a loss of bases, especially potassium, in gut secretions of abnormally large volume resulting from the presence of urine in the bowel. The first suggestion was advanced by Ferris and Odel (1950) who noted that acidosis did not develop in patients who remained rectally incontinent, but might arise soon after sphincter control was obtained. The experiments of Parsons, Powell and Pyrah (1952) and Parsons, Pyrah, Powell, Reed and Spiers (1952) indicated that there was a greater net uptake of chloride from the bowel than of the fixed bases sodium and potassium. The effect of impaired renal function, particularly of the renal tubular capacity to reabsorb bicarbonate, and manufacture and excrete hydrogen ions and ammonia, was suggested by Kekwick, Paulley, Riches and Semple (1951) and Lapides (1951); the latter suggested that the electrolyte imbalance followed renal damage due to recurrent attacks of pyelonephritis. Jacobs and Stirling (1952) found that although hyperchloraemic acidosis could arise in patients with normal excretory pyelograms, it was much more frequent when function was considered to be moderate or poor. Whether the biochemical changes which follow ureteo-colic anastomosis are ever solely due to impairment of renal function is doubtful, but the two mechanisms may reinforce each other for colonic absorption of urinary solutes is more likely to lead to disturbances of homeostasis if renal function is impaired. In experimental studies with dogs, Hayward, Wakin, ReMine and Grindlay (1961) showed that hyperchloraemic

acidosis might be expected following urinary diversion to the colon if a s sufficiently large area of mucosa was exposed. Unless renal function was greatly impaired, the kidney itself did not contribute to the imbalance of electrolytes.

Although Ferris and Odel (1950) did not record plasma potassium concentrations, two other studies that year reported hypokalaemia with hyperchloraemic acidosis following bilateral uretero-colic anastomosis (Foster, Drew and Wiss, 1950; Diefenbach, Fisk and Gibson, 1950). Since then the relationship has been reported on many occasions and hypokalaemia is not recognized as a serious complication requiring treatment as well as correction of the hyperchloraemic acidosis. Slight or moderate hypokalaemia is frequently found and Jacobs and Stirling (1952) found with routine plasma potassium estimations, that 34.6 per cent of 142 patients had some degree of hypokalaemia at some time after operation.

(b) Aetiology: Several reasons have been advanced for potassium deficiency following uretero-colic transplantations. It is well known that abnormal potassium loss in the urine accompanies acidosis whether the latter is due to abnormal production or ingestion of acid radicles in the presence of normal renal function, or to the impairment of the renal tubular capacity to form ammonium and hydrogen ions. The renal tubules appear to conserve sodium at the expense of potassium by exchange of potassium for sodium ions carried to it in the glomerular filtrate. In addition, it is well established that various forms of chronic diarrhoea can lead to abnormal potassium losses from the bowel and it may be that potassium loss through the large bowel is produced

when it acts as a urinary reservoir (Wilkinson, 1952). Lastly, potassium deficiency may follow acute dehydration for water deficiency may be partly borne by mobilization of intracellular water with subsequent liberation of intracellular potassium.

Previous studies have reported potassium deficiency in terms of the serum concentration, but while hypokalaemia indicates a deficiency of whole body potassium, such a state may exist before the extracellular potassium concentration falls. In the present study, whole body potassium was measured by gamma ray spectrometry and the value compared with the expected normal value for that patient estimated from the total body water and the regression equation (10) established in Section VIII. Twenty observations were made on 18 male patients, aged 22 to 71 years, on 15 of whom uretero-colic anastomosis, with or without cystectomy, had been performed at time intervals varying from 14 days to 27 years before measurement. In the remaining 3 patients left iliac colostomy had been established and a recto-sigmoid bladder constructed. Operation had been performed on 14 patients for bladder tumour; on 2 patients for the results of severe trauma with irreparable rupture of the urethra; on 1 patient who had a contracted tuberculous bladder and on another patient with congenital bladder exstrophy. Clinical histories are summarized in Appendix 3. At the time of measurement all patients were active, ambulant and taking a normal diet and free fluid intake. No patient was measured during an episode of acute electrolyte imbalance. Blood chemistry was performed on the day of whole body potassium measurement. Most patients had been recommended to take sodium bicarbonate in doses of 2 to 4 g daily

following the operation, though some of them may have been unreliable in taking the medication.

(c) Results and Discussion: The results are shown on Table With 2 exceptions, all patients showed a potassium deficiency which ranged from 1.9 per cent to 24.6 per cent with a mean value of 11.9 per cent. The whole body potassium deficiency was not reflected in the serum potassium concentrations, thus agreeing with the findings of Flear, Cooke and Quinton (1957), nor was there any apparent correlation with the duration of uretero-colic anastomosis and the degree of uraemia, acidosis, or hyperchloraemia. Three of the patients, (H.M., J.A. and A.R.), had been admitted on previous occasions with severe electrolyte imbalance requiring urgent replacement therapy. Two of them (H.M. and J.A.) had recurrent pyelonephritis with renal failure and in the third (A.R.) the deficiency was precipitated by a bout of diarrhoea. These 3 patients were among those with the greatest potassium deficiency. Hyperchloraemia was present in 11 patients and some degree of acidosis in all patients except one; however, most patients were taking sodium bicarbonate in a dosage proportional to the degree of acidosis. Potassium deficiency was also present in 3 patients on whom a recto-sigmoid bladder had been constructed.

Two patients did not have a potassium deficiency. One of these (F.F.) was a well-developed, muscular farm labourer who might be regarded as having an extreme body composition in which the large muscular element would hide a mild potassium deficiency (VIII. 4.p.97). The other patient (W.H.) had total cystectomy and uretero-colic anastomosis performed for bladder carcinoma. He was admitted to

hospital 4 years later as an emergency with a large bowel obstruction due either to recurrent tumour or to the effects of radiotherapy. Obstruction was relieved by a left descending colostomy and the body potassium was measured 21 days later. Apart from any possible effect of this recent episode, there was no apparent reason why the condition of his whole body potassium should differ from those of the other patients described.

Erroneous results could be obtained if there was an absolute increase in the total body water following uretero-colic anastomosis. There is clinical evidence that these patients are unable to conserve water normally, and this was confirmed by the studies on water balance by Kekwick et al. (1951). In the present study, a very large increase in the absolute value would be required to produce a discrepancy of the order found. In addition, the total body water was estimated in 2 patients before operation and again at a later date following operation. In one patient (W.R.) the total body water decreased by 3.7 litres and in the other it increased by 1.4 litres. These results do not suggest a large absolute increase in body water.

Ansell, Geist and Creevy (1961) measured exchangeable body potassium by ^{42}K dilution in 4 children, 4 adult males and 4 adult females, on whom uretero-sigmoidostomy had been performed. They expressed the results in terms of body weight and compared them with those for a group of normal controls. They concluded that the average deficit in body potassium store was about one-third of the store in normal individuals in similar age groups and observed that there was no apparent correlation between potassium deficiency and the duration

of uretero-sigmoidostomy and the degree of uraemia, acidosis, hyperchloraemia or hypokalaemia. The present study used more accurate methods for assessment of potassium deficiency and showed that most patients have a deficiency though not as great as that reported by Ansell et al. (1961).

If the abnormal potassium losses are renal and solely due to acidosis then the prevention of acidosis by a high fluid intake, frequent voiding, restriction of dietary chloride and medication with sodium bicarbonate, should prevent excessive loss of potassium. All these methods were employed on the patients who were studied yet significant potassium deficiences were still present. It is suggested that, unless there is impairment of glomerular function, routine potassium supplementation by oral administration of 1 to 2 g potassium citrate daily is advisable.

IX. 3. Miscellaneous Conditions.

The whole body potassium was measured in a few patients suffering from a variety of conditions in which potassium deficiency might be found. This was not a full investigation into the problem in the conditions concerned but was more of a pilot study to outline future investigations.

(a) Crohn's Disease and Malabsorption Syndrome: Potassium deficiency may arise in relation to gastro-intestinal disease, either from decreased intake or decreased absorption from the gastro-intestinal tract or as the result of increased losses from it. Decreased potassium intake alone does not lead usually to severe potassium

depletion, but it may become a major contributing factor when some other mechanism is also operating. Absorption from the intestinal tract can be severely interfered with in some of the malabsorption states; potassium deficiency has been described in Crohn's disease (Heitzman, Patterson and Stanley, 1962), in sprue (Harrison, Tompsett and Barr, 1943) and in other states associated with a malabsorption syndrome. Ariel (1954) described potassium loss from diarrhoea and Schwartz and Relman (1953) showed potassium deficiency induced by the use of laxatives.

The patient studied, J.C., had suffered from recurrent Crohn's disease for eight years. Multiple and extensive resection of small bowel had been performed and he was first admitted to The General Infirmary at Leeds for investigation of malabsorption syndrome. Before admission he had been advised to limit himself to a light diet and on this regime he had failed to thrive. When first seen in July 1963, he was thin but active; he showed no clinical evidence of potassium deficiency. The results of repeated measurement of whole body potassium over a six-month period are shown in Table On first attendance in July 1963, the serum potassium concentration was 4.2 mEq/l. Although this was within the normal range, whole body potassium measurements showed a deficiency of 39.2 per cent of the expected value. At that time the concentration of calcium in the serum was 6.8 mg/100 ml and of magnesium 1.1 mEq/l. Milk of magnesia and a more substantial diet were prescribed and by the second measurement three months later, the whole body potassium had increased by 14.5 per cent although body weight had increased

by only 2.0 kg. At this time the serum concentration of potassium remained in the normal range, magnesium had increased to 1.5 mEq/l, but calcium remained low at 7.2 mg/100 ml. By the following month the potassium deficiency was only 22.1 per cent of the expected value. Although the serum concentration of magnesium was now within the normal range, that of calcium remained low and treatment with vitamin D was started. At the last measurement, six months after the initial one, whole body potassium was 69.7 g an increase of 18.6 g from the original measurement. Serum concentrations of magnesium and potassium were normal and that of calcium had increased to 8.9 mg/100 ml. There had been little alteration in body weight throughout.

This patient represents a state of multiple deficiencies due to inadequate absorptive area of the small bowel and aggravated by a period on restricted diet. Although potassium deficiency has been reported in Crohn's disease (Heitzman et al. 1962) there was no evidence that the disease was active in this patient during the period of measurement.

(b) Renal Failure: The potassium to water relationship was examined in three male patients suffering from renal failure who were admitted for treatment by haemodialysis. The results are shown in Table 12. Patient J.L., aged 34 years, suffered from chronic glomerulo-nephritis and had a urinary output of about 500 ml/day. Patients P.L., aged 38 years, and S.B., aged 56 years,

had congenital polycystic disease of the kidneys and their urinary outputs were satisfactory at the time of examination. All had blood urea nitrogen concentrations in excess of 100 mg/100 ml and patient J.L. was kept alive only by repeated dialyses.

As the normal water content of these patients was in some doubt, the results of the whole body potassium measurements could not be interpreted with confidence. Deviations of the total body water from normal could be estimated if the whole body potassium remained a stable parameter, but in renal failure this would be an unwarranted assumption.

(c) Hypertension: De Wesselow and Thomson (1939) demonstrated that many hypertensive patients have a low serum potassium concentration and Hilden and Krogsgaard (1958) confirmed this finding; both found that hypokalaemia was particularly common in patients with malignant hypertension. Wrong (1961) found a definite hypokalaemia in 20 per cent of 64 patients with severe hypertension, the greatest incidence being in those patients with papilloedema. He considered that the term "primary" aldosteronism was not applicable to this syndrome but that the hypokalaemia appeared to be due to hyperaldosteronism caused by renal ischaemia, the hypertension itself, or some closely related factor. The potassium-losing effect of the hypertensive diuretics, chlorothiazide, hydrochlorothiazide and trichlormethiazide, is well known and replacement therapy with potassium is recognized practice. Remenchik and Miller (1962) measured whole body potassium in hypertensive patients and demonstrated potassium deficiencies resulting from treatment with antihypertensive drugs.

