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ACUTE GLOMERULONEPHRITIS:
A STUDY OF CERTAIN ASPECTS

by

John C. Maclaurin
M.B., Ch.B., M.R.C.P. (Glas. & Edin.)

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# CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>PROLOGUE</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>SECTION ONE - THE TOOLS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHAPTER ONE</td>
<td>The body water compartments in childhood: their definition and measurement.</td>
<td>8</td>
</tr>
<tr>
<td>CHAPTER TWO</td>
<td>The selected methodology of body water distribution.</td>
<td>25</td>
</tr>
<tr>
<td>CHAPTER THREE</td>
<td>Body water distribution in normal children:</td>
<td>61</td>
</tr>
<tr>
<td></td>
<td>I. The age group 1½-12 years.</td>
<td></td>
</tr>
<tr>
<td>CHAPTER FOUR</td>
<td>Body water distribution in normal children:</td>
<td>79</td>
</tr>
<tr>
<td></td>
<td>II. The first two weeks of life.</td>
<td></td>
</tr>
<tr>
<td>CHAPTER FIVE</td>
<td>Other methods.</td>
<td>121</td>
</tr>
</tbody>
</table>
# SECTION TWO - THE PROBLEM

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHAPTER SIX</strong></td>
<td>Acute glomerulonephritis: A short review of existing theories concerning oedema.</td>
<td>135</td>
</tr>
<tr>
<td><strong>CHAPTER SEVEN</strong></td>
<td>Clarifying the problem: A study of the case records from 170 children with acute glomerulonephritis.</td>
<td>147</td>
</tr>
<tr>
<td><strong>CHAPTER EIGHT</strong></td>
<td>Body water distribution in acute glomerulonephritis.</td>
<td>168</td>
</tr>
<tr>
<td><strong>CHAPTER NINE</strong></td>
<td>The diuresis of acute glomerulonephritis</td>
<td>195</td>
</tr>
<tr>
<td><strong>CHAPTER TEN</strong></td>
<td>Other aspects: I. Electrophoretic patterns.</td>
<td>234</td>
</tr>
<tr>
<td><strong>CHAPTER ELEVEN</strong></td>
<td>Other aspects: II. The chest X-ray picture and changes in the radiological heart volume.</td>
<td>249</td>
</tr>
<tr>
<td><strong>CHAPTER TWELVE</strong></td>
<td>The synthesis.</td>
<td>264</td>
</tr>
<tr>
<td><strong>SPECULATIVE EPILOGUE</strong></td>
<td></td>
<td>283</td>
</tr>
<tr>
<td><strong>REFERENCES</strong></td>
<td></td>
<td>288</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>AGN</td>
<td>Acute glomerulonephritis.</td>
<td></td>
</tr>
<tr>
<td>BV</td>
<td>Total blood volume (ml.)</td>
<td></td>
</tr>
<tr>
<td>B.Wt.</td>
<td>Body weight (kg.)</td>
<td></td>
</tr>
<tr>
<td>CV.</td>
<td>Total red cell mass (ml.)</td>
<td></td>
</tr>
<tr>
<td>Delta = C</td>
<td>Change of.</td>
<td></td>
</tr>
<tr>
<td>ECW</td>
<td>Extracellular water volume (litres).</td>
<td></td>
</tr>
<tr>
<td>ICW</td>
<td>Intracellular water volume (litres).</td>
<td></td>
</tr>
<tr>
<td>ISW</td>
<td>Interstitial water volume (litres).</td>
<td></td>
</tr>
<tr>
<td>Ke</td>
<td>Total exchangeable potassium (meq.)</td>
<td></td>
</tr>
<tr>
<td>Na_{e}</td>
<td>Total exchangeable sodium (meq.)</td>
<td></td>
</tr>
<tr>
<td>Na_{ec}</td>
<td>Total extracellular sodium (meq.)</td>
<td></td>
</tr>
<tr>
<td>Na_{is}</td>
<td>Total interstitial sodium (meq.)</td>
<td></td>
</tr>
<tr>
<td>Na_{is}</td>
<td>Concentration of sodium in interstitial water (meq./l)</td>
<td></td>
</tr>
<tr>
<td>Na_{s}</td>
<td>Serum or plasma sodium concentration (meq./l.)</td>
<td></td>
</tr>
<tr>
<td>PV</td>
<td>Plasma volume (ml.)</td>
<td></td>
</tr>
<tr>
<td>PW</td>
<td>Plasma water volume (ml.)</td>
<td></td>
</tr>
</tbody>
</table>
**S.D.** Standard deviation = \( \sqrt{\frac{\sum (x - \bar{x})^2}{n - 1}} \)

**S.E.** Standard error of mean = \( \sqrt{\frac{\sum (x - \bar{x})^2}{n(n - 1)}} \)

N.B. In both of the above the Bessel correction has been used in all samples in which \( n \geq 30 \).

**TBW** Total body water (litres).
On the evening of the 23rd of September 1960 a ten-year old girl was brought by her parents to the Admission Hall of the Royal Hospital for Sick Children, Glasgow. The history of her illness was one of very short duration, and distinctly unusual. Until two hours previously she had been in apparently normal health. She had then become acutely breathless, in which state she had remained until her arrival at Hospital. She had not been coughing, and there was nothing in her past history to suggest heart-disease.

On clinical examination she was seen to be tachypnoeic, somewhat pale, and her face was minimally puffy. Her heart-rate was 62/minute, cardiomegaly was readily detectable, but a short Grade 2 murmur at the base of her heart did not suggest the presence of an organic heart lesion. The blood pressure was normal. A few fine crepitations were audible throughout both lung-fields, but no other abnormality was clinically obvious, and the urine was chemically and microscopically normal.

The appearances of a chest X-ray (Figure 1.a.) were striking: considerable enlargement of the heart was combined with appearances which the radiologist
Patient A.G. See text for details
considered to be those of pulmonary oedema due to left ventricular failure.

Here was an urgent clinical problem: a ten-year-old child presenting with apparent heart failure of obscure origin and recent, acute onset. She was put to bed forthwith and within half an hour some improvement was evident before any empirical treatment had been administered. In a further two hours she was no longer breathless. The following morning she looked well, and X-ray of her chest (Figure 1.b.) showed that her heart was reduced somewhat in size, although not yet normal; on the other hand, the appearances of acute pulmonary oedema visible on the previous evening had quite disappeared.

Three days later a trace of proteinuria together with minimal microscopic erythrocyturia was observed. These signs persisted for a few days, the girl being thereafter well. The normal chest X-ray appearances at this time are shown in Figure 1.c.

The final clue to the solution of this puzzle was the level of the anti-streptolysin 'O' titre. At 2,500 units/ml., this left no doubt that an active streptococcal infection had been recently present.
This child was therefore retrospectively diagnosed as having an unusual variant of acute glomerulonephritis. Unusual, since signs were present indicative of considerable abnormality of body fluids in the absence of urinary evidence of renal disease. The demonstration that these abnormalities could occur and return towards normal with such speed was to me fascinating, and posed numerous questions:

To what extent were these caused directly by the kidneys, and by what multitude of mechanisms?

In the absence of the usual evidence of renal disease, could extrarenal mechanisms be playing a part? If so, of what nature?

The rapidity of the apparent changes could suggest fluid shifts to a profound degree within the subject, although as a general phenomenon evidence in the literature to support such a concept was scanty.

Acute glomerulonephritis presenting in this way is of course by no means unknown (37, 15); it was well described, for example occurring in soldiers of the Austrian army fighting on the Italian front during World War I. These men frequently presented, acutely ill, with oedema and initially normal urine. For a time they were referred to as cases of
"Kriegssoezema" until the truly nephritic nature of the disease was demonstrated (85).

The problem thus presented was clear-cut; namely, the investigation of the nature and cause of the fluid-disturbances in acute glomerulonephritis. The most obvious direct approach to such a problem was to undertake the measurement of body water in its three fundamental compartments - the intracellular, the interstitial and the intravascular. This Thesis is an attempt to follow the natural and logical trend of investigative thought which such an objective has dictated.
CHAPTER 1

THE BODY WATER COMPARTMENTS IN CHILDHOOD:
THEIR DEFINITION AND MEASUREMENT

Water has possessed a considerable fascination for man throughout the ages. By its nature indispensable and irreplaceable, water has provided man with one of the essential elixirs of life, with a substance vital to his crops, with an object of worship, and with an object of fear. Water has given man a medium for trade and travel, a bulwark for his safety, the driving force for his machines, a means of waging aggressive war, and has been the subject of some of the greatest masterpieces of his artistic genius.

In science, and especially in the biological sciences, water might well be regarded as the paramount substance. It is the medium which makes electrolytic dissociation possible, and in which enzymatic reactions take place. This is true also in the mammalian body in which the place of water is certainly unique and its functions legion. It acts as a solvent and as a lubricant. It protects the brain from injury and
transports solutes, cells and heat. Grave illness may be caused by its relative absence or by its superabundance. Consequently a scientific study involving measurements of body water possesses at the outset a fundamentality which invests this type of work with a special fascination.

While the ancient literature contains scattered references alluding to water metabolism, it is only within the past century that actual measurements of the amount of water present in the body have been carried out. The earliest investigators in this field used desiccation of cadavers for this purpose, and it is noteworthy that their results bear comparison with those from the most sophisticated techniques in use today. However, to the physician the measurement of water in the living body naturally presents much greater possibilities; with the introduction of indicator dilution substances in the present century the possibility has become reality, and it is no accident that the whole science of fluid replacement therapy has developed concurrently. At the same time it must be remembered, and will be made clear in the coming chapters, that the study of body
water is even now in its infancy. This is particularly true of pathological states, where concepts hitherto regarded as fundamental are undergoing constant modification until they have frequently become unrecognisable.

This thesis, it is felt, reflects one such change.

The Body Water Compartments.

It is conventional, convenient, and more or less accurate to divide body water into compartments. The main subdivision is into the intracellular and extracellular phases, and the latter is further divided into the intravascular (plasma) and the interstitial compartments.

The term total body water refers to all the water in the body, including that in the bladder and in the gastro-intestinal tract. Tissues vary greatly in their proportion of water, from 93% in blood plasma to less than 10% in body fat.

Intracellular water refers to all body water surrounded by the cell-membrane of an individual cell; it does not therefore include water situated within the acini of endocrine glands. The vital distinguishing
point of this compartment is that due to the remarkable properties of cell-membranes its electrolytic composition differs profoundly from that of the extracellular space. Freely permeable to water, these membranes are likewise permeable to most undissociated small molecules while maintaining critical separation of ionic particles.

The definition of the extracellular space is considerably more complex. Anatomically, it defines the volume of fluid which surrounds the cells. As well as plasma water and interstitial water (e.g., lymphatic fluid), this term includes the connective tissue water in bone and cartilage. These three major components collectively make up about 20% of the body weight in the mature animal (26). Furthermore, it includes fluid in the gastro-intestinal tract, the cerebro-spinal fluid, and water in the eye, peri-cardium, pleura and joints. Since extracellular water must cross cell membranes other than those of capillaries to reach these last named areas, the term "transcellular water" has been proposed (26) to cover these rather small volumes of fluid.

The intravascular division of the extracellular space is the blood plasma. The red cells, surrounded
by this medium, form part of the intracellular space but differ from the great bulk of body cells in containing water with a relatively high chloride content (110).

In recent years it has become increasingly evident that the concept of such a clear-cut division between the intra- and extracellular compartments may well need extensive revaluation. As knowledge of the nature of cell membranes progresses, a stronger impression of dynamism is gained. Thus not only water but extracellularly situated ions are in continuous circulation (232), a balanced state which may be readily altered in the presence of disease (67). A more modern concept, and one strongly supported by the revelations of electron microscopy is of enormous numbers of "membranes" throughout the cell and not merely at its surface. Those appear infinitely reduplicated and of fantastic complexity and surface area. They may well be giant molecules of living protoplasm exchanging water and electrolytes throughout their structure and according to their needs. The necessary supply of energy is presumably derived from local transfer of electrons (136). Such a view must appeal greatly to those who have constantly pointed out
that a single cell membrane has to have so many attributes that it should be inches thick and slaving as fiercely as the interior of a nuclear reactor (137).