Patient E.C., a male aged 42 years, suffered from malignant hypertension with blood pressure 170/120 mm Hg. He was first admitted in October 1963, having had no previous investigations or treatment. On admission he complained of symptoms of potassium deficiency such as weakness, malaise, and was easily tired. At that time the serum potassium concentration was 2.6 mEq/l and the electrocardiograph showed the typical changes of potassium deficiency. He was treated for three weeks with oral potassium supplementation and the diagnosis of primary aldosteronism was eliminated. Whole body potassium measurement was made six weeks after oral supplementation of potassium had been discontinued and at that time no antihypertensive drug was being used. The results are shown in Table 12. Although the serum concentration of potassium was now within the normal range, a whole body potassium deficiency of 21.1 per cent of the expected value was found.

From the observations of Wrong (1961) and this observation it would appear likely that severe deficiency of potassium may accompany hypertension. A further full clinical study is indicated. Again a considerable deficiency in whole body potassium was present with a normal serum concentration of potassium.

(d) The Nephrotic Syndrome: Two female patients with nephrotic syndrome were studied. Although sufficient data are not available to calculate a regression equation for normal females and the expected body potassium cannot therefore be estimated accurately, examination of the ratio of measured potassium to whole body water shows a gradual

response to treatment in these two women (Table 13).

Patient S.B., a girl of 21 years, had an influenza-like illness which was followed two weeks later by a marked diminution in urinary output and the onset of generalized oedema. During six weeks of treatment with restricted fluid intake, Aldactone and Cortisone, there was a considerable improvement in her condition. The body weight decreased by 8.9 kg and the total body water by 11.3 litres. The whole body potassium decreased to a lesser extent and the potassium to water ratio increased.

Patient R.H., a woman of 32 years, noted the sudden onset of generalized oedema accompanied by a decreased urinary output. She had no previous symptoms. She responded well to treatment with Aldactone and Prednisolone. The potassium to water ratio increased from 2.19 to 3.12. While the measured body potassium remained more or less constant, the total body water decreased by 13.6 litres and the body weight by 9.8 kg over a period of two months.

It is appreciated that Cortisone and Prednisolone have a potassium - losing effect and that Aldactone tends to conserve potassium, but these pharmacological effects do not appear to have affected the measurements that have been made. More accurate deductions will be possible when further data on normal females are available. It would appear that some estimate of excess fluid may be made in the nephrotic syndrome, when the whole body potassium is measured.

IX. 4. Summary.

(a) Most patients with ureteric transplantation into the rectum have a considerable deficiency in whole body potassium.

(b) The whole body potassium deficiency is not reflected in the serum concentration of potassium.

(c) Whole body potassium deficiency is present if a recto-sigmoid bladder is constructed.

(d) Marked deficiency of whole body potassium has been demonstrated in a patient with malabsorption and in another with hypertension.

Table 12. Comparison of Measured and Expected Values for Whole Body Potassium in Miscellaneous Conditions.

Name	Disease	Date	Wt. kg	Ht. cm	Age Yrs.	Total Body Water l.	Whole Body Potassium Measured g	Whole Body Potassium Expected g	Deficiency per cent	Serum K mEq/l
	Mal-absorption Syndrome									
J.C. 1		17. 7.63	40.1	163	47	29.2	51.1	84.7	39.2	4.2
2		22.10.63	42.1	"	"	-	65.6	-	-	4.1
3		27.11.63	39.6	"	"	29.0	65.5	84.1	22.1	4.4
4		3. 1.64	43.2	"	"	-	69.7	-	-	3.9
	Chronic Glomerulo-Nephritis									
J.L.		24. 8.62	67.7	175	34	40.1	121.4	119.1	- 1.9	5.1
	Polycystic Kidneys									
P.L. 1		22. 8.62	62.5	176	38	40.4	113.4	120.1	5.6	4.7
2		2.10.62	59.3	"	"	33.3	106.7	97.6	- 9.3	4.1
S.B.		20. 2.63	66.0	173	56	41.6	117.8	123.9	4.9	4.0
	Malignant Hypertension									
E.C.		9.12.63	78.3	174	42	49.0	116.2	147.3	21.1	4.1

Table 13. Alteration in Potassium to Water Ratio in Two Women with Nephrotic Syndrome

Name	Date	Wt. kg	Ht. cm	Age Yrs	Whole Body Water l.	Whole Body Potassium g	WBK / TBW
S.B.1	8.3.63	70.6	163	21	52.5	84.7	1.61
2	26.4.63	61.7	"	"	41.2	76.0	1.84
R.H.1	4.1.63	79.1	168	33	42.0	92.1	2.19
2	28.1.63	69.5	"	"	31.4	88.1	2.81
3	5.3.63	69.3	"	"	28.4	88.7	3.12

Table 14. Comparison of Measured and Expected Whole Body Potassium Measurements in Patients with Bilateral Uretero-Colic Anastomosis and with Recto-Sigmoid Bladder.

Subject	Body Weight	Height	Age	Total Body Water	Whole Body Potassium Measured	Expected	Deficiency	Na	K	Cl	CO_2	Urea N
	kg	cm	yrs	l.	g	g	%	mEq/l	mEq/l	mEq/l	mEq/l	mg/100
T.E.1	49.8	178	24	35.3	81.8	104.0	21.3	138	3.4	106	27	12
T.E.2	51.9	178	24	32.3	84.1	94.5	11.0	133	3.5	110	14	17
H.M.1	60.3	150	61	26.9	65.7	77.4	15.1	139	4.0	107	22	36
H.M.2	55.2	150	62	30.9	72.5	90.1	19.5	140	4.4	112	14	55
A.D.	45.0	170	37	29.9	80.7	86.9	7.1	138	4.2	106	24	16
A.W.	59.9	181	22	42.5	121.2	126.7	4.5	142	4.4	111	18	34
J.A.	57.8	157	68	33.4	83.4	98.0	14.9	140	3.8	110	17	60
J.R.	62.5	163	53	38.3	98.7	113.4	13.0	140	3.9	92	28	15
B.G.	51.9	164	71	35.0	92.8	103.0	9.9	139	4.5	104	23	35
A.W.	59.6	180	34	36.6	92.8	108.1	14.2	131	4.9	93	22	19
W.R.	77.5	161	63	38.0	105.9	112.5	5.9	138	4.3	103	16	15
F.F.	84.4	170	50	45.4	147.0	135.9	(Excess 8.2)	135	3.7	108	22	20
W.H.	59.0	158	60	37.7	126.1	111.6	(Excess 13.0)	144	4.5	116	25	30
L.T.	56.4	163	62	35.2	92.9	103.7	10.4	141	4.8	116	24	54
H.H.	62.0	180	58	39.6	108.5	117.6	7.7	143	4.2	111	25	30
M.A.	53.3	172	59	36.3	105.3	107.3	1.9	142	4.8	115	23	19
A.R.	52.9	166	63	36.9	82.2	109.0	24.6	135	4.2	109	21	32
Recto-Sigmoid Bladder												
R.I.	68.5	172	65	39.6	106.1	117.6	9.8	139	3.7	103	25	27
R.C.	74.1	170	58	40.0	109.6	118.8	7.7	135	4.1	118	10	31
A.L.	69.1	173	60	43.6	110.4	130.2	15.2	138	4.4	100	26	45

X. EPILOGUE

X. EPILOGUE

The experiments and studies which have been described demonstrate that tritiated water, in our hands, has proved a satisfactory method for the measurement of total body water. With careful technique, satisfactory results can be obtained after the administration of dosages small enough to allow several repeat estimations to be made. In experimental animal work, urea and creatinine have been shown to have volumes of distribution similar to the total body water as measured by the dilution of NAAP and tritiated water. Clinical studies have shown that the total body water can be estimated from the extraction of urea and creatinine during haemodialysis. The total body water provides a useful parameter for whole body potassium measurement in health and disease.

The basic problems of calibration having been solved, further avenues of investigation by whole body potassium measurement have been opened up. Further studies on the normal female are indicated first. Thereafter a variety of conditions can be examined to give results of value in the clinical management of these patients. The conditions include pyloric stenosis, Crohn's disease, ulcerative colitis, primary aldosteronism, hypertension and hyperthyroidism. An opportunity is available for the study of the loss of potassium produced by various drugs given over a long period of time; the anti-hypertensive drugs in particular should be investigated.

Although the apparatus used for whole body potassium counting in these studies is the only one with sufficient sensitivity located in a general hospital in this country, similar apparatuses are now being made available. Over the next ten years this technique will become established in many centres where it will provide a more accurate and less troublesome method of body potassium measurement than exchangeable studies.

Appendix 1. Tritium Counting Technique established by Mr.A.H.Smith, Department of Medical Physics, University of Leeds.

Apparatus and Materials:

A Nuclear Enterprises (G.B.) Ltd. Tritium Counter (NE 8301) was used with a dioxane based scintillator fluid (NE 220) which can dissolve up to 20 per cent by volume of water. Conditions for optimum efficiency were obtained by maximizing $\frac{S^2}{B}$ where S and B are counting rates for tritiated water sample and background respectively. All specimens were counted in quartz ampoules.

From tritiated water of activity 200 mC per ml, supplied by the Radiochemical Centre, Amersham, a stock solution of 1 mC per ml was prepared and of this 0.3 to 0.5 ml was diluted in 50 ml 0.9 per cent saline to provide injection solutions.

Method:

The plasma samples whose radioactivities were to be assessed were subjected to a vacuum distillation process (Vaughan and Boling, 1961) yielding pure water, an aliquot of which could be added directly to the scintillator fluid. 1.5 ml plasma was pipetted into each distillation flask, and 0.4 ml of the distillate added to 5.5 ml scintillator fluid.

Standards, in duplicate, were prepared from dilutions x 200 of the injection solution and distilled in the same way.

Both standard and background ampoules included 0.4 ml of distillate obtained from pre-injection plasma samples and the contents (dispensed gravimetrically) of the three categories of

ampoule are summarized in the table.

Counting Procedure:

All distillates, including the standards, were counted in duplicate. Standard ampoules were interspersed among sample ampoules and the background ampoule was counted at the beginning and end of a series. The preparation of samples preceded counting by at least fifteen hours and the ampoules were kept in darkness as completely as possible to minimize any effect of phosphorescence.

Derivation of Results:

It was noted that a complication in this method of tritium counting was that the observed response to a given activity of tritiated water depended on the particular quartz ampoule used. However, by cross calibrating the ampoules with standard solutions it was possible to derive correction factors to bring them all to a comparable basis. By comparing the corrected counting rates of sample and standards, the activities of the sample were derived and hence the radioactive concentration (μc/l) of the plasma water. For a plasma sample taken at time t

$$\text{Volume of distribution (litres)} = \frac{\text{Radioactivity of injection solution (μc)}}{\text{Radioactive concentration of plasma water (μc/l)}}$$

At least 10,000 counts per sample were obtained to give a statistical counting accuracy of not less than 1 per cent. The total experimental standard deviation including dispensing, calibration, counting and reproducibility, was not greater than ± 3 per cent.

The mean calculated efficiency was 10.5 per cent with a background of 30 c.p.m.: the tritiated plasma distillates had activities of the order 30 to 40 μc/l.

	NE 220 scintillator (ml)	Post-injection plasma (ml)	Diluted injection solution (ml)	Pre-injection plasma (ml)	Water (ml)
Sample	5.5	0.4	-	-	0.1
Standards	5.5	-	0.1	0.4	-
Background	5.5	-	-	0.4	0.1

Appendix 2. Summary of Clinical Histories of Patients with Total Body Water Measurement during Haemodialysis.

Name	Age	Sex	Dialyses (days from onset of renal failure)	Remarks
Traumatic:				
M.F.	74	F	7	Acute renal failure following cholecystectomy and choledochotomy with post-operative collapse. Oedematous. Died 4 days after dialysis with purulent bronchopneumonia.
F.O.	53	F	6,8 & 11	Radical mastectomy for carcinoma of breast with post-operative collapse followed by acute renal failure. Died 3 days after last dialysis. Obese. Urinary output less than 500 ml/day.
R.B.	21	M	10, 12 & 15	Fractured skull with prolonged concussion following vehicle accident. Multiple soft tissue injuries. Recovered fully, apart from right 3rd cervical nerve palsy.
F.S.	44	M	7,9 & 13	Acute renal failure following cardiac arrest during cholecysteotomy. No recovery of renal function. Died.
R.A.	17	M	3,6,9,12 & 16.	Motor cycle accident. Fractured pelvis, both femora, compound right tibia and fibula, right Colles' fracture and laparotomy for intra-abdominal injuries. Above knee amputation, right leg, and below knee amputation, left leg, performed on day after accident. High rate of protein breakdown. Urinary output returned just prior to last dialysis. Recovered.
E.J.	45	F	5,8 & 12	Self-inflicted gun-shot wound of abdomen. Left nephrectomy, splenectomy and colostomy performed. Urinary secretion gradually increased following last dialysis. Recovered.

Name	Age	Sex	Dialyses (days from onset of renal failure	Remarks
Obstetric:				
J.D.	38	F	14.	A 28-week still-born foetus was followed by profuse haemorrhage. Thereafter urinary output decreased to nil. Plasma urea nitrogen started to fall 12 days after dialysis. Probably incomplete cortical necrosis.
P.D.	27	F	7	Severe pre-eclamptic toxaemia, surgical induction of labour and post-partum haemorrhage. Diuresis started the day after dialysis. Full recovery.
Pyelo- and Glomerulonephritic Renal Failure.				
H.W.	59	M	9	Previous retrograde pyelograms had shown appearances of chronic pyelonephritis. Admitted with progressive uraemia and convulsions. Plasma urea nitrogen concentration settled at 95 mgm/100 mm after dialysis. Died 6 months later. Autopsy confirmed chronic pyelonephritis.
I.L.	52	F	12 & 25	Sub-acute glomerulonephritis produced oedema and fibrinous pericarditis. Unexplained sudden death following second haemodialysis. Autopsy confirmed diagnosis.
G.A.	61	M	16	Admitted with oliguria, oedema and hypercalaemia. A needle renal biopsy confirmed sub-acute glomerulonephritis. Dialysized once only.
M.B.	54	F	7, 9, 26 & 60	Mild, chronic uraemia for 4 years, with rapid deterioration for 1 week. First dialysis for 3 hours only. Collapsed and died after 4th dialysis. Autopsy showed chronic pyelonephritis and fibrinous pericarditis.
J.L.	37	M	7,9,22, 38 & 51	Symptoms of chronic uraemia for 2 months. Worse in the week prior to admission. First dialysis 4 hours only, others for 6 hours. Open renal biopsy confirmed diagnosis of chronic glomerulonephritis. After 5 dialyses cadaveric renal transplantation performed. Dialysized 3 times after transplantation but condition deteriorated. Died.

Name	Age	Sex	Dialyses (days from onset of renal failure	Remarks
J.M.	22	M	9	Nephrotic syndrome with superadded pyelonephritis. Urinary output started to decrease 9 days before dialysis. Remained oliguric and died from pulmonary oedema 8 days after dialysis.
W.O.	62	F	14,15 & 20	Throat infection followed by haematuria and oliguria. First dialysis 2 hours only, was performed for hyperkalaemia. Renal biopsy showed haemorrhagic glomerulonephritis. No recovery of kidney function; patient died during 3rd dialysis.
F.H.	61	M	9 & 11	Total cystectomy for carcinoma of bladder with ureterosigmoidostomy 10 years previously. Oliguria developed about 1 week before first dialysis. Patient died during second dialysis. Autopsy showed suppurative pyelonephritis and acute pancreatitis.
Miscellaneous:				
J.C.	36	M	11,13 & 17	Copious vomiting and diarrhoea for 11 days led to dehydration and renal failure. First dialysis 3 hours only. Copious replenishment of fluid given and gradually urinary output increased. Recovered.
M.A.	30	F	4	Copious vomiting and diarrhoea progressing to circulatory failure. Recovered on antibiotics, fluid replenishment and haemodialysis.
A.B.	37	M	4 & 9	Collapsed dorsal spine thought to be tuberculous. Some return of renal function after 2 dialyses. Collapsed and died 18 days after second dialysis. Autopsy showed multiple myelomatosis.
P.L.	36	M	9 & 18	Progressive uraemia for 2 months. Worse in 9 days prior to admission. Recovered after 2 dialyses. Retrograde pyelogram showed bilateral polycystic kidneys. Subsequently this patient had further haemodialyses performed followed by cadaveric renal transplantation and is still alive 1 year after transplantation.

Appendix 3. Summary of Clinical Histories of Patients on whom Uretero-Colic Anastomosis had been Performed:

Name	Age	Time Since Operation	Clinical History
T.E.	24	9 months and 13 months	Motor-cycle accident in September 1960 with fractured pelvis, ruptured urethra with urethro-rectal fistula and multiple soft tissue injuries. Developed acute renal failure and haemodialysed 8 times. Colostomy established and multiple attempts made to close the urethro-rectal fistula. These failed and in December 1962 bilateral uretero-colic anastomosis was performed and the colostomy closed. He has mild hyperchloraemic acidosis and takes sodium bicarbonate regularly.
H.M.	62	20 years	Left nephrectomy in 1931 for tuberculosis and later developed incontinence with small contracted bladder. In 1942 right ureter transplanted into colon. Developed moderate hyperchloraemic acidosis and in 1952 started on sodium bicarbonate 2 g per day and has continued on varying dosages ever since. Then developed severe biochemical osteomalacia. In past 5 years has had recurrent pyelonephritis and has required treatment for severe hyperchloraemic acidosis with serum potassium concentration as low as 1.9 mEq/l. Died December 1963.
A.D.	37	27 years	Born with exstrophy of bladder and bilateral uretero-colic anastomosis performed when 10 years of age. Anal sphincter unsatisfactory and he was incontinent of urine and faeces through the anus for 27 years, refusing to attend for medical treatment. Admitted November 1962, whole body potassium measured and then ileal loop diversion of urine performed. Did not take sodium bicarbonate.
A.W.	22	7 years	Vehicle accident 1956 gave fractured right femur, fractured pelvis and ruptured urethra. Bilateral uretero-colic anastomosis performed. Took sodium bicarbonate regularly.

Name	Age	Time Since Operation	Clinical History
J.A.	68	2 years	Total cystectomy and bilateral uretero-colic anastomosis had been performed 2 years previously for bladder carcinoma. A degree of renal failure was present then with back pressure and infection, this gradually progressed and he finally died from renal failure and electrolyte imbalance in October 1962. Sodium bicarbonate taken regularly.
J.R.	53	14 days	Total cystectomy and bilateral uretero-colic anastomosis had been performed 14 days previously for bladder carcinoma.
B.G.	71	9 years	In 1954 total cystectomy and bilateral uretero-colic anastomosis had been performed for bladder tumour. Mild hyperchloraemic acidosis developed; he took sodium bicarbonate regularly. The patient remains well.
A.W.	34	4 months	Total cystectomy and bilateral uretero-colic anastomosis had been performed for carcinoma of bladder. Hyperchloraemic acidosis had not developed. Died December 1963 from metastatic tumour.
W.R.	63	5 months	The patient suffered recurrent bladder tumours from 1956. Partial cystectomy May 1961. Bilateral uretero-colic anastomosis performed January 1963 as a palliative measure for recurrent tumour. Developed mild hyperchloraemic acidosis.
F.F.	50	10 years	Total cystectomy and bilateral uretero-colic anastomosis performed in 1953 for bladder carcinoma. Takes bicarbonate regularly and has mild hyperchloraemic acidosis. This patient is a heavy, muscular man who works as a farm labourer.
W.H.	60	4 years	Total cystectomy and bilateral uretero-colic anastomosis for bladder carcinoma in 1959. Admitted 21 days before whole body potassium measurement for urgent intestinal obstruction due to either recurrent tumour in the pelvis or to adhesions following radiotherapy. Left sigmoid colostomy performed. Condition deteriorated and he died December 1963. This patient took sodium bicarbonate regularly.

Name	Age	Time Since Operation	Clinical History
L.T.	62	9 years	Total cystectomy and bilateral uretero-colic anastomosis performed in 1954 for bladder tumour. Patient takes sodium bicarbonate regularly and has mild hyperchloraemic acidosis. Remains fit and well.
H.H.	58	7 years	Total cystectomy and bilateral transplantation of ureters into sigmoid colon in 1956 for bladder carcinoma. Takes bicarbonate regularly, has mild hyperchloraemia and remains fit and well.
M.A.	59	5 years	Total cystectomy and bilateral uretero-colic anastomosis performed for bladder tumour in January 1959. Takes sodium bicarbonate regularly and has mild hyperchloraemia.
A.R.	63	2 years	Total cystectomy and bilateral uretero-colic anastomosis performed in July 1961 for bladder carcinoma. Since then patient troubled with recurrent pyelonephritis and has been admitted on several occasions with potassium depletion and severe hyperchloraemic acidosis. Takes sodium bicarbonate regularly.
<u>Rectal Sigmoid Bladder</u>			
R.I.	65	7 years	Total cystectomy and recto-sigmoid bladder established in 1956. Takes sodium bicarbonate regularly, has mild hyperchloraemia but remains fit and well.
R.C.	58	3 years	Total cystectomy and recto-sigmoid bladder established in April 1960. Patient has mild hyperchloraemic acidosis but this was more marked on the day of whole body potassium estimation. He takes sodium bicarbonate regularly and remains fit and well.
A.L.	60	1 year	Total cystectomy and recto-sigmoid bladder performed in October 1962 for bladder carcinoma. Takes sodium regularly. Developed strangulated incisional hernia followed by a small bowel fistula, his condition gradually deteriorated and he died January 1964.