**Measurement of the Water Compartments.**

Desiccation of cadavers as carried out by classical experimenters such as von Besold in 1857 (15) naturally gave results for total body water only. Several approaches have been made to the problem in the living subject, the most generally practical involving the use of indicator dilution substances. The principal of such a method is to inject a known quantity of a substance which is distributed only in the space to be measured. Once equilibration has taken place the concentration of the indicator is estimated. The equation governing such a relationship is:

\[ A_1 = V \times C_{eq} - A_2 \]

where:
- \( A_1 \) = Amount of indicator injected.
- \( A_2 \) = Amount lost during equilibration period.
- \( V \) = Volume of space to be measured.
- \( C_{eq} \) = Concentration of indicator at equilibrium.
For an indicator to be of value it must possess certain characteristics:

1. Complete safety and freedom from side effects.

2. A volume of dilution which can be accurately assessed, and of which the basic limits do not alter with disease.

3. Uniform distribution

4. Rapid equilibration.

5. Slow excretion.

6. Capable of accurate measurement in plasma.

It is evident that such an exacting list of requirements will never be entirely satisfied in practice. In general it is those available for measuring extracellular volume which fail shortest of this ideal standard. It is also evident that radioactive indicators will a priori possess certain of these characteristics. With modern techniques tracer doses have been reduced to a level which may well carry no risk to the paediatric patient. Since factual evidence on this point is lacking it was decided at the outset to choose stable indicators. The only exception to this rule has been the measurement of total exchangeable sodium in some patients using
exceedingly small amounts of $^{24}\text{Na}$, (see Chapter 7)

**Total Body Water.**

Urea was the first substance to be used as an indicator for the estimation of total body water, by Marshall and Davis in 1914 (134). Other indicators used have been: deuterium oxide (heavy water), (96), sulphonamide (158), thiourea (41), tritium oxide (radioactive water), (157), and antipyrine (phenazone), (201).

Those most generally used have been antipyrine and the isotopes of water. The latter suffer from the disadvantage that an error is introduced due to exchange of labile hydrogen atoms from the solid constituents of the body, mainly protein (74). While this error is relatively small, and can be allowed for (188), it has been shown that the antipyrine volume of dilution represents the closest approximation to the values obtained by desiccation (167).

Antipyrine was originally introduced as a drug with mild anti-pyretic properties. It is non-toxic, the only recorded side effect being on very rare occasions a mild, transient, erythematous rash (80). Its renal excretion-rate is negligible
(0.3-0.6% of the dose in 4 hours), but it is metabolised by the liver at a rate of approximately 6% per hour (201). Since equilibration takes about one and a half hours, it is necessary to obtain several blood samples at specified times thereafter. The plasma concentrations are then plotted on semi-logarithmic paper, when extrapolation gives the theoretical concentration at zero time, had mixing been instantaneous.

Penetration of antipyrine into oedema fluid in adults is rather slow. If the degree of oedema is severe, this fact renders antipyrine unsuitable for the purpose (100).

The estimation of antipyrine in plasma originally presented some difficulties (23,42), but these have been removed with the method devised by Mendelsohn and Levin in 1960 (135).

This method has been used throughout the present series. The term total body water thus refers to the volume of dilution of antipyrine.

**Extracellular Water.**

No ideal indicator has yet been found for the estimation of extracellular water. For a number
of reasons it is likely that such a substance never will be discovered. The biggest problem resides in the difficulty in defining the precise volume of dilution of any given indicator. Some penetrate to a significant degree into cells, a tendency which may alter in disease, while others fail to distribute themselves throughout the entire anatomical extracellular compartment.

A number of saccharide non-electrolytes have been used, for example mannitol (148), inulin (113), and sucrose (44). It has been shown however that these substances fail to penetrate to any extent the "trans-cellular water", and only to a limited extent the extracellular water contained in connective tissue and in bone (34). Thus a considerable under-estimate of the extracellular volume may be expected on using these indicators.

Electrolytes which have been used most are: thiocyanate (35), sulphate (116), bromide (222A) and thiosulphate (78). Radioactive electrolytes frequently used have been sodium (106), and chloride (235).

It was at one time considered (45) that 20-40% of total body chloride was intracellular, and
that estimates of extracellular volume based on
the distribution of chloride or bromide would
therefore be much too high. From the results of a
series of painstaking studies, Cheek (26) concluded
that no more than 10% of chloride is situated in
cells. It is generally considered that the bromide
space corrected for this phenomenon, together with
corrections for serum water and Donnan equilibrium
represents the most accurate theoretical estimate of
extracellular water. The main disadvantage of using
stable bromide is that the estimation of the ion in
plasma is difficult and time-consuming.

Thiosulphate is very rapidly excreted from the
body, and for this a large correction factor is
necessary.

The use of thiocyanate has certain obvious
advantages. In low concentration, the ion is entirely
non-toxic. It equilibrates very rapidly, even in
oedematous subjects (116), and its rate of excretion
is low, something of the order of 1% per hour. It
suffers, however, from certain disadvantages. The
thiocyanate ion has been shown to be bound to plasma
lipids (181). It is also loosely bound to proteins
(187), a property which is shared to a smaller extent
by other univalent ions including bromide (186). Agreement has nevertheless been found between the thiocyanate volume of dilution and that of chloride (235), and it will be shown in Chapter 3 that wellnigh perfect agreement between the thiocyanate space and the 'corrected' bromide space can be demonstrated in normal children. Since any method for estimating extracellular water can only approximate to the truth, the important point, as Ely and Sutow (61) have pointed out, is one of proportionality, i.e. whether the results obtained lend themselves to valid inter-subject comparison. A large volume of literature suggests this to be true of the thiocyanate space in the normal subject (116, 235).

It was demonstrated many years ago by Overman (154) that in monkeys injected with two types of malaria parasite the thiocyanate space could rise to approach that of total body water. A similar discrepancy was found in patients with septicaemia and certain other infective conditions, and it was suggested that this was a general phenomenon accompanying febrile states. Overman inclined
to the view that abnormal permeability of cell
membranes was resulting in excessive intracellular
penetration by the thiocyanate ion. Maegraith
and Leithead in 1962 (132) suggested that an
alternative explanation could be that the rise of
extracellular volume was real and due to a shift of
water from the intracellular space. Some evidence
bearing on this point will be presented in Chapter 7.

In spite of these shortcomings the thiocyanate
method has been selected for the estimation of
extracellular water. Using a modification of the
method of Bowler (20) the determination of the
concentration in plasma is easy and can be done on
capillary blood. The only correction applied has
been for the concentration in plasma water.

**Blood Volume**

The earliest determinations of blood volume
were carried out by post mortem exsanguination (226).
The first indicator dilution substance to be used was
Congo Red in 1915 (111), but this substance gave
highly inaccurate results in the presence of haemolysis.
The blue azo-dye T-1824 (Evans Blue) was introduced
by Gibson and Evans in 1937 (75) and has been easily
the most widely used of the non-radioactive indicators. Other dye-substances used for measuring blood volume have been: Rose Bengal (193), and Geigy Blue (14). Coomassie Blue, much used in dye-dilution studies of heart-output etc., is quite unsuitable for blood volume determination since a high proportion of the injected dose is eliminated from the intravascular compartment within ten minutes (160).

Apart from dyes, certain other non-radioactive indicators have been recommended: polyvinylpyrrolidone (P.V.P.) (166), a substance of large molecular weight with a similar volume of distribution to plasma albumin. The colour reaction used in estimating this compound unfortunately does not obey the de Beer-Lambert Law, rendering the method cumbersome and relatively inaccurate. P.V.P. is however of value for a number of purposes when tagged with $^{131}$Iodine. Yamaguchi (237) used sodium para-amiino hippurate for the simultaneous determination of plasma volume and renal plasma flow. Using Sjostrand's (195) method for estimating total body haemoglobin, Karlberg and Lind (107) measured blood volume in children with a neat apparatus utilising measurement of carbon
monoxide absorption from samples of expired air. This method has the great advantage of requiring no sample of blood other than for estimation of haemoglobin concentration. Intravenous iron-dextran ('Imferon') has also been used (130), but the occurrence of occasional allergic reactions caused the method to fall into disfavour (191).

In recent years radioactive indicators have been widely employed in all save paediatric patients. Those most often used have been albumin labelled with $^{131}\text{I}$ (77), and red cells labelled with either $^{32}\text{P}$ (7) or $^{51}\text{Cr}$ (206).

**T-1824 (Evans Blue).**

The concentration of T-1824 was originally estimated by direct colorimetry of plasma, but the presence of turbidity in many samples rendered this generally quite unsatisfactory. A number of early attempts at extracting the dye gave variable results and were mostly time-consuming (88, 38, 141). A notable advance came in 1951, when Allen (3) obtained consistent recoveries by adsorbing the dye on shredded tissue paper. This principle formed the
Basis of the method of Bedwell et al. (13) which is highly accurate and not unduly lengthy. Some more recent modifications of these techniques have failed to display any further significant advantages (97,104).

A vast literature exists on the behaviour of T-1824 in the body. This has been ably summarised by Gregersen and Rawson (84) who have done a high proportion of the work. The dye is known to become attached to the lysine groups of the plasma albumin molecule (174). Binding is extremely rapid and appears to be complete up to a concentration of 4 mg/100 ml. If this concentration is exceeded extravasation into the interstitial space occurs and the patient turns clinically blue (174). This is more likely to occur when serial blood volume determinations are carried out at daily intervals, as is the rare toxic manifestation of vomiting (178).

Freinkel et al. (71) found that the total distribution compartment and the biological half-life of T-1824 in normal adults did not differ significantly from those of 131I-labelled albumin ( ). The results of Zipf et al. (240) showed a slightly higher mean plasma volume when T-1824 was used, and in
their hands 131I-labelled albumin gave more consistent results. Jegier et al. (105) have shown good correlation between the two methods in the neonate.

It is therefore probable that for children the use of T-125 with extraction of the dye by Edwell’s method remains the most accurate way to estimate plasma volume and has been used throughout the present work.

To compute total blood volume from plasma volume an accurate measurement of the haematocrit is obviously required. Using Vintrobe tubes spun at 3,000 r.p.m. a correction for the amount of plasma trapped between the red cells was necessary (25). Modern micro-haematocrit centrifuges spinning at 15,000 r.p.m. have eliminated the need for this (211). The ratio of total body to venous haematocrit is usually taken as 0.91 (76) or in the newborn as 0.87 (140).

Conclusion

The foregoing discussion has given in outline some of the problems concerning estimations of body water distribution together with the reasons for choosing the specific indicators used in the present work.
CHAPTER 2
THE SELECTED METHODOLOGY OF
BODY WATER DISTRIBUTION

Introduction.

In the previous chapter the reasons for choosing T-1824, thiocyanate and antipyrine to estimate blood volume, extracellular water and total body water have been discussed. In general, all these methods, depending as they do on the concentration of the indicator dilution substances at equilibration have certain features in common. Thus the absolute accuracy of such a method depends on the following factors:

1. Administration of dose.

2. Estimation of the concentration of the indicator in plasma.

3. The characteristic behaviour of the individual indicator with regard to its rate of elimination and the time taken to reach equilibrium with its ultimate volume of dilution.

These headings will now be discussed in detail.
Administration of dose.

When radioactive indicators are used, measurement of the given dose is a simple matter; the activity of the administering syringe is measured before and after use and the dose obtained by subtraction.

With radio-stable indicators the problem is somewhat harder to solve satisfactorily. The three substances used here have all been given intravenously throughout. It is indeed feasible to give sodium thiocyanate and antipyrine orally, but the additional variable incurred by slower distribution makes this inadvisable.

Doses given from a syringe may be measured by:

1. Weighing.
2. Colorimetry.

To weigh a syringe before and after use has theoretical advantages. In practice this method is satisfactory when injection is made directly through a rigid needle, as when working with adult subjects. With children, the inevitability of young subjects moving during the procedure makes it imperative to employ a flexible system such as a polythene scalp-vein needle attachment.

The method evolved in the present investigation has been as follows:
A set of all-glass Luer-Lock syringes of various sizes were individually selected for tightness etc., and suitably marked for identification purposes. Fine scratch-marks were made on both piston and cylinder to allow accurate alignment. This is particularly essential in the case of T-1824, the deep colour of which obscures the end of the piston. These scratch-marks were placed to allow giving a close approximation of the required dose. Other apparatus required consisted of a polythene scalp-vein set with 23-Gauge needle, a carefully selected Luer-Lock two-way tap, and a "Braunule" polythene cannula, size 0.

Procedure.

Under local anaesthesia, the "Braunule" cannula was placed in any suitable vein. All blood samples were obtained through this, avoiding stasis and further venepuncture. After removal of each sample a drop of heparinised saline was put into the cannula before sealing with the stopper provided. The scalp-vein set was attached to the two-way tap and the system filled with sterile isotonic saline from a 20 ml syringe on the direct arm of the tap. This needle was introduced into any suitable vein other than in the limb with the cannula. Thus for each set of determinations
the child was subjected to a total of two needle-
punctures, both of which were from extremely small
needles. On the majority of occasions, the
investigation was carried out when the routine
management of the child demanded a venepuncture.
On humane grounds, it therefore appears to the author
that the technique for body water determination carried
out in this way is unexceptionable.

The syringes containing antipyrine, sodium
thiocyanate and T-1824 were then successively
discharged in that order through the side-arm of the
two-way tap. The dose of indicator was injected,
ever pushing the plunger right home. The indicator
remaining in the side-arm was then washed back into
the syringe with approximately 2 ml. saline from the
reservoir syringe and the indicator syringe removed.
With the tap closed, the next indicator syringe was
then attached and the process repeated. Finally after
the T-1824 had been discharged the polythene tube and
needle were flushed with saline until no blue colour
was visible; approximately 2 ml. of saline were
required for this condition to be fulfilled.

For the sake of reproducibility it was
considered an essential part of this technique to use
the same syringes throughout. Several doses of the indicators were selected to give, in children of widely differing weight, the following approximate plasma concentrations:

**TABLE 2.1.**

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Required plasma concn. mg/100ml</th>
<th>Dose mg/kg</th>
<th>Solution available</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1824</td>
<td>0.4</td>
<td>0.2</td>
<td>Aqueous 0.1%(G.T.Gurr)</td>
</tr>
<tr>
<td>Thiocyanate</td>
<td>7</td>
<td>15</td>
<td>Aqueous 6% (T &amp; H Smith)</td>
</tr>
<tr>
<td>Antipyrene</td>
<td>5</td>
<td>25</td>
<td>Aqueous 20% (B.D.H.)</td>
</tr>
</tbody>
</table>

**Standardization of doses**

The individual doses so derived were standardized by repeating precisely the same process of injection into volumetric flasks. The actual quantity injected was then determined colorimetrically with its standard deviation of the error involved, the figure being calculated from ten such injections into the same volumetric flask. T-1824 was used throughout to standardise all the doses, it being assumed that the volume of fluid injected would not vary with the indicator. Using the dye naturally meant that the
only intrinsic error of this part of the standardization procedure lay with the colorimeter (Hilger 'Uvispek'); it was quickly verified that no significant variation came from this source.

The average S.D. of these administered doses was ± 1% giving an error in practice of ± 2%. The error was in general less for the greater volumes and slightly greater with the smaller volumes. Individual correction factors could thus be calculated for each dose, and the results rechecked at the end of the series when no significant differences from the original factors was found. An example of one such set of results and the calculation of the derived correction factor is set out below:

Experiment 1.

Indicator: T-1824.

Approximate dose administered: 6 mg. (6ml. of 0.1% solution) This quantity injected into 500 ml. volumetric flask and made up to mark with distilled water.

Mean of 10 colorimeter readings (± S.D.): 0.290 (± 0.003) Standard (triplicated): 6 ml. pipetted into same flask: 0.301

\[ \text{Actual dose administered = } 6 \times \frac{0.290}{0.301} \text{ mg.} = 5.79 \text{ mg.} \]
The full table of corrected doses used in the series is set out below:

**TABLE 2.2**

Syringe calibrations (all quantities in mg.)

<table>
<thead>
<tr>
<th>T-1824</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe dose</td>
<td>6</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Corrected</td>
<td>5.79</td>
<td>3.95</td>
<td>2.86</td>
<td>1.97</td>
<td>0.98</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sodium thiocyanate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe dose</td>
</tr>
<tr>
<td>Corrected</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antipyrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Syringe dose</td>
</tr>
<tr>
<td>Corrected</td>
</tr>
</tbody>
</table>

The above procedure may sound somewhat cumbersome; once these calibrations were available, however no further checks were necessary until the completion of the experiment. Close reproducibility has been throughout the paramount object; thus when serial observations were
made on the same child, the same dose (and therefore the same syringe) was utilised.

Estimation of indicators in plasma.

Blood samples were withdrawn through the polythene cannula at the following times:

1. Pre-injection: 2 ml., as controls for thiocyanate and antipyrine estimations.


3. and 4. Post-injection, 3 and 5 hours: each 1 ml. for anti-pyrine estimation only.

In a few subjects larger volumes were withdrawn at 5 hours post-injection for T-1824 and thiocyanate elimination studies.

All samples were withdrawn into tubes containing dried heparin and sealed with paraffin paper. Plasma was immediately separated by centrifugation. If it was not directly practicable to carry out the estimations, the samples were stored at 4°C. This is particularly important in the case of T-1824: if plasma albumin, to which the dye is attached, undergoes significant
autolysis column-extraction of the dye becomes incomplete.

Blood volume.

The estimation of T-1824 in plasma.

T-1824 has been estimated throughout using either the method of Bodwell et al., (13) or a semi-micro modification of the same method. The former was designed to utilise 4 ml. of plasma although half this amount may be used without significant loss of accuracy. The modified method uses 0.5 ml. of plasma. The further minor modifications suggested by Muldowney (145) were incorporated into both methods. The principle of both is the extraction of dye in a column of specially prepared cellulose, and then to elute with 35% acetone. The whole operation is carried out at 50°C.

Macro-method

Apparatus. The extraction column is made from two glass tubes fused together, the internal diameters being 15 mm. and 4 mm. This is surrounded by a glass water-jacket through which water is syphoned from a water-bath at 52°C. When three such columns are used simultaneously, as in Figure 2.1, the temperature of the
Columns for extracting T-1824 from plasma. The small-bore column for semi-micro quantities is on the right. Water at 50°C syphons from the water-bath (left) through the jackets surrounding each column.
third jacket is around 46°C. This gradient does not appear to be material.

Reagents.
1. 50% Teepol-water
2. 1/200 Teepol-saline. 1 ml. of Teepol diluted to 200 ml. with isotonic saline.
3. 35% acetone-water.
4. 25% salicyl sulphonic acid. 25 Gm. of salicyl sulphonic acid diluted to 100 ml. with water.
5. Cellulose. 1 Gm. of degraded amorphous cellulose (Grade PM.36) is dissolved in 200 ml. of 95% acetone. It is then precipitated with 10 ml. of 0.5 NaOH, and allowed to stand for 5 mins. This is centrifuged in 6 x 1" test tubes for 2 - 3 mins. at 1500 r.p.m. It is washed three times with isotonic saline, loosening from the bottom of the test-tubes each time to make sure the cellulose does not become stuck together, since T-1824 tends to stick to lumps. It is then decanted into a 100 ml. volumetric flask with saline and made up to the mark. This reagent cannot be used if more than a week old.
7. Chemical cotton, Type 13.
Procedure. The following tubes are placed in a water-bath at 52°C:

1. A tube containing about 1" of Celite and half filled with 1/200 Teepol-saline.

2. Three tubes each containing Celite sufficient to cover the bottom of the tube and 8 ml. of cellulose reagent.

3. For each plasma sample a tube containing 4 ml. of plasma with 2 ml. of 50% Teepol.

4. A tube containing 1/200 Teepol-saline only.

5. A tube containing 35% acetone only.

Nos. 4 and 5 are refilled as required. The above quantities are sufficient to carry out three extractions.

The column is now plugged at the narrow neck with chemical cotton to allow 30 drops per minute. Three "Pasteur pipettefulls" from tube No 1 are added and allowed to settle and then, very carefully, the contents of tube No. 2 are added, using a Pasteur pipette round the sides of the filter-column.

This layer is allowed to settle and the sides of the column are washed down with the contents of Tube No.4 to remove all traces of cellulose. When the solutions in the column have settled and the level of
the 1/200 Teepol is about $\frac{1}{4}$" above the surface of the filtering medium. The contents of tube No. 3 are carefully added and the tubes washed out to give a quantitative addition. The column is washed several times with the Teepol solution until the eluate is free from protein as tested with salicyl sulphonic acid.

When the column has very nearly dried, 35% acetone is added. This carries the dye before it, to be collected in a 10 ml. measuring cylinder.

**Standard.** 1 ml. is taken from the dye remaining in the ampoule used for the test and diluted to 50 ml. with distilled water; 1.5 ml. of this is made up to 10 ml. with 35% acetone.

Since all traces of plasma are washed out during extraction, no plasma-blank determination is required unless blood volume has been measured within the previous two weeks.

**Semi-micro modification**

The method depends on the fact that since a reduction of the volume of fluid in the column will not pass through spontaneously, pressure is applied to the column. This is carried out either as gentle
suction from below or by applying cautiously pressure from an air-filled syringe to the top of the column. The rate of flow can thus be very easily regulated. The internal diameters of the two tubes forming the column are 4 mm. and 2 mm.

All reagents are the same as used in the macro-method. 0.5 ml. of plasma with 0.35 ml. of 50% Teepol are put through and the eluate made up to 3 ml. The same standard is used.

All readings have been made on a Hilger 'Uvispek' photo-electric colorimeter at 620 A.U.

Haematocrit determinations were made with a micro-haematocrit centrifuge (Hawksley), spinning at 15,000 r.p.m. for 5 minutes. When this machine is used, it is generally agreed that packing of red cells is complete and that no correction for trapped plasma is required (211). Total body haematocrit has been calculated using the factor 0.91 (763).

The computation of blood volume is as follows:

Dose of T-1824 administered = D mg.

Volume of plasma extracted = V ml.

Plasma reading (colorimeter) = R

\[ \text{1 ml. Plasma} = \frac{R}{V} = T \]
30 µgm. standard (colorimeter) = S

... concentration in 1 ml. plasma \( \frac{T}{S} \times 30 = C \)

Plasma volume \( \frac{D \times 1,000}{C} = P \) ml.

Total body haematocrit = (Observed hct.) \( \times 0.91 \)

Cell volume = \( \frac{P \times H}{(100-R)} \) = Ce ml.

Blood volume = \( (P + Ce) \) ml.

Control Experiments

1. Linearity.

Experiment 2. A solution of T-1824 in aqueous 35% acetone obeyed the de Beer-Lambert Law (Figure 2.2).

2. Recoveries.

Experiment 3. 0.25 ml. of 0.1% T-1824 added to:

1. 50 ml. of aqueous 35% acetone,
2. 50 ml. of plasma (reconstituted, dried).

4 ml. of 1. made up to 10 ml. with 35% acetone as standard.
4 ml. of 2. extracted and made up to 10 ml. with 35% acetone.

Results: (Readings from Uvispek).

Standard: 0.136 (duplicated)
FIG. 2.2.

T-1824 — COLORIMETRIC PROPERTY

COLORIMETER READING

0.280

0.240

0.200

0.160

0.120

0.080

0.040

CONCENTRATION (μgm./ml.)

0.4 0.8 1.2 1.6 2.0 2.4 2.8 3.2 3.6 4.0
Test: Mean of 10 such estimations

\[ = 0.1348 \pm \text{(S.D.)} 0.00278 \]

This represents a mean recovery of 99.3\% \pm \text{(S.D.)} 2.0\%.

No correction has been made for this small mean loss of dye.

The total error of estimating blood volume by this method is therefore estimated at approximately \( \pm 6\% \).


Experiment 4: 0.5 ml. of 0.1\% T-1824 made up to 100 ml. with: 1. Distilled water

2. Plasma.

of these solutions,

1. Standard: 0.5 ml. made up to 3 ml. with 35\% acetone.

2. Test: 0.5 ml. extracted and made up to 3 ml. with 35 acetone.

Results: (Readings from Uvispek)

Standard: 0.0885 (duplicated).

Test: Mean of 10 such estimations

\[ = 0.0879 \pm \text{(S.D.)} 0.0018 \]

This represents once again a mean recovery of 99.3\% \pm \text{(S.D.)} 2.0\%. 

h. Coexistence of T-1824 with sodium thiocyanate and antipyrine in plasma.

Experiment 5. Injection of T-1824, thiocyanate and antipyrine into reconstituted dried human plasma (which contains 0.4% sodium acid citrate.)

Doses administered:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Volume</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-1824</td>
<td>3 ml.</td>
<td>3 mg.</td>
</tr>
<tr>
<td>Sod. thiocyanate</td>
<td>1 ml.</td>
<td>60 mg.</td>
</tr>
<tr>
<td>Antipyrine</td>
<td>0.4 ml.</td>
<td>80 mg.</td>
</tr>
</tbody>
</table>

Suitable quantities of plasma mixture were then taken, concentrations of the three indicators estimated and the apparent total volume computed for each substance.

Results: Measured volume of plasma mixture = 1,000 ml. (For thiocyanate and antipyrine results see under appropriate heading). T-1824 volume of dilution = 996 ml. (mean of two).