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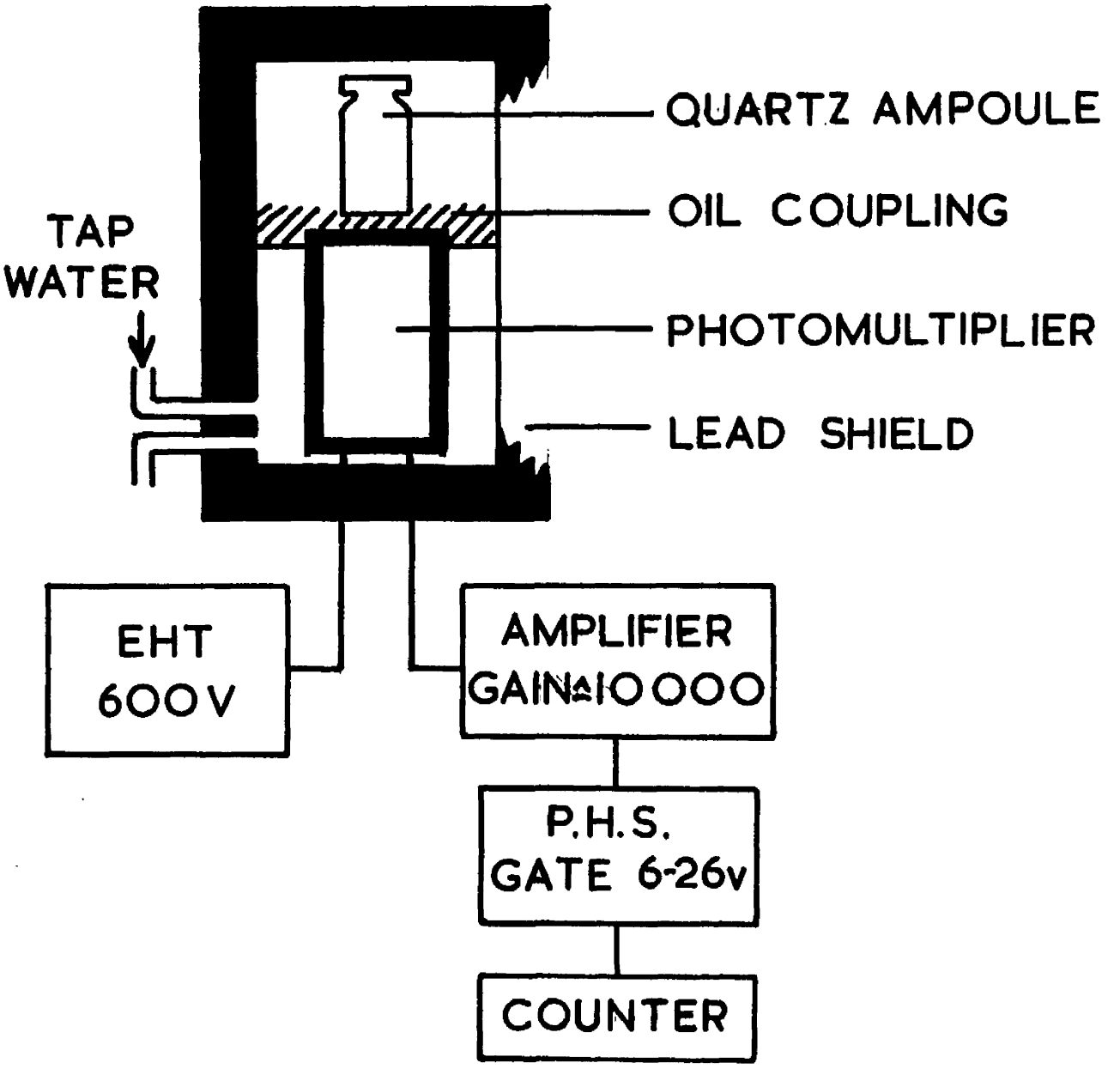

Fig. 1. Schematic diagram of tritium counting apparatus.

Fig.2. Quartz ampoule, held in calipers, being loaded into counting head.

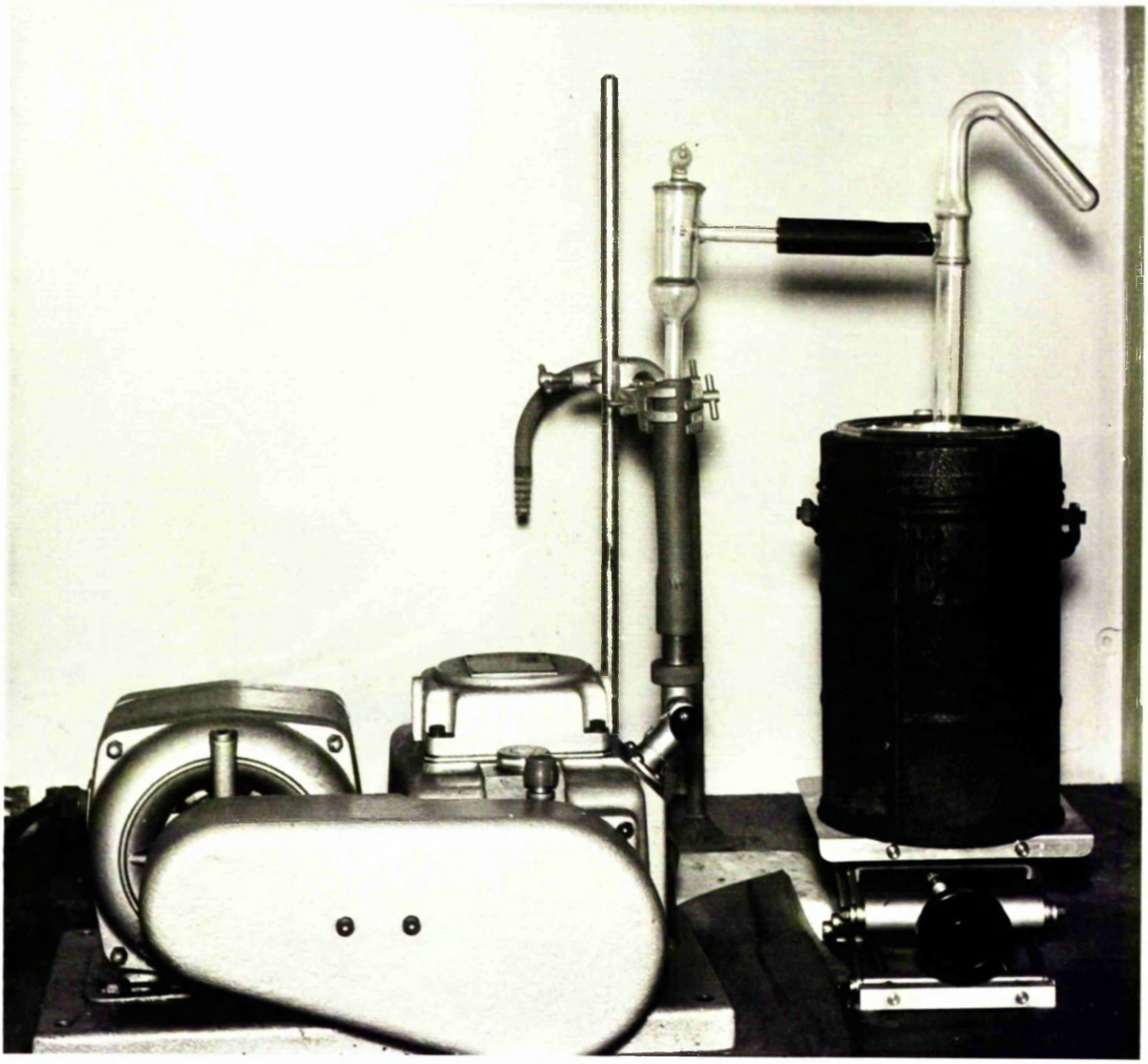

Fig.3. Thumberg flask, with bulb immersed in liquid nitrogen, placed under vacuum by a rotary oil pump.

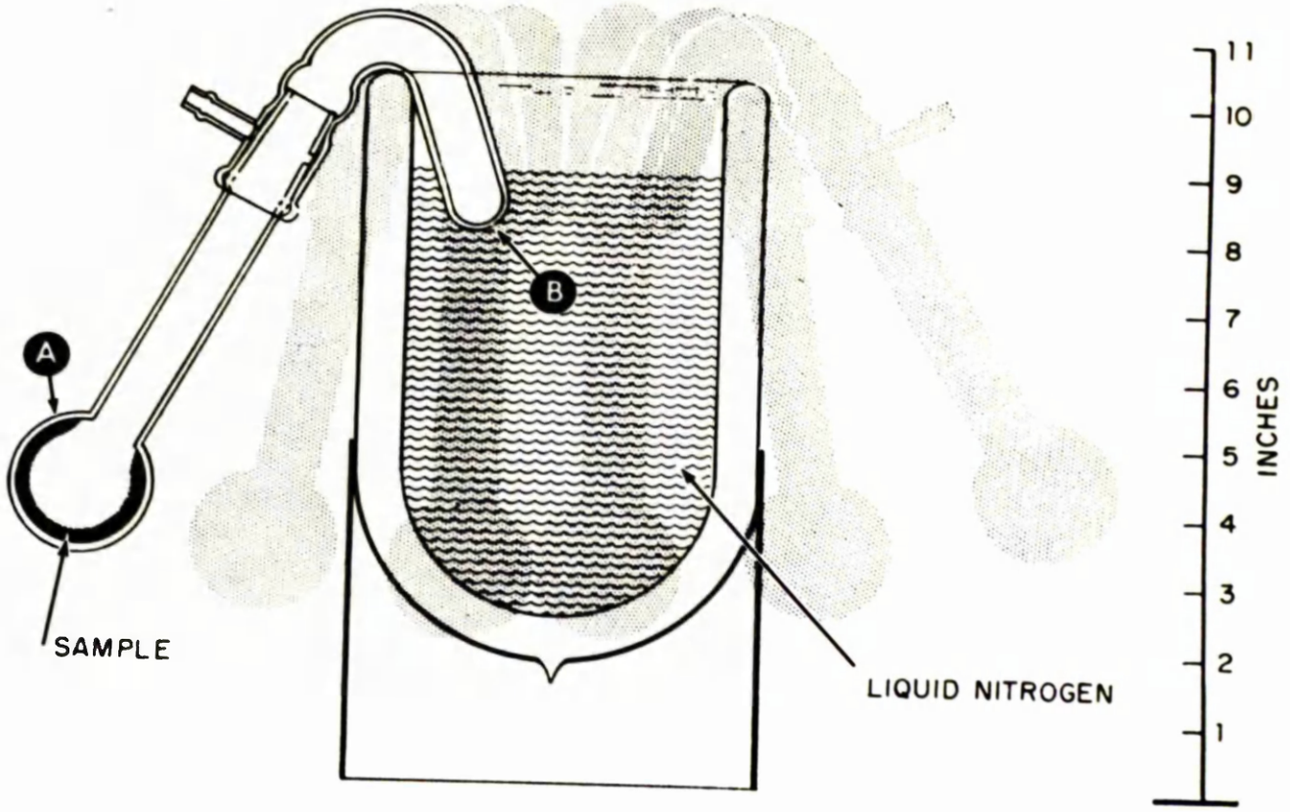

Fig. 4. Diagram of Thumberg flask with beak immersed in liquid nitrogen during distillation.

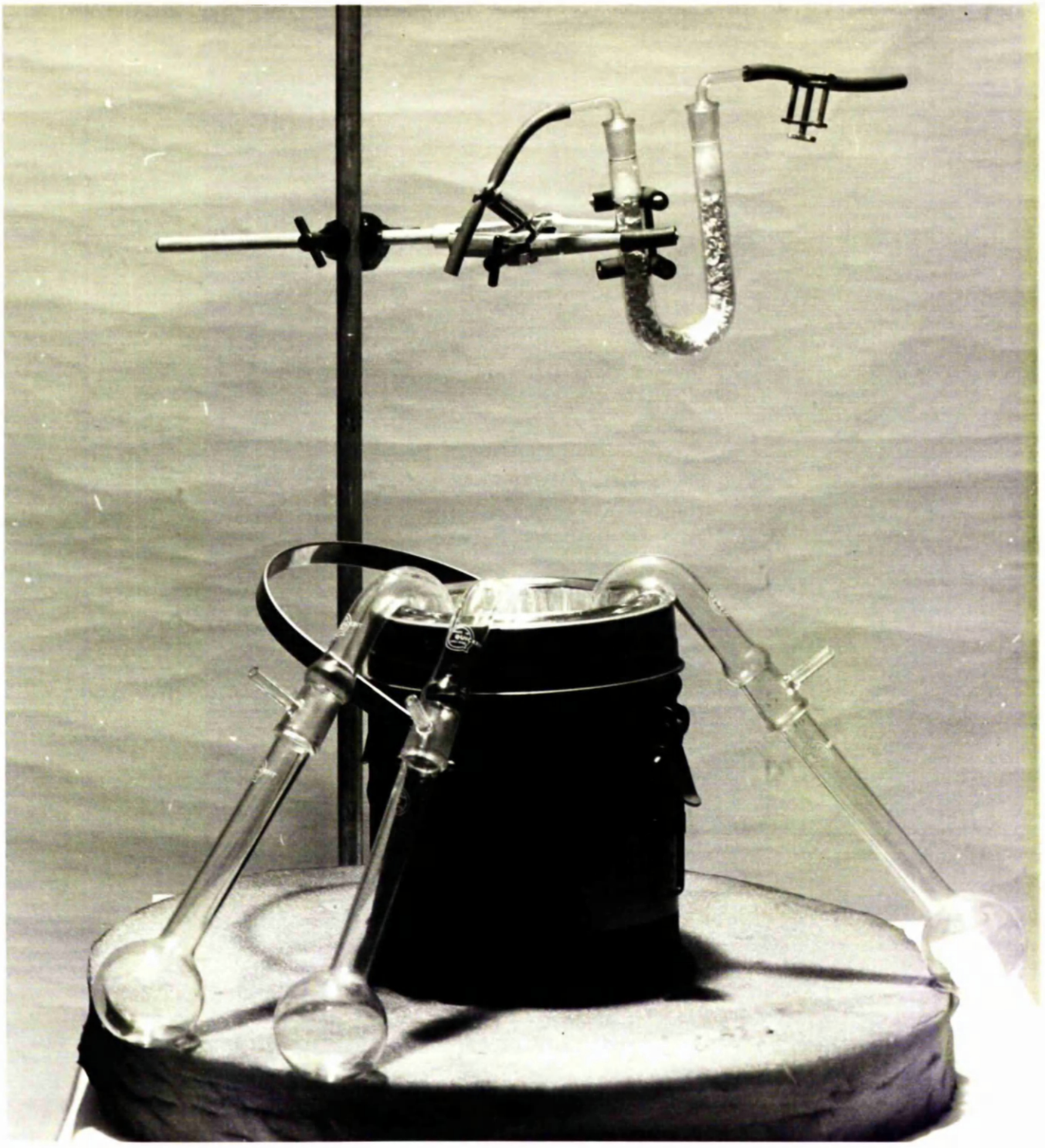

Fig. 5. Thumberg flasks, with beaks immersed in liquid nitrogen, during distillation. Calcium chloride column dries air used to release vacuum.