Interpretation: There is no evidence that the presence of thiocyanate and antipyrine in plasma interferes with the extraction of T-1824.

5. Evaporation of 35% acetone at 50°C

This experiment was carried out to see if an occasional failure to elute could be accounted for by a change of concentration of the eluting solution placed
in a water-bath at 50°C.

**Experiment 6.** 10 ml. of 35% aqueous acetone solution in a graduated centrifuge tube were maintained at 50°C in a water-bath. No significant alteration of the volume was apparent after 30 mins.

**Extracellular Water**

**The Estimation of Thiocyanate in Plasma**

Thiocyanate has been estimated using the method of Bowler (19), or a simple micro-modification of this method devised by the author. The former requires 1 ml. of plasma, the latter 0.2. Heparin should be used to anticoagulate the samples, since citrate is known to interfere with the colour-reaction in this estimation. This finding has been checked (see Expts. 9 and 10.)

**Reagents**

1. 30% trichloracetic acid.
2. Ferric nitrate-nitric acid reagent.

80 Gm. of ferric nitrate $\text{Fe(NO}_3\text{)_3}$· $9\text{H}_2\text{O}$ are dissolved in 250 ml. of 2N - nitric acid, made up to 500 ml. with distilled water and filtered.
Procedure

To 1 ml. of plasma in a test-tube is added 6.5 ml. of distilled water followed by 2.5 ml. of trichloracetic acid. The tube is inverted several times and allowed to stand for 5 minutes. It is then filtered using Whatman No. 43 papers and 5 ml. of the clear supernatant pipetted into a further tube. In subdued artificial (fluorescent) light 5 ml. of the ferric nitrate reagent are added and the colour read as soon as possible at 470 A.U. together with plasma and water blanks.

Wherever feasible automatic pipettes have been employed.

The micro-modification has consisted simply of using 1/5 quantities throughout. Separation of the supernatant aliquots was by centrifuge rather than by filtration. This method has given results throughout indistinguishable from the original.

The computation of extracellular water is as follows:

Dose of sodium thiocyanate administered = D mg.
Plasma reading (colorimeter) = P
Plasma blank reading = B
Standard (3 mg./100 ml.) = S
After the work of Puranen (170), the concentration in plasma water is given by:

\[ C_w = C \times \frac{100}{100 - Pr} \]

where \( Pr \) = the concentration of plasma proteins in Gm./100 ml. In normal subjects this has throughout been assumed to be 7 Gm.%.

Thiocyanate space = \( \frac{D}{C_w \times 10} \) litres.

Control Experiments

1. Linearity

Experiments. Within wide limits (0-20 mg./100 ml.) the colour produced from thiocyanate by this method obeyed the de Beer-Lambert Law.

2. Recoveries.

Experiments. 10 ml. of 6% sodium thiocyanate were made up to 50 ml. with distilled water. 0.2 ml. of this solution was then made up to:

1. 25 ml. with distilled water.
2. 25 ml. with heparinised plasma (obtained from a normal adult.)
Both of these solutions were put through the thiocyanate method with water and plasma blanks.

Results: (Unispek readings.)

Standards: Mean of three = 0.1603

Test: Mean of twelve estimations:

\[ = 0.1698 \pm (s.e.) \times 0.0032.\]

Plasma blank (duplicated) = 0.012

Test minus plasma blank = 0.1578.

This represents a mean recovery rate of 98.4\% \pm (s.e.) 1.9\%.

The total error of estimating thiocyanate space in this way is thus approximately \pm 5\%.

3. Presence of citrate in samples - 1.

Experiment 2. Aqueous solutions were made up containing 3 and 6 mg./100 ml. of thiocyanate, with and without 0.4% of sodium citrate.

When the thiocyanate in the samples was estimated, no difference was detected between the results with and without citrate.


Experiment 10. The results for thiocyanate from Experiment 5.
Actual volume of plasma = 1000 ml.

Apparent volume computed from estimated
concentration of thiocyanate = 828 ml.

Interpretation of Experiments 9 and 10

Citrate does not interfere with the
thiocyanate method when both are in aqueous solution.
When in plasma, however the chromogenicity of the
thiocyanate-complex is significantly enhanced.
(N.B. The possibility of interference due to the
presence of T-1824 and antipyrine was eliminated by
repeating a modification of this experiment using
heparinised plasma; the results for the three indicators
then showed close correlation.)

Total body water

The Estimation of Antipyrine in Plasma.

Antipyrine has been estimated throughout using
the method of Mendelsohn and Levin (135), which
utilises 0.2 ml. of plasma. It does not matter which
anticoagulant is used, but it is important that the
child should not have received any form of sulphonamide
drug during the previous fortnight. Since antipyrine
is eliminated relatively rapidly from the body, the
estimation was carried out on 2 hr., 3 hr., and 5 hr.
samples together with a plasma blank.

The principle of the method is to nitrosate the antipyrrine in a protein-free aliquot with sodium nitrite, and to couple the resulting compound with $N(1$-naphthyl)$)-ethylenediamine dihydrochloride in strong acid to form a blue azo-dye.

Reagents.

1. Cadmium reagent: 5.2 Gm. of cadmium sulphate (3CdSO$_4$.8H$_2$O) are dissolved in 25.4 ml. of 1.0 N sulphuric acid and made up to 50 ml. with distilled water.

2. 1.0 N sodium hydroxide.

3. Glacial acetic acid, analytical grade.

4. 15% aqueous sodium nitrite, freshly prepared.

5. 75% aqueous ammonium sulphamate, freshly prepared.

6. Concentrated hydrochloric acid, analytical reagent grade.

7. $N(1$-naphthyl)$)-ethylenediamine dihydrochloride (chemically pure): 0.5 Gm. made up to 100 ml. with distilled water. Kept in dark bottle, may be used for one week.
Procedure

In a centrifuge tube are placed 0.2 ml. plasma, 0.2 ml. cadmium reagent and 0.2 ml. NaOH, and thoroughly mixed. It is then centrifuged at 3,000 r.p.m. for 5 minutes. 0.2 ml. clear supernatant fluid are transferred to a stoppered tube; 0.4 ml. of glacial acetic acid are added, then 0.1 ml. of sodium nitrite, and the tube allowed to stand for 4 minutes. 0.1 ml. of ammonium sulphamate are then added and the mixture and the tube is shaken very vigorously until no more gas is evolved. It is of crucial importance that this step is carried out thoroughly. 0.08 ml. conc. HCl are then added, then 0.4 ml. of the ethylenediamine reagent, and the tubes are mixed and placed in a water-bath at 80°C for 20 minutes. After cooling they are read at 590 A.U. against water and plasma blanks. The standard has in each case been prepared from the ampoule of the particular test to give a solution of antipyrine 4 mg./100 ml. water.

Reproducibility was probably improved by using a marked set of micro-pipettes, most of which were self-levelling and each used only for its particular reagent (Figure 2.3).

The computation of total body water has been as follows:
Set of micro-pipettes used throughout for estimation of antipyrine
The colorimeter reading for the plasma blank was subtracted from each of the plasma test readings and the results plotted on semi-logarithmic paper against time.

i.e. \( \log(\text{conc.}) \cdot \text{time} \), an exponential relationship which is illustrated in Figure 2.5. Extrapolating back to zero time gives the theoretical reading \((T)\) had complete mixing of the indicator throughout its volume of dilution been instantaneous.

Then, where dose administered \(= D\ mg.\)

4\ mg./100\ ml. standard reading \(S\).

\[
\text{plasma concentration } C_p = \frac{T}{S} \times 4\text{mg.}/100\text{ ml.}
\]

Plasma water concentration \(C_w = C_p \times \frac{100}{100-\Pr}\)

\[
\text{Antipyrine space } = \frac{100}{C_w \times 10} \text{ litres}
\]

From the Pace-Rathbun equation (156), (q.v.), we can say that:

\[\text{Lean body mass } = \text{Antipyrine space } \times \frac{100}{73} \text{ kg.}\]

Control Experiments.

1. Linearity

Experiment II. Obedience to the de Beer-Lambert Law was verified for concentrations 0.10 mg./100 ml.
2. Recoveries.

**Experiment 12.** 2 ml. of 20% antipyrine were made up to 50 ml. with distilled water. 0.2 ml. of this solution were made up to:

1. 10 ml. with distilled water.
2. 10 ml. with heparinised plasma (from normal adult).

Both of these solutions were put through the method with water and plasma blanks.

Results: (Uvispek readings.)

Standard = 0.417 (Mean of three).
Plasma blank = 0.000 (Duplicated).
Test: Mean of seven plasma estimations

\[
= 0.417 ± (S.D.) 0.005
\]

This represents a mean recovery of \(100\% ± (S.D.)\) 1.25%. The total error of estimating antipyrine space by this method is thus rather less than 5%.

3. Presence of T-1824 and thiocyanate in samples.

**Experiment 13.** The results for antipyrine from Experiment 5.

Actual volume of plasma = 1,000 ml.

Apparent volume computed from estimated concentration of antipyrine = 990 ml.
Elimination of Indicators from Plasma.

It can readily be shown (Figures 2.4, 2.5, and 2.6) that once T-1824, thiocyanate and antipyrine have equilibrated with their respective volumes of dilution, the disappearance of each from plasma with time is exponential.

\[ \text{Log } C = \text{plasma concentration} \]

\[ T = \text{time after injection} \]

then \( \text{Log } C \propto T \)

or \( \text{Log } C = \kappa T \)

where \( \kappa \) is a constant. This constant must be dependant on a number of factors such as the nature of the indicator, the presence of pathology such as abnormal capillary permeability, etc. In other words, varies with different subjects and also at different times within the same subject.

T-1824.

A typical elimination curve for this substance is shown in Figure 2.4. Disappearance becomes exponential after approximately 15 minutes, indicating that complete mixing has taken place. Thereafter the moderate rate of disappearance is similar to that occurring in adults, and also to that of \( ^{131} \text{I}-\text{labelled I.H.S.A.} \), (82, 71). The disappearance rate in newborn
A typical plasma elimination curve for T-1824, becoming exponential after 15 mins.
infants is somewhat faster, but that of I.H.S.A. considerably more so (105,103).

Clearly the most accurate way to evaluate the T-1824 space is to plot the concentrations after 15 minutes and extrapolate back to zero time. The volume of blood required prohibits such a procedure in children, and Gregersen (83) demonstrated that a single concentration at ten minutes would give a satisfactory estimate of plasma volume. Figure 2.3 indicates the relationship of such a sample to the general elimination-curve, and this procedure has been used throughout the present series. Jegier et al. (105) found that a single ten minute sample would yield comparable results in the newborn period.

**Thiocyanate**

The rate of excretion of thiocyanate is extremely slow, the figure usually quoted being less than 1% per hour (145). This slow rate has been confirmed (Figure 2.5). At the same time equilibration with its volume of dilution is relatively fast, all the evidence pointing to complete mixing in under two hours, even in the oedematous subject (Figure 2.5). This is confirmed by the exponential
Elimination of thiocyanate from an oedematous subject with AGN
character of the curve at two hours in such a
subject, since if equilibration with oedema fluid
were still taking place the concentration plotted at
this time would lie above the straight line drawn
through the later values.

Thus a single two-hour sample has been used
throughout the series, no correction being made for
the small loss which will have occurred by this time.

Antipyrine.

Antipyrine is excreted faster than thiocyanate,
necessitating the use of the extrapolation technique
already referred to. Elimination is exponential by
two hours, even in the mildly oedematous subject
(figure 2.6). This is in general not true of the
grossly oedematous subject, in whom the slow rate of
equilibration renders antipyrine unsuitable for
determining total body water (100). This finding was
confirmed here in three children with nephrotic
syndrome, in none of whom had the exponential curve
straightened by four hours, and in one case, by eight
hours.

In the normal subject, and the mildly oedematous
acute nephritic, samples taken at two, three and five
hours post-injection have invariably produced a
ACUTE NEPHRITIS
ANTI-PYRINE ELIMINATION

CASE JB.

Exponential elimination over 24 hrs. of antipyrine from oedematous subject with AGN
straight line when plotted.

The newborn is characterised by very slow excretion of antipyrine (131). A single sample taken at two hours is therefore probably satisfactory during the first few days of life. In the present series of newborn infants (Chapter 4), however, three samples have been used throughout.

**Derivation of intracellular and interstitial volumes.**

Since the values for both thiocyanate and antipyrine space determinations have been expressed in terms of volumes of water, it is permissible to subtract one from the other to obtain an estimate of intracellular water (I.C.W.)

i.e. throughout this series:

I.C.W. = Antipyrine space - thiocyanate space.