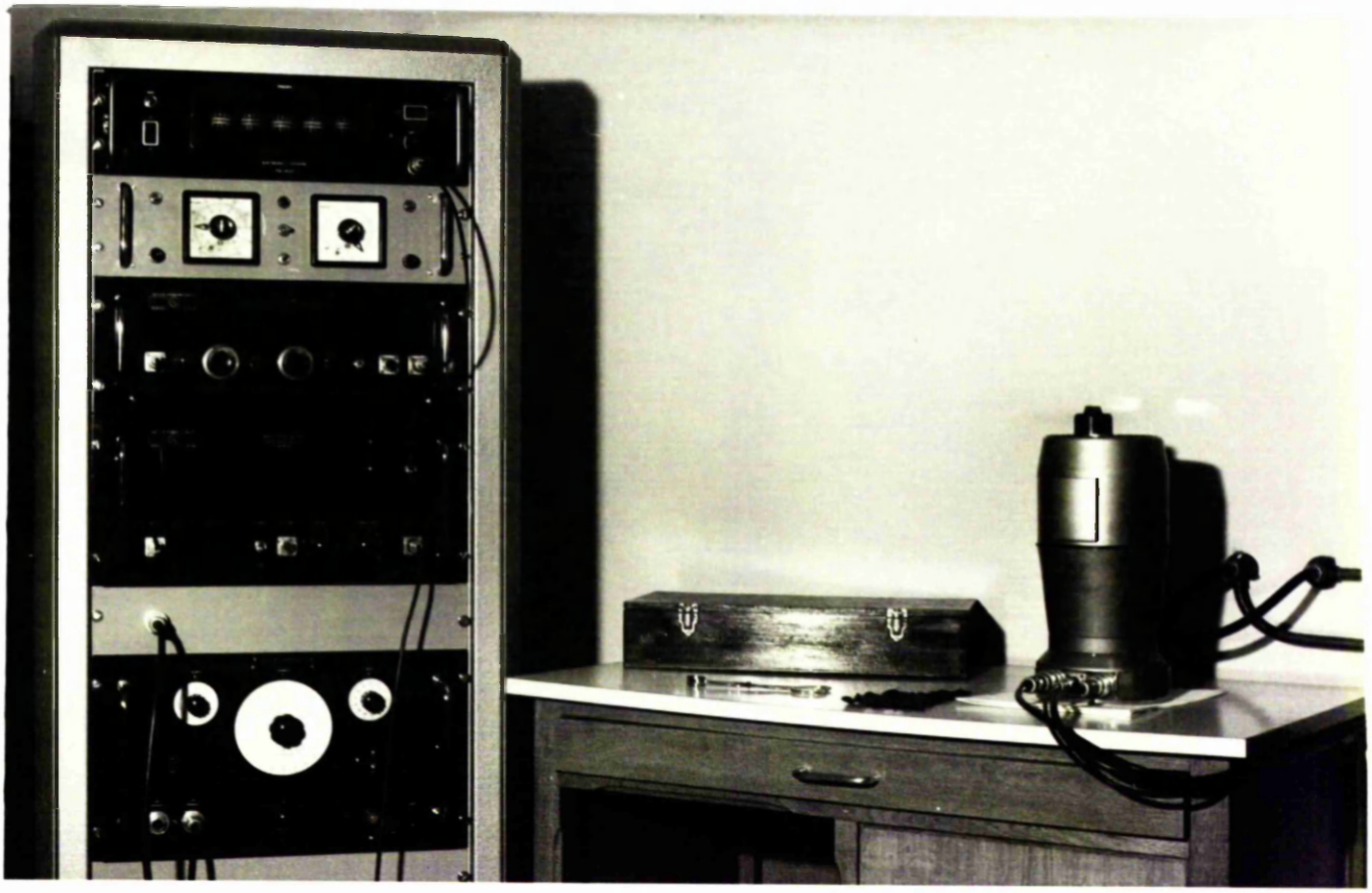

Fig. 6. Nuclear Enterprises (G.B.) Ltd. Tritium Counter (NE 8301) with counting head.

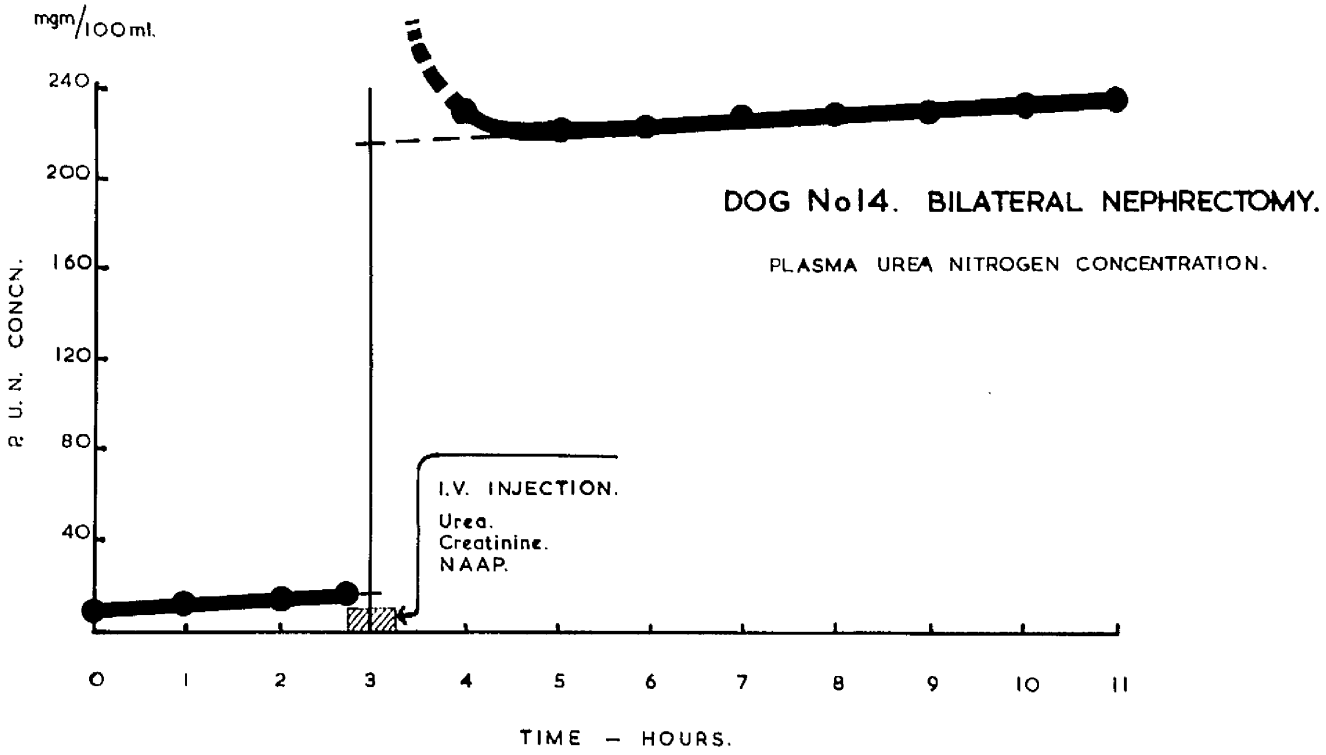

Fig. 7. Concentration of urea nitrogen in plasma plotted against time in a bilaterally nephrectomized dog. Values extrapolated to mid-point of time of injection.

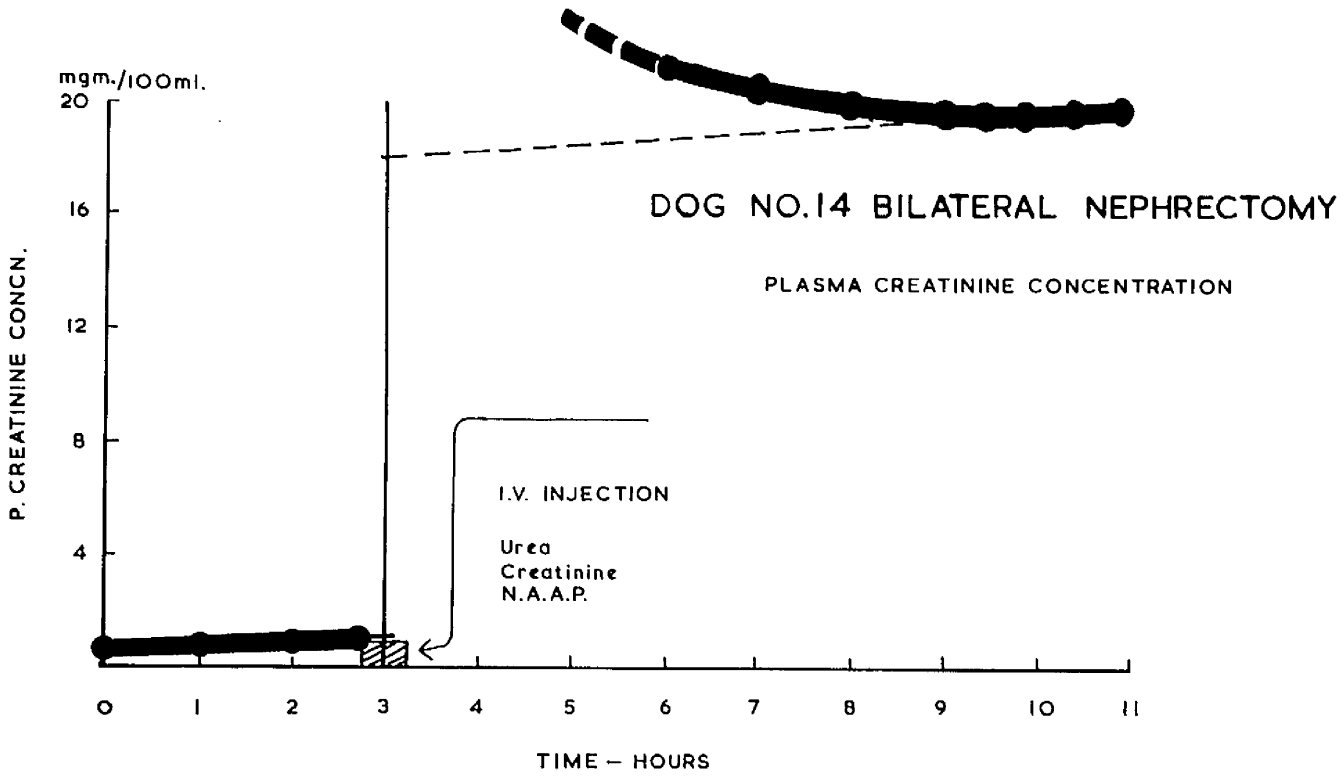

Fig. 8. Concentration of creatinine in plasma plotted against time in a bilaterally nephrectomized dog. Values extrapolated to mid-point of time of injection.

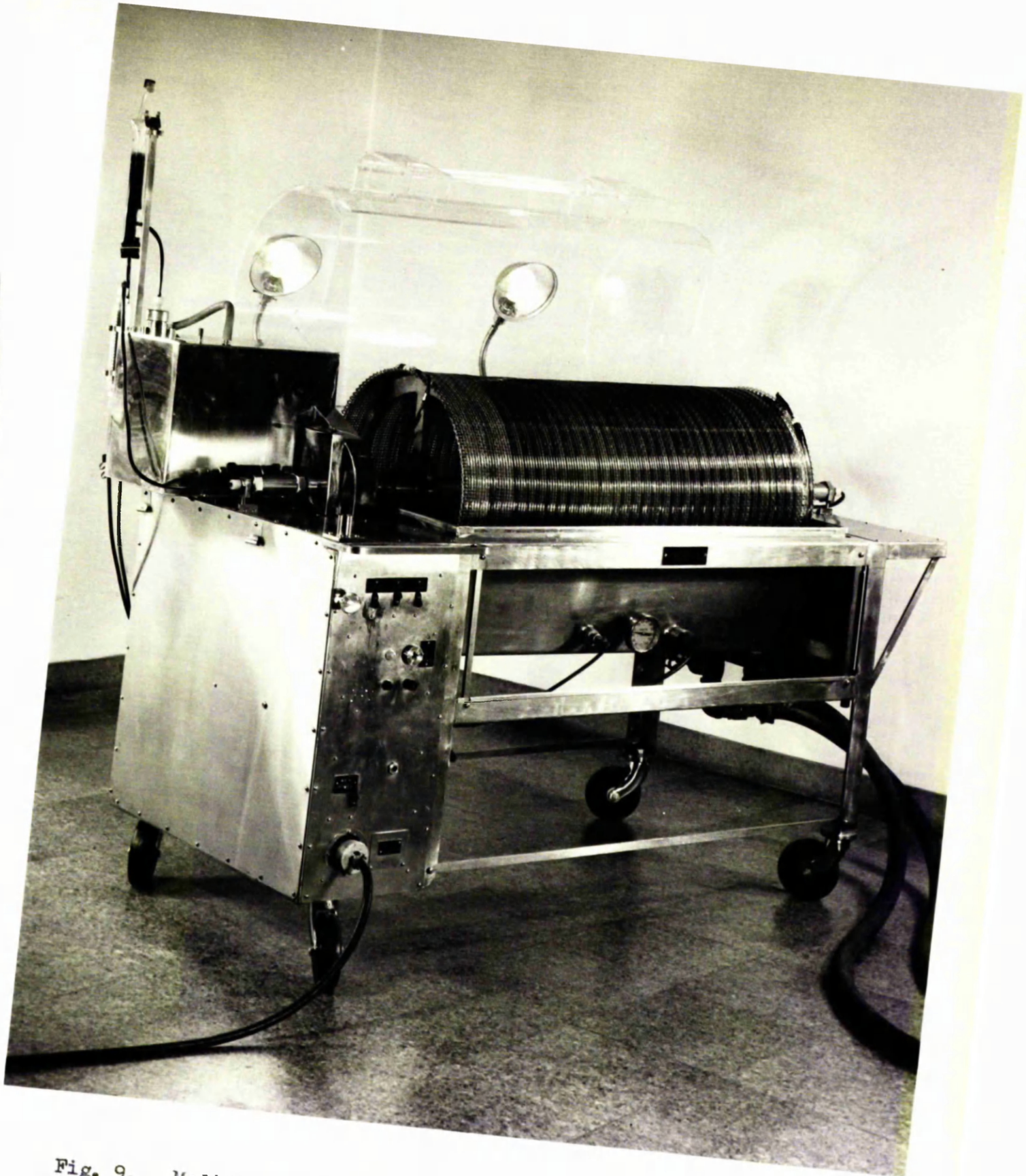

Fig. 9. Modified Kolff Rotary Drum Artificial Kidney.

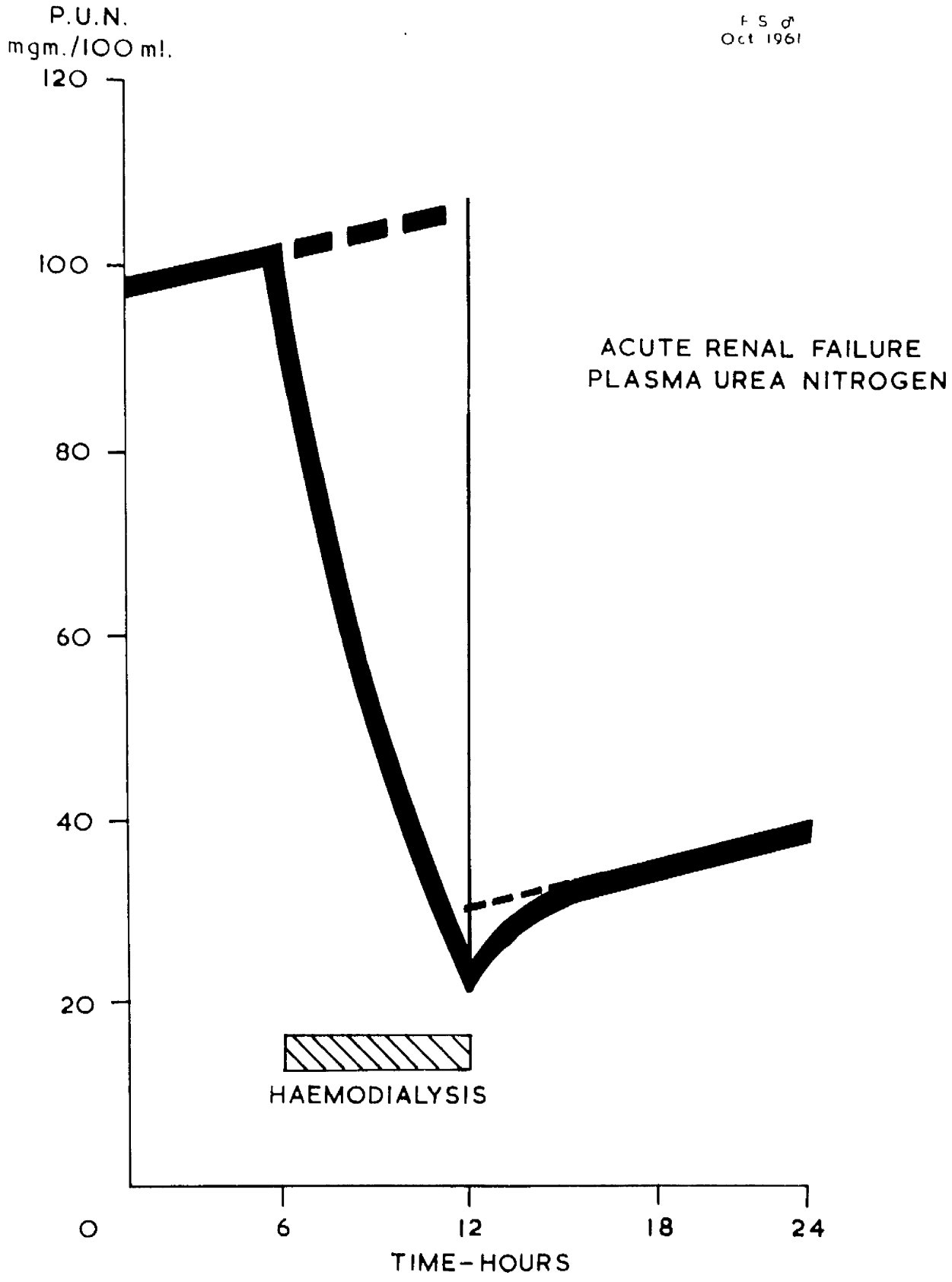

Fig.10. Fall in plasma concentration of urea nitrogen during haemodialysis in the treatment of renal failure. Values extrapolated to correct for endogenous production of urea during dialysis and its redistribution after dialysis.

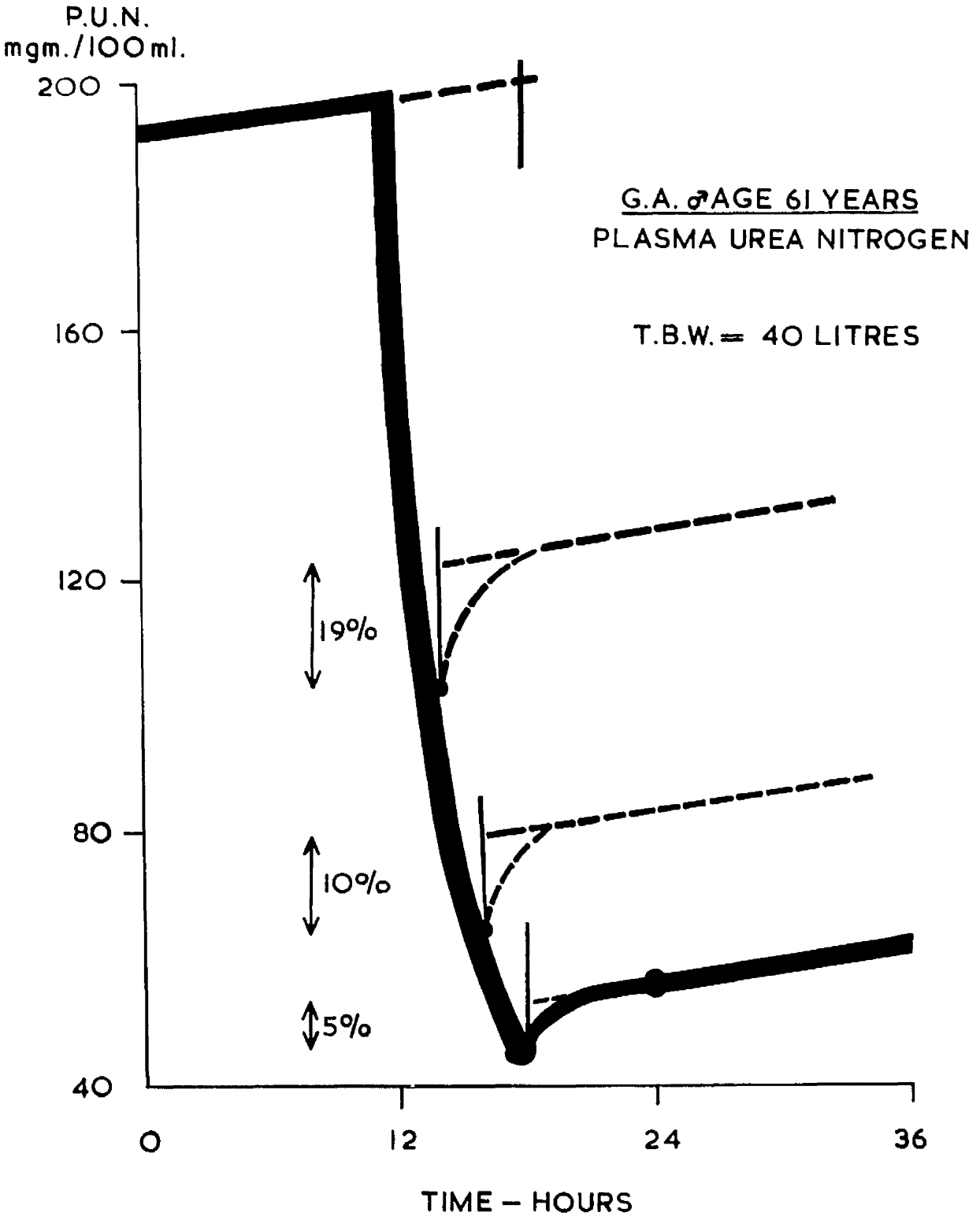

Fig.11. Fall in plasma concentration of urea nitrogen during haemodialysis in the treatment of renal failure. The effect of redistribution of urea is shown to be proportionately greater in a shorter dialysis.