Intracellular space can be subdivided by subtracting one part of it which has been measured separately, namely, the red cell mass. Since the latter has not been expressed in terms of cell water, the two are not strictly comparable, but the difference between red cell volume and red cell water must be rather small. No obvious additional information has
become apparent from studying the results of such a division in the present work.

**Interstitial water.**

To derive a value for interstitial water (I.S.W.), the plasma volume (T-1824 space) must first be converted to plasma water:

\[
\text{Plasma water} = \text{T-}1824 \text{ space} \times 0.93,
\]

assuming a plasma protein concentration of 7 Gm./100 ml. In diseased subjects the observed value for plasma proteins has been substituted in this equation.

Then, \( I.S.W. = \text{Thiocyanate space} - \text{plasma water} \)

**Body "solids".**

The difference between body weight and total body water has been referred to throughout as representing body "solids". The two largest single components of this are body fat, with an average water content of 10%, and the inorganic component of bone. The ratio of solids to water varies between subjects and between tissues. Further theoretical aspects of this parameter have been discussed in Chapter 1.
CHAPTER 3

BODY WATER DISTRIBUTION IN NORMAL CHILDREN

1. THE AGE GROUP 1½ - 12 YEARS

Much fundamental work on body water distribution throughout childhood has been carried out in the last twenty years. In this context the work of Friis-Hansen is pre-eminent. This worker, in a series of ninety three children ranging from birth to sixteen years, showed a decrease in the proportion of both extracellular water and total body water to body weight occurring throughout childhood. The greatest rate of decrease was during the first year of life (74). The results of other workers have tended to confirm these observations (142,123,26). The whole subject of the growth and development of these and other parameters has been extensively discussed by Forbes (69).

The decrease in the proportion of extracellular water is confined to the interstitial compartment, since the plasma volume remains remarkably constant throughout growth at around 50 ml./kg. body weight (184,61,143).

In the present work, the study of body water
distribution in normal children has been carried out with three aims in view:

1. The formation of a control group to provide a basis for comparison with the changes observed in acute nephritis.

2. In order to gain personal experience of the practical methodology of determining body water distribution.

3. The addition of a small contribution to the general literature on the subject. As stated above, this is extensive, but it has appeared to the author that information concerning certain relationships in children is deficient. For example, Muldowney showed in 1957 (145) that in normal adults there is an extremely close linear correlation between lean body mass and red cell mass; in the same subjects the correlation between red cell mass and total body weight was relatively poor. A relationship of this kind has not so far been demonstrated in childhood. In calculating lean body mass, Muldowney used the relationship: Lean body mass = Total body water \( \times \frac{100}{73} \), suggested by Pace and Rathbun (156). The proportion of water in the lean body mass in infancy is considerably greater than 73% (233), but the adult
figure is thought in this respect to be reached around 2 years of age. (74).

The Series.

Twenty children have been studied. Since the group was intended primarily as a control for the nephritic series, no children under eighteen months old have been included; acute nephritis is rare in children younger than this. Although referred to throughout as a "Normal" series, the basis for this designation must be called in question.

Definite criteria for selection.

1. The height and weight to lie above the third percentile of mean for age (190).

2. A haemoglobin level within the accepted normal range for age (214).

Less Definite Criteria

Most of the children were investigated following recovery from the disease leading to admission to hospital, and therefore immediately prior to going home. Any child whose disease had been associated with oedema or other obvious abnormality of body water distribution (e.g. diabetes mellitus, dehydration,
children on steroid drugs), was excluded. Three cases presented as behaviour problems without demonstrable disease, but in this category encopretics were excluded in view of the possibility of abnormal extracellular volume due to the presence of large amounts of bowel water.

Results and Comment

The basic data for the group is given in Table 3.1. The individual results have been expressed throughout in absolute terms, together with the arithmetical means for the group. Also indicated are the mean values (with Standard Error of the mean) of the results expressed in ml./kg. body weight.

From these mean absolute values a block diagram (Fig. 3.1.) has been constructed to represent the body water distribution of an average normal child of this age group.

Reproducibility

A second determination of body water distribution was carried out on three of the subjects one week later. The mean deviation between results was for total body water $+0.8\%$ (range $-1.2 - +2.1\%$) for extracellular water $+0.3\%$ ($-0.5 - +1.3\%$), and for plasma volume $-0.8\%$ ($-2.2 - +1.6\%$). These deviations are
### TABLE 3.1

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age (Years)</th>
<th>Weight (kg)</th>
<th>T.B.W. (litres)</th>
<th>E.C.W. (litres)</th>
<th>P.V. (ml)</th>
<th>E.V. (ml)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>11.34</td>
<td>33.0</td>
<td>20.90</td>
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<td>1203</td>
<td>1965</td>
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<td>2</td>
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<td>23.6</td>
<td>14.54</td>
<td>6.35</td>
<td>1292</td>
<td>2010</td>
</tr>
<tr>
<td>3</td>
<td>8.97</td>
<td>20.4</td>
<td>12.33</td>
<td>5.12</td>
<td>933</td>
<td>1377</td>
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<td>4</td>
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<td>2.79</td>
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<td>688</td>
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<td>7</td>
<td>11.97</td>
<td>23.8</td>
<td>17.36</td>
<td>6.46</td>
<td>1074</td>
<td>1739</td>
</tr>
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<td>8</td>
<td>2.08</td>
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<td>1817</td>
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<td>22.6</td>
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<td>1019</td>
<td>1623</td>
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<td>3490</td>
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<td>2640</td>
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<tr>
<td>15</td>
<td>7.92</td>
<td>27.2</td>
<td>18.64</td>
<td>8.21</td>
<td>1220</td>
<td>1912</td>
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<tr>
<td>16</td>
<td>7.41</td>
<td>25.6</td>
<td>16.60</td>
<td>7.89</td>
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<td>2111</td>
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<td>12.17</td>
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<td>9.82</td>
<td>1693</td>
<td>2474</td>
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<tr>
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<td>11.04</td>
<td>25.6</td>
<td>17.58</td>
<td>7.67</td>
<td>1735</td>
<td>2750</td>
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<tr>
<td>19</td>
<td>5.75</td>
<td>16.5</td>
<td>9.33</td>
<td>4.73</td>
<td>796</td>
<td>1180</td>
</tr>
<tr>
<td>20</td>
<td>8.50</td>
<td>20.1</td>
<td>11.37</td>
<td>5.59</td>
<td>1060</td>
<td>1586</td>
</tr>
</tbody>
</table>

| Mean        | 7.66        | 2357        | 13.97          | 6.24           | 1120      | 1732      |

| Mean Values in ml./kg. | 598 | 270 | 47.4 | 72.9 |

| S.D.         | 17.9 | 6.55 | 1.53 | 2.43 |
Body Water Distribution
Average of 20 Normal
Children 2-11 Years

Block diagram representing mean percentage values for body water and solids. The volumes of distribution of the three indicators utilised are shown.
considerably less than the theoretical maximum error of the methods, described in Chapter 2.

Regression of Measured Parameters

The linear regression of red cell mass on lean body mass, and red cell mass on total body weight are shown in Fig. 3.2. The overall correlation represented in the two graphs is similar, with a slightly higher value for the correlation coefficient pertaining to total body weight (0.9085 as compared with 0.8813).

Using scattergrams (not illustrated), the regression of all the parameters measured, both with each other and also with body weight, has been shown to be roughly linear. That with the highest correlation coefficient of those tested was E.C.W. against body weight (r = 0.9696).

A list of all these linear correlation coefficients is shown in Table 3.2., together with regression equations for all the values of 'r' greater than 0.9. It is clear that with such a table a number of calculations are possible; the individual scatter of results however makes it in practice more realistic to regard these equations as a general guide.
The regression of red cell mass on lean body mass and total body mass in normal children. The similarity of the patterns is evident.
<table>
<thead>
<tr>
<th><em>y</em></th>
<th><em>x</em></th>
<th>r</th>
<th>Regression equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.C.W.</td>
<td>T.B.W.</td>
<td>0.9775</td>
<td>$y = -0.419 + (0.583) x$</td>
</tr>
<tr>
<td>E.C.W.</td>
<td>B.W+</td>
<td>0.9696</td>
<td>$y = 0.654 + (0.237) x$</td>
</tr>
<tr>
<td>I.C.W.</td>
<td>T.B.W.</td>
<td>0.9683</td>
<td>$y = -0.402 + (0.538) x$</td>
</tr>
<tr>
<td>I.S.W.</td>
<td>B.W+</td>
<td>0.9644</td>
<td>$y = 0.637 + (0.194) x$</td>
</tr>
<tr>
<td>I.S.W.</td>
<td>T.B.W.</td>
<td>0.9599</td>
<td>$y = 0.400 + (0.344) x$</td>
</tr>
<tr>
<td>E.C.W.</td>
<td>T.B.W.</td>
<td>0.9572</td>
<td>$y = 0.419 + (0.417) x$</td>
</tr>
<tr>
<td>P.V.</td>
<td>E.C.W.</td>
<td>0.9516</td>
<td>$y = -87.29 + (193.48) x$</td>
</tr>
<tr>
<td>B.V.</td>
<td>E.C.W.</td>
<td>0.9444</td>
<td>$y = -139.9 + (299.9) x$</td>
</tr>
<tr>
<td>T.B.W.</td>
<td>B.W+</td>
<td>0.9421</td>
<td>$y = 1.53 + (0.528) x$</td>
</tr>
<tr>
<td>P.V.</td>
<td>B.W+</td>
<td>0.9339</td>
<td>$y = 27.37 + (46.36) x$</td>
</tr>
<tr>
<td>B.V.</td>
<td>B.W+</td>
<td>0.9335</td>
<td>$y = 25.55 + (723.8) x$</td>
</tr>
<tr>
<td>P.V.</td>
<td>I.S.W.</td>
<td>0.9274</td>
<td>$y = -72.43 + (229.31) x$</td>
</tr>
<tr>
<td>B.V.</td>
<td>I.S.W.</td>
<td>0.9193</td>
<td>$y = -115.3 + (355.25) x$</td>
</tr>
<tr>
<td>C.V.</td>
<td>B.W+</td>
<td>0.9085</td>
<td>$y = -1.61 + (26.02) x$</td>
</tr>
<tr>
<td>C.V.</td>
<td>E.C.W.</td>
<td>0.9072</td>
<td>$y = -52.4 + (106.49) x$</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>y</th>
<th>x</th>
<th>r</th>
<th>y</th>
<th>x</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.V.</td>
<td>T.B.W.</td>
<td>0.8944</td>
<td>I.C.W.-C.V.</td>
<td>I.S.W.</td>
<td>0.8646</td>
</tr>
<tr>
<td>P.V.</td>
<td>T.B.W.</td>
<td>0.8835</td>
<td>I.C.W.-C.V.</td>
<td>B.W+</td>
<td>0.8519</td>
</tr>
<tr>
<td>C.V.</td>
<td>T.B.W.</td>
<td>0.8813</td>
<td>C.V.</td>
<td>I.C.W.</td>
<td>0.6151</td>
</tr>
<tr>
<td>I.S.W.</td>
<td>I.C.W.</td>
<td>0.8809</td>
<td>B.V.</td>
<td>I.C.W.</td>
<td>0.8099</td>
</tr>
<tr>
<td>E.C.W.</td>
<td>I.C.W.</td>
<td>0.8745</td>
<td>P.V.</td>
<td>I.C.W.</td>
<td>0.7949</td>
</tr>
<tr>
<td>I.C.W.</td>
<td>B.W+</td>
<td>0.8714</td>
<td>C.V.</td>
<td>I.C.W.-</td>
<td>0.7829</td>
</tr>
</tbody>
</table>

* Values for x and y are expressed in litres or kilogrammes, except for plasma, red cell and blood volumes, which are expressed in ml.
Variation with age.

There is no significant correlation between T.B.W. expressed in ml./kg., either with age or with Log (Age) \( r = -0.0403 \). The same is true of plasma volume (ml./kg.) against age \( r = 0.1612 \), and of I.C.W. \( r = 0.1181 \).

When E.C.W. is correlated with Log (Age), there is a negative trend which with this small number of cases is not significant \( r = -0.3864, P>0.1 \).

With I.S.W. the correlation with Log (Age) becomes significant \( r = -0.4484, P<0.05 \). This is a clear log-linear relationship which is depicted in Fig. 3.4. The appropriate regression equation for this is:

\[
y = 251.4 - 3.4 (\log_{e} x).
\]

Where \( y = \) Interstitial Water (ml./kg.) and \( x = \) Age in years, (2-12 inclusive.)

The standard deviation of regression, however is such (14,80) that the equation is of little practical value for calculating interstitial water. For this purpose the equation linking this parameter with body weight the equation linking this parameter with body weight (Table 3.2) is more suitable.
20 NORMAL CHILDREN

Log-linear regression of interstitial water and plasma water on age. The slope of the upper regression line is significant.
In general, the patterns of regression demonstrated between the various components of body water distribution and body weight are approximately linear, although the small size of the group make absolute verification of this unsatisfactory. Various workers have compared body water distribution with other parameters, such as height, surface area, and a combination of the latter with body weight. (61, 74, 184). For practical purposes, body weight in childhood appears to be as satisfactory a reference as any. It has been shown that for both total body water (74) and for extracellular water (26), there is close agreement with body weight from early foetal life to around six weeks post-natal age. After that age the slope of the regression line is slightly less but once again straight, and extrapolation no longer passes through the point of origin of the graph. It is thought (26) that body composition matures and approximates much more closely to the adult pattern from that time on. Since the age-range of the present group is older than this critical value, a break in the linearity would not be expected and has not been found.
It has also been suggested (142) that one of the aspects of this maturing process is the changing of the proportion of water in the lean body mass to the adult figure of 73% - i.e. the time whence the Pace-Bathbun equation becomes operative. It will readily be appreciated that if this equation is applied to young infants the values for "lean body mass" so obtained will frequently exceed the infant's total weight. It will be shown in the next chapter of this work that the mean total body water in the newborn infant is around 715 ml./kg. Accepting Widdowson's figure (233) for body fat as comprising 16% of body weight at this age, the proportion of water in the lean body mass can immediately be calculated at around 83%. In other words, the Pace-Bathbun equation should at this age be modified to:

\[ \text{Lean body mass} = \text{Total body water} \times \frac{100}{83} \]

The fact that the proportion of total body water to body weight throughout childhood is greater than in adults suggests either - 1. that children have less body fat than adults, or 2. that the maturation process is a gradual one extending at least
to puberty. Muldowney's results (145) showing well-nigh perfect correlation between red cell mass and lean body mass but poor correlation with body weight in normal adults appears to illustrate a quite fundamental principle. The fact that such a distinction is not at all brought out in childhood by the results of the present series seems strong evidence of basic differences in body composition between the pre-pubertal and the mature.

The practical use of equations for calculating an unknown component of body water composition is tempered by the fact that any given equation refers only to the particular indicator dilution substance used in deriving the results. Thus whether or not the volumes of dilution measured represent an accurate determination of the true water-spaces is clearly fundamental. There is general agreement that in the cases of T.1824 and antipyrine, the representation of the plasma volume and of total body water is a fair one, within the limits mentioned in Chapter 1. With thiocyanate there is less agreement, due to the peculiar properties of this ion, already described. Cheek (26), in a scholarly discussion of the definition and measurement of extracellular volume,
came to the conclusion, based on much evidence, that the most accurate determination of this parameter is represented by the volume of dilution of stable bromide, corrected for 10% intracellular penetration. For thirty children whose weights ranged from 10-30 kg., he derived the regression equation:

Corrected bromide space = \frac{28.67 \text{ weight}}{(Cl)_e} + 35.5

Where \((Cl)_e\) is the concentration of chloride per litre of water in a serum ultrafiltrate.

Assuming an average normal value for \((Cl)_e\) of 110 meq., this equation becomes:

Corrected bromide space = 0.323 + 0.261 weight

This compares with the comparable equation for thiocyanate space given in Table 3.2:

Thio. space = 0.654 + 0.237 weight

These two regression equations are shown graphically in Figure 3.4. The close agreement in this weight-range is obvious, and supplements evidence pointing to the same conclusion from the early work of Lavietes et. al. (116).

Similarly it will be shown in Chapter 4 that reasonably close agreement between the corrected bromide space and the thiocyanate space holds also in the
Regression lines illustrating estimation of extracellular water from the 'corrected' bromide space - from results of Cheek (26), and from the thiocyanate space (present series.)
newborn infant.

The presentation of this list of regression equations for practical use in, for example, the planning of intravenous therapy is therefore felt to be fully justified.

It was stated early in this Chapter that the prime reason for investigating a series of normal children was to form a control picture with which to compare the results obtaining in acute nephritis. The block diagram appearing as Figure 3.1 is the outcome of this. It is of course a composite picture, representing the mean values for children of widely differing sizes. However, the fact there is no alterations with age in the proportion of plasma volume or total body water justifies the application of a single mean value. The values for interstitial water do indeed decrease proportionately with age; this decrease is of the order of some 10 ml./kg., occurring between two and twelve years and has been therefore ignored.

Summary

1. The mean values for body water distribution measured in twenty normal children were as follows:

   Total body water: 598 ml./kg.
Extracellular water  270 ml./kg.
Plasma volume  47.4 ml./kg.
Blood volume  72.9 ml./kg.

2. A list of regression equations linking the components of body water distribution with body weight has been drawn up for practical use. The theoretical accuracy of such equations is discussed.

3. The fundamentally close correlation between red cell mass and lean body mass obtaining in normal adults was not found in the present series. The significance of this finding is discussed.
CHAPTER 4

BODY WATER DISTRIBUTION IN NORMAL CHILDREN

II. THE FIRST TWO WEEKS OF LIFE

Introduction

In order to render more complete the picture of body water distribution in normal children, a study of the newborn period has been undertaken. In the older child, it seems that, disease-states apart, water-distribution remains fairly static. The immediate post-natal period however represents a time of generalised body-changes, the degree of which is certainly unequalled at any other age. It would be surprising therefore if body water did not play a large part in these alterations, some of which have indeed been documented. It is noteworthy that existing studies of body water distribution tend to show at this age wide subject-to-subject variation, both in full-term infants (68, 73, 64, 86) and in the premature (28,200).

It has appeared to the author that some premature infants accepted in the literature as 'normal', may in fact by virtue of an immature inability to control their own body water, readily become abnormal
in this respect. It is possible that such changes might be associated with excessive alteration of water-distribution, always assuming such changes to be part of the normal picture. Shifts of fluid between the intravascular and the interstitial compartments have been shown to occur during the first twelve hours of life (29,216). The possibility of shifts occurring between the intracellular and the extracellular spaces has not however been adequately investigated. A pointer towards such a possibility lies in the clinical observation that an infant with little or no demonstrable oedema at birth may become oedematous during the first twenty four hours (212). During this period weight-loss will certainly have occurred, and the infant may well have had no fluid-intake.

Case Material and Methods.

Forty six apparently healthy infants born by spontaneous vertex delivery in the Royal Maternity Hospital, Glasgow, were the subjects of the study. No standardization of time-interval regarding clamping of the cord was introduced. The birth weights varied from 1.62 kg. to 3.65 kg and the ages from 4 hours to
13 days. Four of the infants (Nos. 10, 14, 18 and 21) were clinically mildly oedematous at the time of the determination. Thirty nine were examined during the first six days of life and form a special part of the investigation.

In each subject the weight was recorded daily for the first week or until body water determination was carried out, whichever was the greater. In forty four of the infants oral fluids were started at twenty four hours; two (Nos. 11 and 20) were started at twelve hours. Water was offered twice, four hours apart, the quantities being decided by the infants. Fifteen or thirty ml. of half cream milk was then offered three or four-hourly according to the individual infant's size, with increments dependant on demand.

Forty-six determinations were made of extracellular space and blood volume; total body water was estimated in 20 of the same infants. The procedure was as previously described (Chap. 2), with the following modifications:

A pre-injection sample of capillary blood was obtained from the infant by heel-stab. A No. 27 gauge scalp-vein Usher needle was introduced into a
suitable scalp vein. The needle was attached to 6 cm. of 40 gauge polythene tubing with a No. 23 intradermal needle pushed into the other end. This was connected to a carefully selected Luer Lock two-way tap and the system filled with isotonic saline prior to use.

The indicator dilution substances were given from the same three 1 ml. Tuberculin syringes used throughout the study. The method of use and standardization of the doses was as previously described. The indicators were employed in the following doses:

<table>
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<tr>
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<th>Antipyrine</th>
<th>Thiocyanate</th>
<th>T-1824</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infants under 2.00 kg.</td>
<td>50 mg.</td>
<td>30 mg.</td>
<td>0.4 mg.</td>
</tr>
<tr>
<td>2.01 – 3.00 kg</td>
<td>60 mg.</td>
<td>40 mg.</td>
<td>0.5 mg.</td>
</tr>
<tr>
<td>over 3.00 kg.</td>
<td>80 mg.</td>
<td>60 mg.</td>
<td>0.7 mg.</td>
</tr>
</tbody>
</table>

A venous sample (5 ml.) was withdrawn 10 minutes post-injection for T-1824, haematocrit and, in 34 subjects, haemoglobin determination. Capillary samples were withdrawn at 2 hours, 3 hours and 5 hours.

Total body haematocrit was calculated using the factor 0.87 as suggested by Mollison et al., (140).
Determination of haemoglobin was by the cyanmethaemoglobin method, the results being read on an 'EEL' photoelectric colorimeter.

It is worth noting that in the great majority of infants the concentration of antipyrine at five hours did not differ significantly from that at two hours, indicating a slow rate of excretion which must be limited to the neonate. Thus a single blood sample at two hours would appear to be sufficient for estimating total body water at this age. Indeed, in four instances the concentration of antipyrine at five hours slightly exceeded that at two, presumably indicating greater loss of water relative to antipyrine during the test period. This phenomenon was only noted in infants less than 48 hours old, and in these cases calculation of the antipyrine space was made from the two hour concentration.

Results

The data obtained for body weight, total water, thiocyanate space, intracellular and interstitial water, plasma volume, haematocrit and haemoglobin determinations are shown in Table 4.1. Absolute values for total water, intra- and extracellular water against weight are shown in Figs 4.1 - 4.3.

Total body water expressed in ml./kg. body weight has been plotted against weight in Fig 4.4.
<table>
<thead>
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<th>1.74</th>
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<th>3.75</th>
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<td>29.9</td>
<td>64.9</td>
<td>69.1</td>
<td>71.7</td>
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<td>69.1</td>
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<td>-</td>
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<td>0.02</td>
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<td>71.7</td>
<td>90.6</td>
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<td>-</td>
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<td>0.02</td>
<td>90.6</td>
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<td>4.75</td>
<td>4.75</td>
<td>4.75</td>
<td>-</td>
</tr>
</tbody>
</table>

**NOTE:** The data table continues on the next page.
Open circles refer to seven subjects with 'abnormal' total body water (see text)
NEONATE SERIES

INTRACELLULAR WATER. 30 Subjects

Open circles - as in Figure 4.1.
FIG. 4.3.

NEONATE SERIES

EXTRACELLULAR WATER. 46 Subjects

EXTRACELLULAR WATER

Litres

r = 0.9127

WEIGHT (Kg.)
The infants have been divided into those of three days and less (closed circles) and those of more than three days old (crosses). It should be noted that the linear regression line with its 95% confidence limits refers only to the 15 infants aged three days and less. These are negatively correlated ($r = 0.8083$), this correlation being highly significant ($P < 0.001$).

Of the infants older than three days, all those heavier than 2.20 kg. at the time of estimation show results within the same confidence limits. Seven of the ten infants below this weight however lie well outside.

The results for intracellular water against weight shown in Figure 4.5 indicate a similar significant negative correlation ($r = 0.7526, P < 0.01$) to total body water. The instability of total body water in the smaller infants over three days old is shown to be mainly associated with alterations in this compartment, five of the same seven infants (open circles) lying outside the 95% confidence limits.

The same treatment of the thiocyanate spaces (Figures 4.6) reveals a different relationship. Here the proportion of extracellular water is constant ($r = 0.0106, P > 0.1$); the coefficient of regression does
The regression line and its 95% confidence limits refer only to infants aged three days and younger (closed circles.) Crosses indicate older subjects. Note the seven 'abnormal' values are all from infants weighing less than 2.2 kg.
Closed circles, crosses, regression line and 95% confidence limits as in Figure 4.4. The open circles apply to the seven infants with 'abnormal' total body water.
Subjects denoted as in Figure 4.5. The ratio of extracellular water to body weight is constant.
FIG. 4.7.

Subjects denoted as in Figure 4.5.
not therefore differ significantly from zero and hence the arithmetical mean with two standard deviations has been interpolated on the graph. Once more these lines refer only to the infants aged three days and less. Within these limits it appears that after three days the extracellular space is more effectively maintained than the intracellular; the values for all the infants shown to have an 'abnormal' total body water lie within two standard deviations from the mean. A very similar relationship applies to plasma volume (Figure 4, 7).