WHOLE BODY COUNTER

LIQUID SCINTILLATOR — 4π GEOMETRY

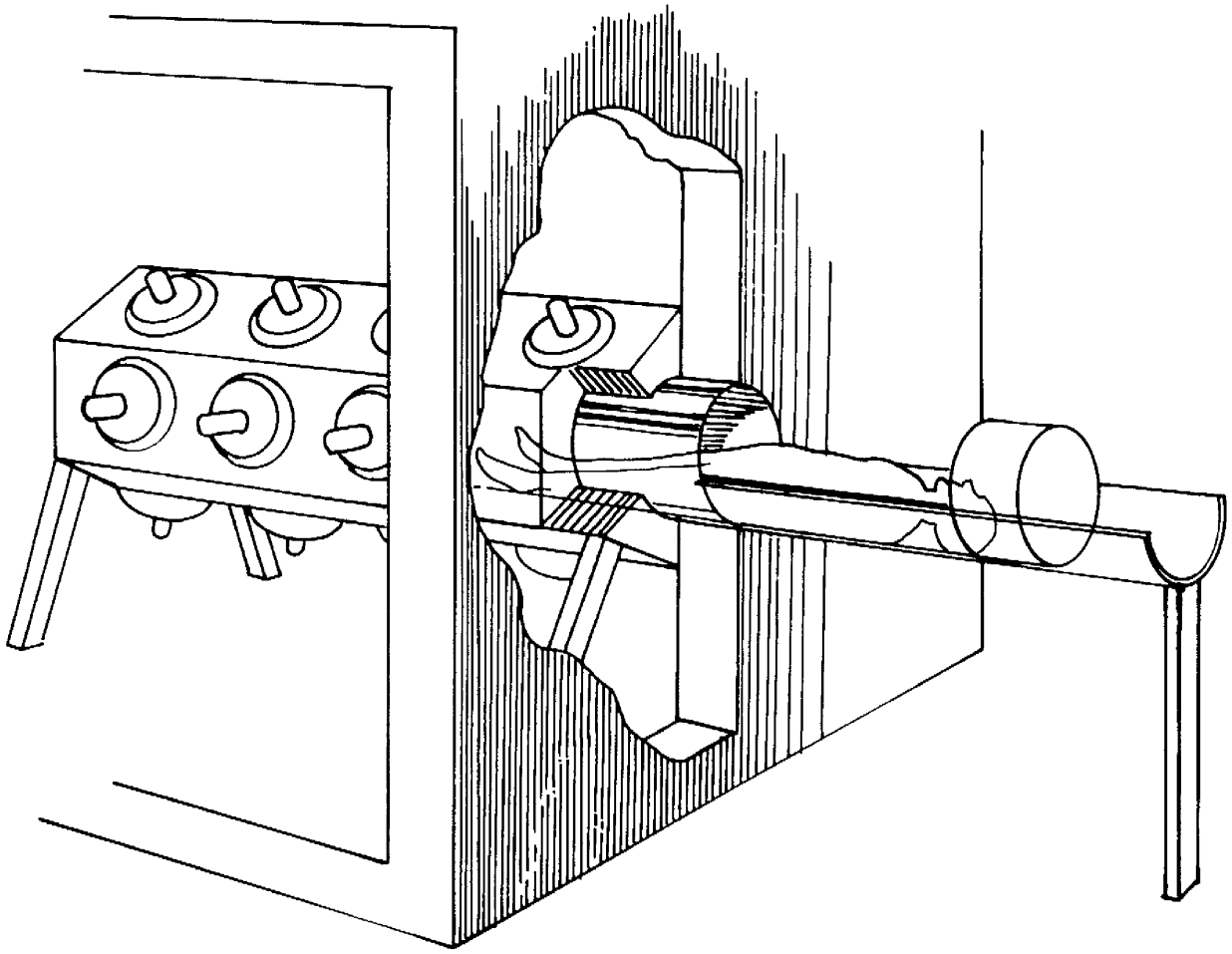

Fig. 12. 4 Pi Geometry: Los Alamos Whole Body Counter with liquid scintillator enclosing the subject.

WHOLE BODY COUNTER

SINGLE NaI(Tl) CRYSTAL – CHAIR GEOMETRY

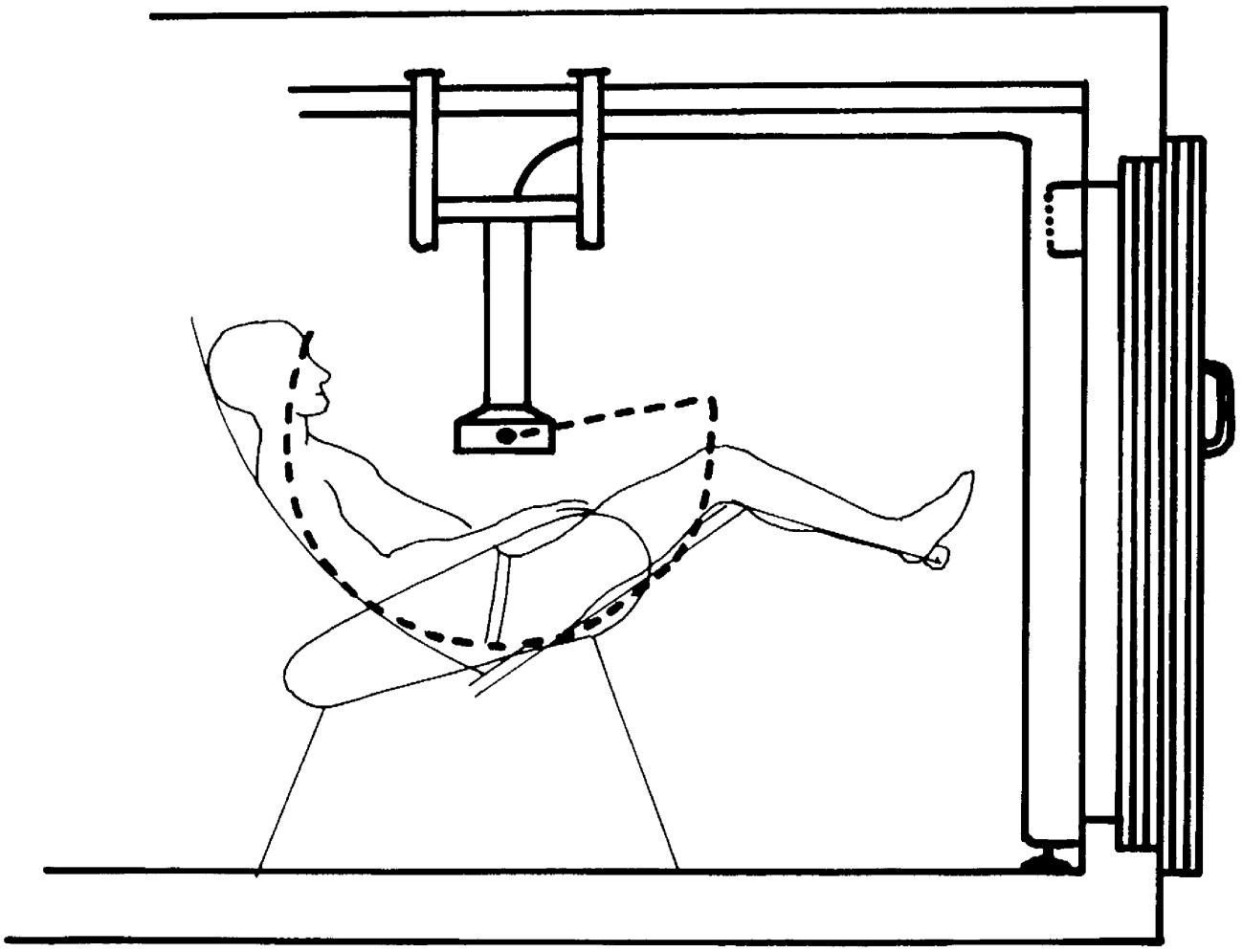

Fig.13. Chair Geometry: Subject's body describes an arc centred on NaI(Tl) crystal.

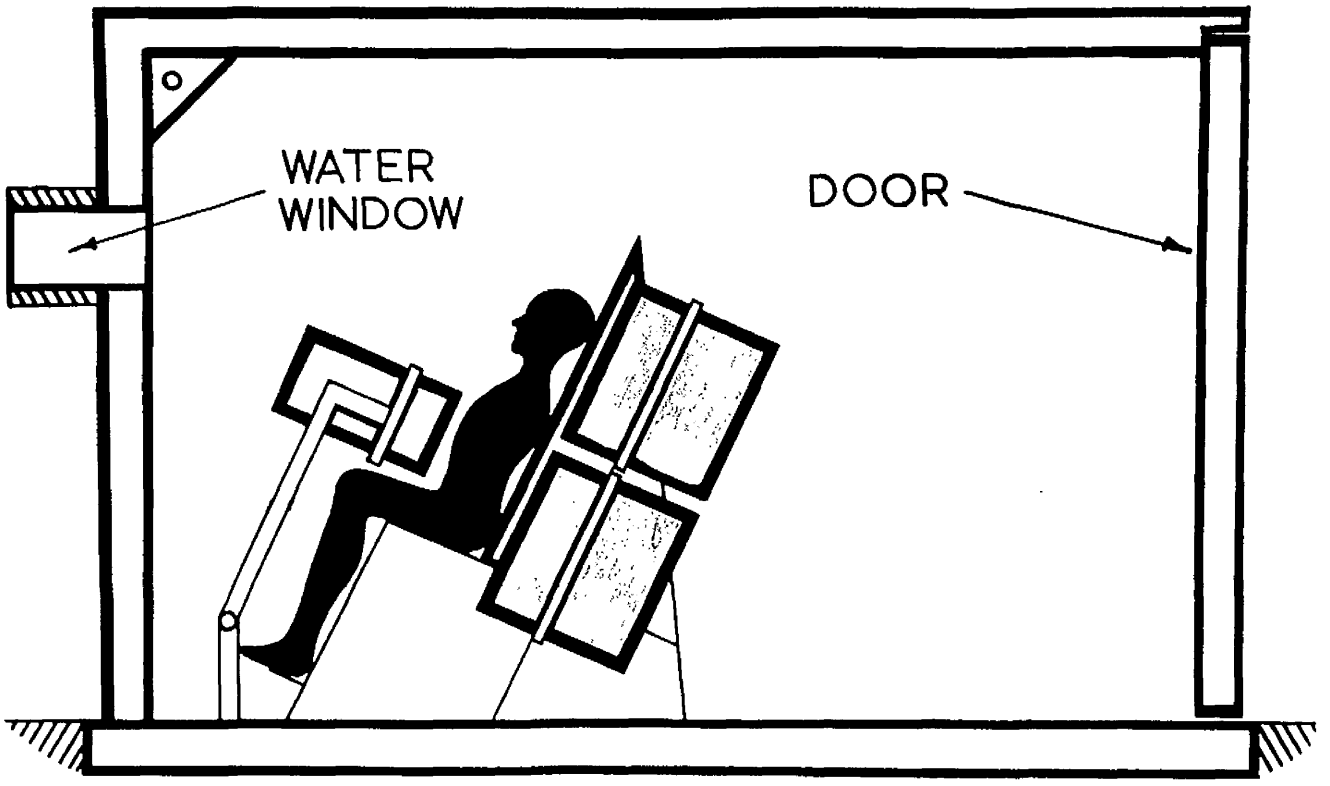

Fig.14. Leeds Plastic Scintillator Geometry: Subject placed in sitting position with two plastic scintillator units behind and one in front of the body.