From the above evidence it seemed reasonable to conclude that certain of the subjects should be considered to have an abnormal proportion of total body water. These infants have therefore been omitted from the remainder of the investigation. Following the same argument, all values for thiocyanate space, and all save one (No. 44) of the results for plasma volume, may be considered to be normal and have therefore been used in the second part of the investigation.

It was clear that in this series the variability of the proportion of extracellular water to body weight was relatively great; the factor of alteration with
time was therefore analysed. Values for extracellular water during the first six days of life were available in 39 infants, the number of observations per day averaging 6.5 (range 5-8). The mean (± S.E.) for each day is seen plotted in Figure 4.8; the connecting line is parabolic in shape, with an immediate rise from the first to the second day of life. The maximum occurs on the fourth day, with return towards the birth-values by the sixth day.

Using the method of least squares, a calculated parabola was fitted to these results. The appropriate regression equation is

\[ Y = 292.32 + 47.204x - 6.359x^2 \]

where \( Y \) = Extracellular water (ml./kg.),

and \( x \) = Day of life within the period under study (1-6).

Analysis of variance indicates that the parabolic component of the above equation is highly significant \((F = 9.90, P < 0.01)\). The calculated parabola has a maximum at 3.7 days, and the closeness of fit to the observed results is seen in Figure 4.8.

During the first six days of life, important changes are taking place in body weight. It does not necessarily follow that the above changes in proportional
Results from 39 infants (mean + S.E.) plotted against time. The broken line represents the calculated parabolic regression curve which has a maximum at 3.7 days.
extracellular water reflect absolute changes occurring in this compartment. Serial alteration of the weights of all 39 infants (mean ± S.E.) are indicated at the top of Figure 4.9. If it is assumed that water-distribution is going to behave in a roughly similar fashion during this period irrespective of birth-weight, then it becomes justifiable to apply the curve derived in Figure 4.8 to these changes in weight. Further, the demonstrated lack of correlation between the extracellular water in ml./kg. and the weight means that no further modification of the values is required for the general concept to be mathematically valid. The results of this are indicated in Table 4.2, and suggest that a significant absolute rise in extracellular water does in fact occur during the first four days. Since only one of the infants measured during the second day of life had been given oral fluids, it follows that the increase at this time can only have been derived by transfer of water from the intracellular space.

These absolute changes in extracellular water are depicted in Figure 4.9. Subtraction of the value for each day from the mean measured weight gives a value which should correspond with the sum of
## Table 4.2

**Changes in Extracellular Water - 39 Subjects**

<table>
<thead>
<tr>
<th>Day of Life</th>
<th>Number of Observations</th>
<th>Observed E.C.W. Mean ± S.E. ml./kg.</th>
<th>Calculated Mean E.C.W. from Regression Equn. ml./kg.</th>
<th>Body Wt. Mean ± S.E. Gm.</th>
<th>Calculated Mean Absolute E.C.W. ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>334 ± 8</td>
<td>333</td>
<td>2400 ± 76</td>
<td>799</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>362 ± 17</td>
<td>361</td>
<td>2310 ± 73</td>
<td>334</td>
</tr>
<tr>
<td>3</td>
<td>5</td>
<td>368 ± 15</td>
<td>377</td>
<td>2250 ± 74</td>
<td>843</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
<td>382 ± 12</td>
<td>379</td>
<td>2260 ± 76</td>
<td>857</td>
</tr>
<tr>
<td>5</td>
<td>6</td>
<td>376 ± 10</td>
<td>369</td>
<td>2290 ± 82</td>
<td>845</td>
</tr>
<tr>
<td>6</td>
<td>7</td>
<td>343 ± 8</td>
<td>347</td>
<td>2320 ± 78</td>
<td>805</td>
</tr>
</tbody>
</table>
Upper curve represents daily changes in observed body weights. Lower curve derived as in Table 4.2. Middle curve represents body weight minus extracellular water.
intraoellular water and 'solids'. Similar curves could not be derived for total body water or intracellular water, since after elimination of a number of the cases for the reasons previously discussed, the scatter of the remaining results was such that they did not correspond to a calculated curve to a statistically significant degree. (For total body water, after correction for variable proportion of body weight, \( F = 2.93, P = 0.10 \)).

Should this general concept of progressive transfer of intracellular water to the extracellular phase be valid, one might expect to find evidence of relative intracellular dehydration affecting also the red blood cells. If the mean corpuscular haemoglobin concentration is determined, the cells can be said to act as simple osmometers. Clearly, osmotic shift of water into the cells will result in depression of the M.C.H.C. and vice versa. Haemoglobin determinations were available in thirty four subjects, and the changes in daily M.C.H.C. readings (mean \( \pm S E. \)) are indicated in Figure 4.10. A clear rise occurs, with the maximum at four days corresponding with the maximum demonstrated extracellular volume. The values then decrease somewhat. From the sixth day the standard error
Results from 34 infants indicate a peak on Day 4 corresponding with the maximum for extracellular water (Figure 4.8.)
becomes small, and the means almost constant, suggesting that the osmotic changes of the first few days are by that time becoming stabilised. The probability that intracellular hydration is not the only factor is suggested by the following reasoning: from the curve drawn for values of extracellular water (Fig. 4.8) it seems that osmotic fluid shifts are occurring from Day 2 - Day 5. The results from before and after this period are unlikely to be affected by this factor. But the means for these two periods, i.e. Day 1 on the one hand, and Day 6 and thereafter on the other, are significantly different; \( t = 2.864, P<0.02 \). The possible implications of this are discussed later.

The daily changes in plasma volume, expressed in ml./kg. (Mean \( \pm \) S.E.), are also shown in Figure 4.8. A slight rise between days one and two occurs, corresponding to the increase in total extracellular water during this period. Thereafter the mean values remain constant, not sharing in the decrease of total extracellular water between days four and six. No statistically significant trend is apparent from these results. It is therefore not justifiable to estimate them in absolute terms in the same way as has been done
with the thiocyanate space. When the ratio
\[
\frac{\text{Plasma water}}{\text{Interstitial water}}
\]
is plotted against time, a distinct profile emerges (Figure 4.11). An increase in the ratio from day one to day two is followed by almost exact constancy until day five, after which the ratio starts to increase towards the values of later infancy and childhood. This ultimate increase is due almost entirely to relative decrease of the interstitial volume, the plasma volume when expressed in ml./kg. remaining fairly constant.

Discussion.

In the analysis of these results, three possible variables have been sought and separated, namely:

1. Variability of proportions of body-water distribution associated with infants of differing weights.

2. Variability of body-water distribution due to extrinsic factors, rendering the values abnormal in this respect.

3. Day to day variability during the period under investigation.

In general the values obtained using these methods agree well with the results of previous investigators.
The increase in the ratio shown here is due to decrease in relative size of the interstitial compartment. Value for older children derived from results given in Chapter 3.
Fink and Cheek (64), using stable bromide space corrected for non-extracellular bromide, Donnan equilibrium, and serum water but uncorrected for binding of the ion by plasma albumin, obtained a mean value for twenty infants in the first day of life of 358 ± 6.5 (S.E.) ml./kg. The results of the present series of eight infants during the first day of life, corrected only for serum water, are 334 ± 8.0 (S.E.) ml./kg.

It has been shown previously by Clapp et al., (28) that in premature infants there is significant negative correlation between total body water, expressed in ml./kg., and body weight. The correlation coefficient obtained by these workers was: 

\[ b = -59.6 \]

In the present series the corresponding value for \( b \) is -59.8, this including some full-term as well as premature infants. A similar relationship holds for intracellular water. While the proportion of extracellular water to body weight is known to decrease throughout childhood (74), there is less unanimity in the literature regarding this correlation in the immediate post-natal period; Cheek (26), by combining four of his own results with five from the work of Finlay and Hare (65), showed a negative
correlation to be present in prematures. However, Finlay and Hare's series of full-term neonates show no significant correlation. Clapp et al., (28) indeed show a slight but significant correlation, but when their results are further analysed it is seen that this is due to the high proportion of extracellular water in the infants less than 1.5 kg. The means of the groups heavier than this are not significantly different. In deriving these correlations, it has been assumed throughout that all the infant subjects without evidence of disease, and given approximately the same fluid regime, could be regarded as normal. In the present series it was arbitrarily surmised that, given reasonable uniformity of handling, it was unlikely that abnormalities of water-distribution would be manifest prior to the age of three days. These correlations were accordingly derived using the results from infants of this age and under. When the older infants are included, those with weights above 2.2 kg, all lie well within the 95% confidence limits from the regression line. The fact that seven out of ten results for total body water from infants below this weight lie outside the same limits suggests that infants of smaller birth weight
lack the same measure of control over the volume of their body water - a fact by no means unexpected.

It is perhaps less immediately comprehensible that this instability is shown to be due to variation in the intracellular compartment rather than the extracellular. The present state of knowledge regarding the factors regulating the volume and composition of extracellular and intracellular fluid has recently been summarised by Bartter (11). Thus extracellular fluid solute concentration is regulated primarily by alterations in the renal excretion of free water, dependant on secretion of antidiuretic hormone. Verney (220) showed that secretion of this hormone responds to changes in total solute concentration, the receptors being sited in the region of the supraopticohypophyseal tract. While this is probably the most important regulator of A.D.H. secretion, there is strong evidence indicating that under certain circumstances alterations in extracellular fluid volume also play a part; furthermore it seems likely that such an effect is mediated primarily by changes in the intravascular volume rather than by the extracellular volume as a whole (117). Control of extracellular fluid volume depends essentially on control of total extracellular fluid solute. The
mechanisms by which this is brought about have been only partly defined. Wesson et al., (229) showed that hypertonic expansion of the extracellular space resulted in increase of the glomerular filtration rate with a rise in urinary sodium. The renal renin-angiotensin axis has recently received much attention in this respect. It is considered that angiotensin acts as the trophic substance for adrenal secretion of aldosterone (8), thus regulating active renal reabsorption of sodium. Renal production of renin, the activator of plasma angiotensin, has been shown to be associated with changes in granularity of the juxtamedullary apparatus (90); and it is fairly certain that alterations of intravascular volume represent the primary initiating factor here.

The control of intracellular fluid volume and solute concentration depends on the fact that under normal circumstances the total solute concentration of intracellular fluid is the same as that of extracellular fluid (133). Thus changes in the latter result in passive osmotic shift of water with alteration of intracellular volume to render the osmolarities once again equal.
Increase in extracellular osmolarity is therefore associated with the following changes:

1. Passive transfer of water from the intracellular to the extracellular compartment. The osmolarity of both phases following this adjustment will lie approximately midway between the two original values, assuming the intracellular and extracellular volumes to be roughly equal.

2. Increased secretion of A.D.H.

3. The result of 1. and 2. above is to cause an increase in the extracellular volume, and hence increase of glomerular filtration rate and presumably inhibition of renin secretion. The nett effect is increase of urinary osmolarity.

4. Following the completion of these adjustments, extracellular osmolarity will be restored to its original level. Inevitably the intracellular osmolarity and volume will likewise have been restored.

The above sequence of events is clearly operative in the maturely functioning human. It is now necessary to examine the situation in the neonate.

Renal function in the newborn infant has been shown to differ from that of the older child and adult.
in certain important respects. It has been demonstrated that excretion of solutes is low, especially in prematures (126, 239, 46). Available data on glomerular filtration rate in the first few days of life indicate that this also is very low (10, 230).

While infants deprived of water during the first three days after birth produce urine of higher osmolality than those given water directly from birth, it seems clear from the results of a number of investigators that the young infant is unable to concentrate urine to a degree comparable to that of adults. Thus, in a study of thirty normal newborn infants deprived of water for the first twenty four hours, Fisher et al. (66) showed a mean urinary osmolality of 309 mOsm./litre at 24 hours, a value only minimally hypertonic to plasma. Similar findings have been reported in newborn rats, when compared with adults of the species (92). On the basis of these and other studies, it is considered (55) that the maximum urinary concentration of which the newborn infant is capable is around 700 mOsm./litre, which compares with the figures of approximately 1400 mOsm./litre attainable by adults. A number of factors
are no doubt involved in causing this situation. Inadequate production of, or response to, antidiuretic hormone represent an obvious possibility. Heller and Zaimis (93) however showed that A.D.H. production in the newborn, although less than that of adults, is probably adequate. Similarly Barnett et al. (10) have noted a definite ability to respond to Pitressin. This response is lower than that of adults, the diminished glomerular filtration rate and low rate of solute excretion probably playing important parts (118). The length of Henle's loop has been found to correlate positively with maximal concentrating ability in various animal species (163). It is known to be short in infancy and to lengthen with age (198). There appear to be no figures available either for plasma angiotensin levels in neonates or for secretion rates of aldosterone during the first few days of life.