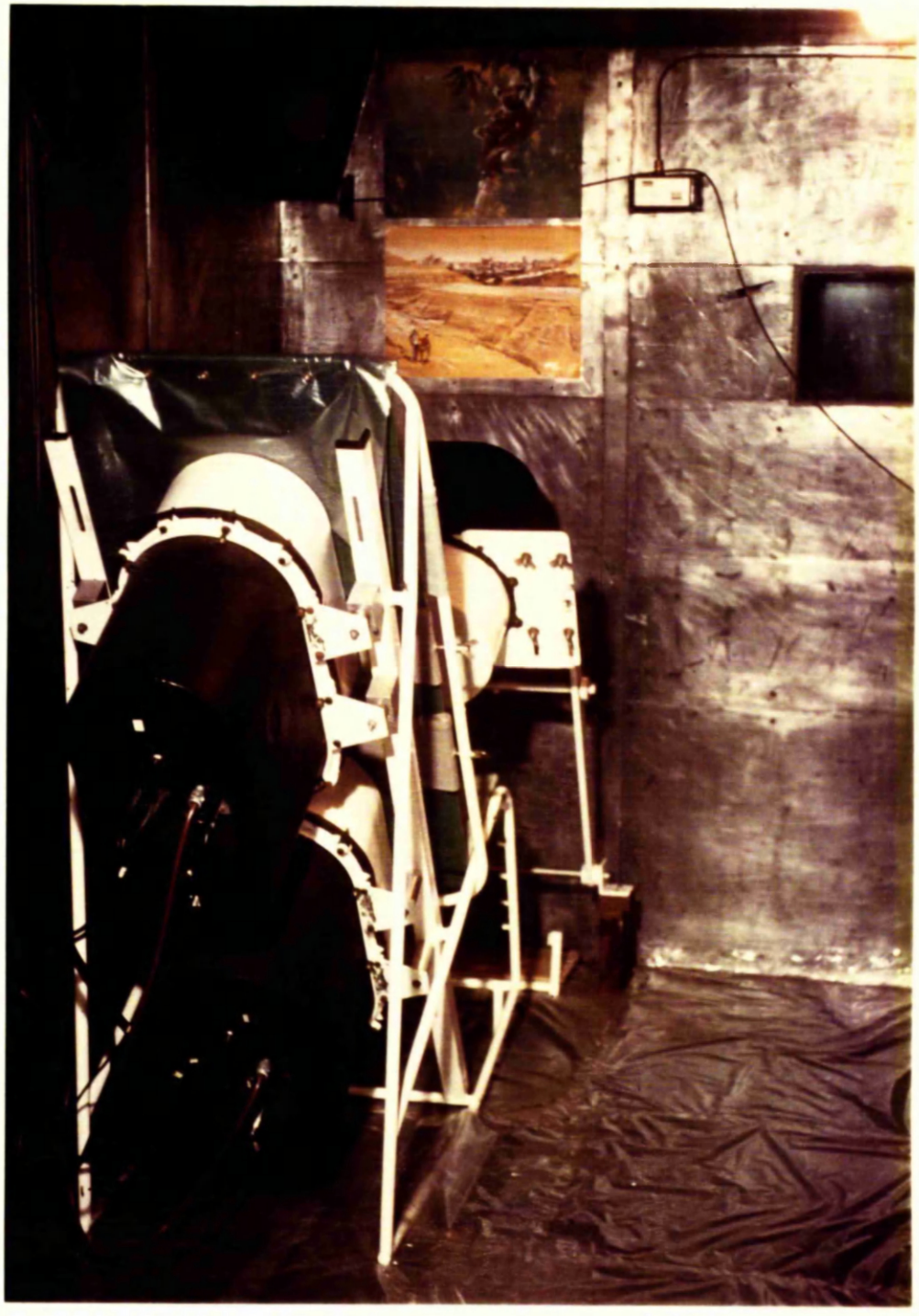

Fig. 15. Interior View of Leeds Plastic Scintillator Whole Body Counter showing chair, scintillator units and loudspeaker in top left corner.

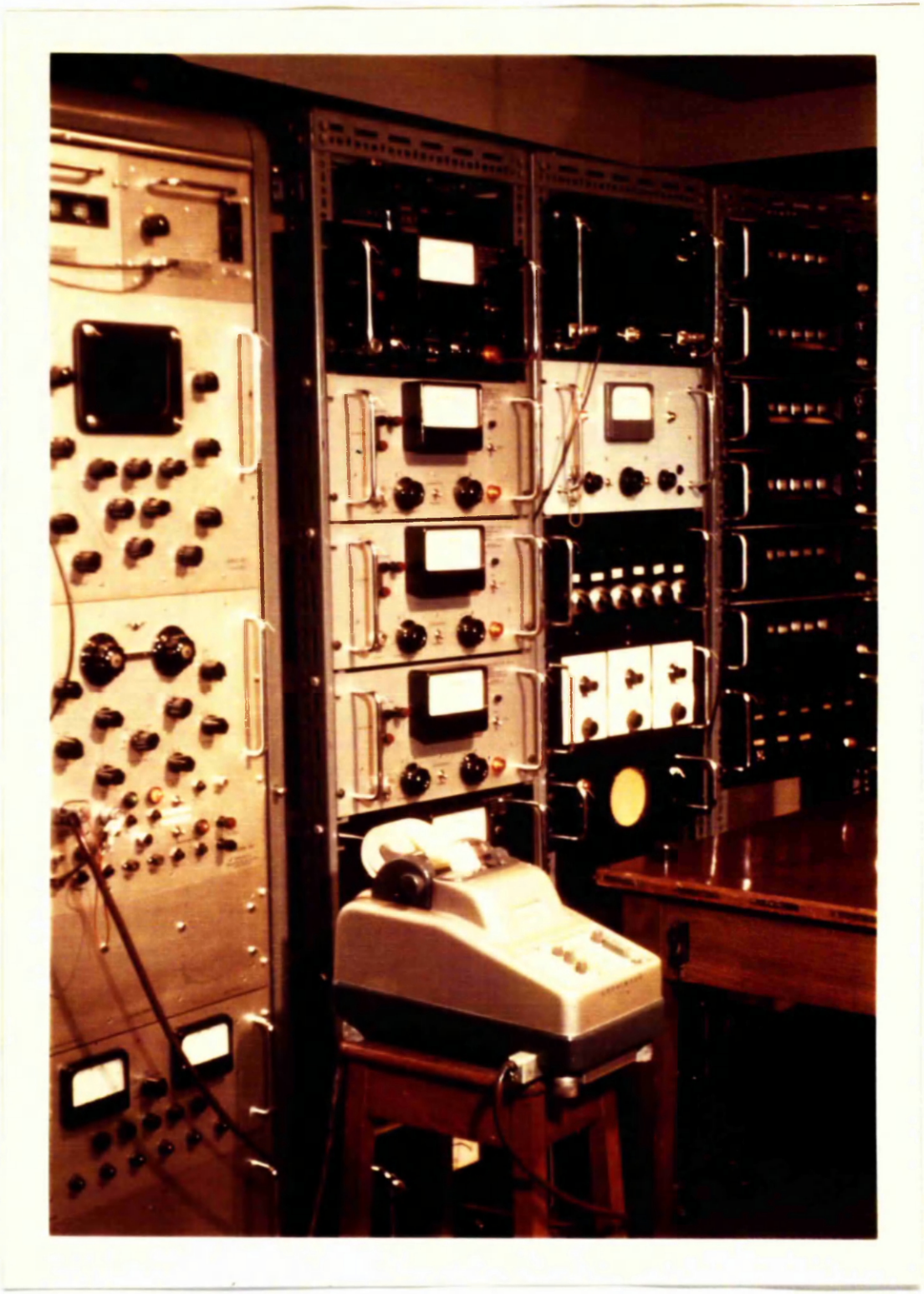

Fig. 16. Counting equipment used with Leeds Plastic Scintillator Whole Body Counter.

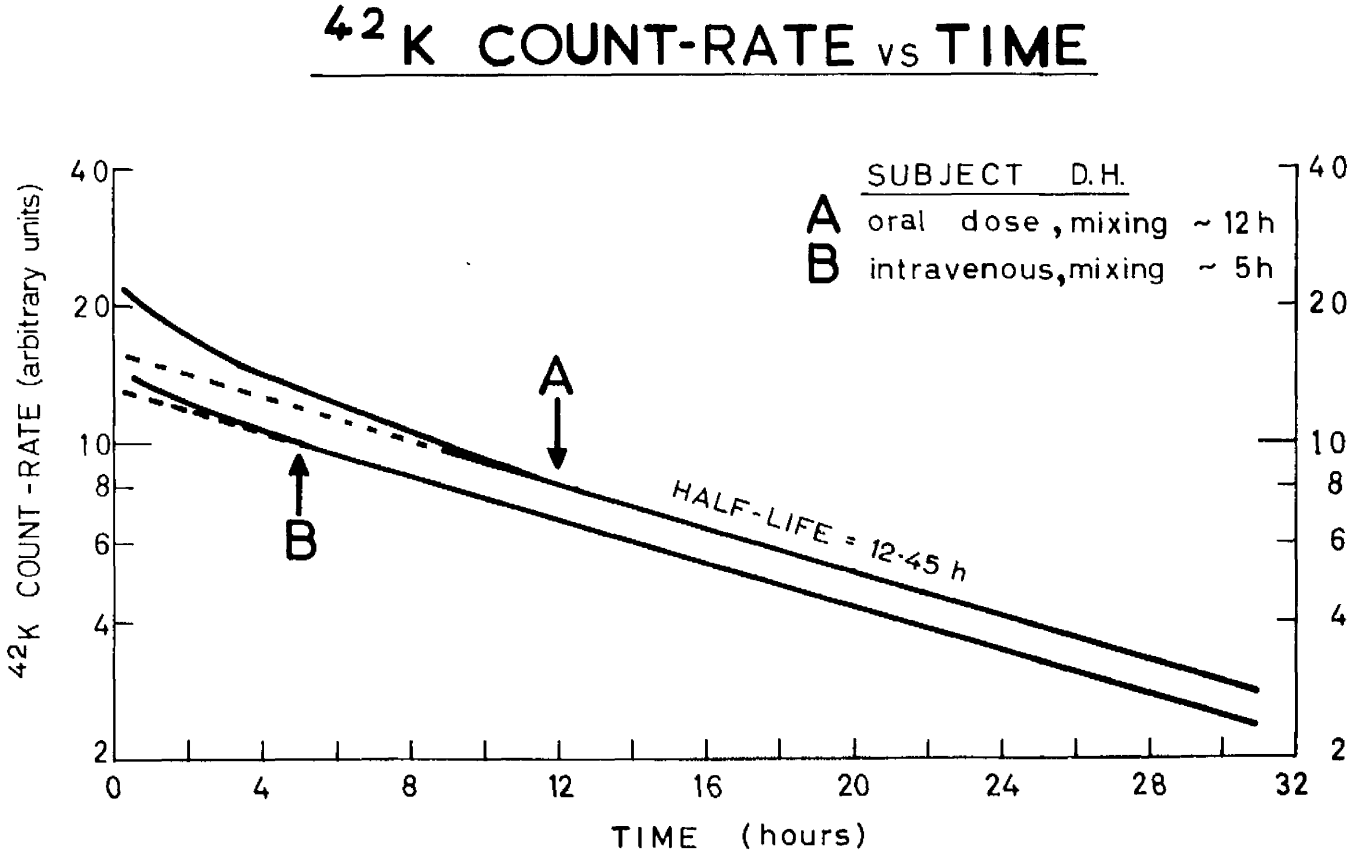

Fig. 17. Logarithmic plot of ^{42}K count-rate, corrected for excretion, against time. Distribution of ^{42}K took about 12 hours following an oral dose and about 5 hours after an intravenous dose.