Response to water-loading in the newborn shows both a smaller and a delayed diuresis when compared to adults (6, 20). It seems likely that this is due both to lower glomerular filtration rates and lower rates of solute excretion (55).

Thus it seems clear that in the maintenance of extracellular volume, the newborn's inability to handle
either salt or water loads as efficiently or as rapidly as older children or adults will result primarily in alterations in extracellular solute concentration. As was made clear above, the maintenance of intracellular volume is entirely dependant on this factor. The newborn is also peculiarly vulnerable on account of the high

\[ \frac{\text{Extracellular volume}}{\text{Intracellular volume}} \]

ratio present at this age, as pointed out by Olmstead (152). In adults this ratio is around 0.6 - 0.7 (145), while in infants it is closer to unity. The greater the relative extracellular volume, alteration of the total osmolarity of this compartment must result in proportionately greater compensatory shift of intracellular water. Hence under these circumstances greater changes of intracellular volume and osmolarity may be expected.

During the first week of life, a number of studies are available of serial alteration of urinary volume and osmolarity and plasma osmolarity. (196, 37, 50, 165). From these and many others it is clear that differing regimes of oral fluid in the first few days result in markedly different patterns of urine concentration and of haemoconcentration. Insensible fluid-loss must also play its part; O'Brien et al. (151) showed that when the respiratory component,
usually considered to represent 60% of the total insensible loss was eliminated by placing infants in atmospheres of 100% humidity, no increase in serum chloride concentration was evident after 72 hours water deprivation.

In the present series, the oral fluid regime has not been rigidly standardised; rather have the infants studied been on a fairly typical day to day neonatal departmental regime. None of the infants became clinically over or under hydrated. Nevertheless the infants whose results after three days lay within the same confidence limits as the younger group show marked day to day fluctuation. This variation, delineated accurately only with regard to the extracellular compartment, presumably represents hypertonic withdrawal of intracellular fluid during the first three days. Increasing the fluid intake has been associated with decrease of the extracellular space during the next three days, but it is noteworthy that a significant time-lag exists here between starting fluids and commencing decrease in extracellular volume; indeed it would seem reasonable to regard infants so handled as 'normal' and it might be postulated that hypertonic withdrawal of fluid from the intracellular to the
extracellular phase is a normal phenomenon during the first 48 hours of life. It would be interesting to know if such a mechanism could prolong the presence of oedema present from birth or indeed play some part in its genesis. Sutherland et al. (212) measured increasing leg-volumes during this early phase in premature infants with hyaline membrane disease. The incidences of both oedema and prematurity are high in this condition and it may be of significance that hypotonic urine passed during water-deprivation has been noted principally in prematures (33). The four oedematous infants in the present series had in general the highest values for extracellular fluid, but in only one of them is a value for intracellular volume available. It is therefore not possible on the present evidence to comment further along these lines. Hypertonic expansion of the extracellular fluid has been previously demonstrated in infants put on salt-loaded diets (127), and in premature infants changed from breast-milk to cow's milk (219), but this appears to be the first time such an expansion has been measured serially in infants under 'normal' nursery conditions. The results of Hanna (86) to some extent confirm this pattern; that
author showed an increase in extracellular water and in blood volume in infants aged three to four days as compared with infants measured shortly after birth. The results are however expressed in percentage body weight and it is therefore not possible to state if the increase is an absolute one as demonstrated here. Serial measurement of body water distribution in the same infant would presumably give a more direct picture but was decided against in the present series on ethical grounds. Hanna was able also to show a diminution over the same period of total body water; once again however the results are expressed as percentages of body weight. In the present investigation, elimination of the results from the subjects regarded as 'abnormal' has meant that the numbers of results available for each day were insufficient to derive a statistically significant pattern. The trend for total body water however indicates as might be expected moderate reduction during the first four days. In view of the increase of extracellular volume, this loss of water must be entirely derived from the intracellular phase. Loss of body weight appears to be greater than that of body water; energy catabolism of fat, protein and carbohydrate renders this inevitable. Some of the
intracellular water lost may indeed be released in this way, as indicated by the studies of Nicolopoulos and Smith (149).

It is interesting to derive support for these findings from analysis of changes in the values for mean corpuscular haemoglobin concentration. The state of hydration of the red cells is almost proportional to their volume, and determination of the M.C.H.C. probably represents the simplest and most accurate way to demonstrate such changes, the correlation, of course being negative (208). While a large literature exists on basic values for M.C.H.C. at all ages, day to day alterations using modern methods do not appear to have been analysed in the first few days of life. Although the red cells at birth tend to be large (36) partly owing to the presence of a high reticulocyte count, the M.C.H.C. at that age is reported to be similar to that of adults (144, 99). Mean red cell volume and M.C.H.C. are also affected by blood pH (161, 70). The correlation is positive in the case of M.C.H.C. and is presumably associated with chloride shift and changes of ionic dissociation within the cells. The known low blood pH at birth (155), and its rapid correction,
could account for the significantly different mean M.C.H.C. readings of Day one on the one hand, and of Day six and subsequently on the other. The higher levels occurring between these times are presumably more related to changes of cell-hydration due to hypertonic withdrawal, and it is significant that the peaks for both M.C.H.C. and extracellular volume (Day four) should coincide.

The factors determining the distribution of fluid between the intravascular and interstitial compartments comprise a subject which in the neonate has received remarkably little attention. In general, the nett fluid transfer across the capillaries depends mainly on plasma colloid osmotic pressure, capillary permeability, and intracapillary hydrostatic pressure. The third of these itself depends on various factors including mean arterial and venous pressures, and also on precapillary and postcapillary flow resistances (176). The latter have been shown to be under neurogenic control (150), and are thought to represent the major control mechanism by which adjustment to relatively acute changes such as haemorrhage, is mediated.
In the newborn, plasma colloid osmotic pressure is similar to the adult level of 20-25 mm Hg (197). Intracapillary pressure in the adult is similar to this (115), and the very few results available for neonates do not suggest this to be any higher (197). Capillary resistance, as measured by the appearance of petechiae during suction applied to the skin, has been shown to be low in newborn, especially prematures (238), and capillary resistance increases during the first week, particularly in the first twenty four hours (221). Further suggestive evidence comes from the fact that both T-

\^{\text{1824}}\text{dye (bound in the plasma to albumin) and radio-iodinated serum albumin, disappear much faster from the intravascular compartment of the neonate than of the adult (105, 103).}

Much work, the results of some of it conflicting, has been done to investigate changes in the plasma and blood volumes during the first twenty four hours of life. Thus Steele (205) and Clark and Gairdner (29) demonstrated reduction of plasma volume during the first hour of life, while the results of Sisson and Whalen (194) suggested the reverse to be true. The latter author's results, however relate to the period 4-6 hours after birth as do the results of Usher et al., (216), who felt that these discrepancies were due to
failure to take into account the presence or absence of placental transfusion. Their results showed an absolute increase in plasma volume occurring between four and twenty-four hours of age, which agrees with the evidence from the present work. They suggested that this increase might be due to local increase in circulation affecting perhaps the gastrointestinal tract. While this may be true, the present findings suggest that at least part of the increase in plasma volume is due to the general expansion of extracellular fluid taking place at this time. It is interesting that after the first 24 hours the ratio \[
\frac{\text{Plasma water}}{\text{Interstitial water}}
\] should be so accurately maintained during the period of osmotic change, and that when the interstitial volume decreases at the fifth to sixth day, this decrease is not apparently shared by the intravascular compartment. Presumably the considerable instability of this compartment during the initial period has by now stabilised. What factor or factors are however operative in causing the subsequent gradual alteration in the subdivision of the extracellular fluid towards the adult picture must remain a mystery under present knowledge.
Conclusion

The major finding of this series relevant to the general theme is the demonstration of fluid shifts from the intracellular to the extracellular space taking place in the first few days of life. The way in which this observation finds a parallel with the changes occurring in acute glomerulonephritis will be made clear to the reader in the second part of this Thesis.
CHAPTER 5
OTHER METHODS

A catalogue of methods used, together with the establishment of normal values: a small investigation into the maintenance of some, and some doubt cast on the value of a well known formula.

It will be made clear in Part 2 of this Thesis that the shifts of body water distribution taking place in acute glomerulonephritis are considerable, and their nature at first sight unexpected. These results will accordingly be seen to pose a problem which the later chapters will attempt to solve. It is the attempt to find a solution that necessitated using a number of standard biochemical methods, a list of which now follows.

Serum and urinary sodium and potassium were done by standard flame photometry, using an 'EEL' instrument.

Serum and urinary chloride were estimated using an 'EEL' chloride meter.

Serum calcium and magnesium estimations were done on the 'EEL' micro-titrator, using murexide as the indicator for calcium, and eriochrome black as the
indicator for the sum of the two ions; the level of magnesium was obtained as the difference between the two readings. No attempt was made to estimate the ionic fraction of calcium.

Serum urea was estimated by the micro-Kjeldahl method, with Nesslerization. The usual modification of this method (217) was used to estimate serum total proteins.

Serum electrophoresis was done on Whatman No.3 mm. paper at pH 8.6, using 0.2 ml. of serum for 16 hours at 300 volts and 0.8 amps. The strips were stained with Lissamine Green and the patterns read on a Loebel 'Chromoscan' which was found to give highly reproducible results.

Serum total osmolarity was estimated by depression of freezing point on a Fiske Osmometer, using standards of 100, 300 and 500 mOsm./kg. water.

Normal Values

The results of estimations carried out on the plasma of twenty-two normal children were as follows:
TABLE 5.1.

<table>
<thead>
<tr>
<th>Estimation</th>
<th>Mean (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>138.7 (4.17) meq./l</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.57 (0.535) meq./l</td>
</tr>
<tr>
<td>Chloride</td>
<td>103.7 (2.80) meq./l</td>
</tr>
<tr>
<td>Urea</td>
<td>32.9 (9.03) mg./100 ml.</td>
</tr>
<tr>
<td>Total protein</td>
<td>6.82 (0.50) Gm./100 ml.</td>
</tr>
<tr>
<td>Total osmolarity</td>
<td>284.5 (4.61) mOsm./kg. water</td>
</tr>
</tbody>
</table>

The normal values for the serum electrophoretic pattern will be detailed in Chapter 9.

Normal biological variation

An important aspect of the investigative procedure detailed in Chapter 8 will be the daily biochemical assessment of acute nephritics over a critical period of around one week. To determine the significance of the changes demonstrated, it was considered a necessary preliminary to gain some information regarding the range of biological variation in normal subjects in a day-to-day study over approximately the same period of time. Or, to put it
another way, an attempt has been made to ascertain the limits within which normal subjects maintain these aspects of their biochemical status.

Material and methods

Four normal adult physicians, three male and one female, whose age-range was 27-33 years, had a venous blood-sample removed with minimal stasis on eight consecutive days. Samples were obtained at random times of day between 0900 and 1630 hrs. The woman was in approximately mid-menstrual cycle.

The following estimations were carried out:
Serum sodium, potassium, chloride, urea, total protein and total osmolarity. Samples were in all cases stored at 4°C, until the last specimens had been obtained. Each set of estimations was then done as a single batch by the same observer.

Results

The mean values with S.D. for eight daily estimations on the four subjects are shown in Table 5.2, together with the averages for the group as a whole.

It can be seen that while there is little difference between the means for each subject, the standard deviations are in general small, indicating
TABLE 5.2.

Mean and S.D. of Eight Daily Readings on Four Normal Adults

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sodium meq./l.</th>
<th>Potassium meq./l.</th>
<th>Chloride meq./l.</th>
<th>Urea mg./100ml.</th>
<th>Protein Gm./100ml.</th>
<th>Osmolarity mOsm./kg. water</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.B.</td>
<td>133.8 (1.39)</td>
<td>5.32 (0.30)</td>
<td>103.5 (1.51)</td>
<td>32.6 (4.05)</td>
<td>7.50 (0.306)</td>
<td>294.0 (4.96)</td>
</tr>
<tr>
<td>B.B.</td>
<td>141.1 (1.56)</td>
<td>4.91 (0.53)</td>
<td>104.9 (1.65)</td>
<td>27.9 (3.41)</td>
<td>7.66 (0.158)</td>
<td>290.1 (5.20)</td>
</tr>
<tr>
<td>J.M.</td>
<td>141.1 (2.28)</td>
<td>4.84 (0.22)</td>
<td>105.4 (1.07)</td>
<td>30.5 (2.91)</td>
<td>7.44 (0.089)</td>
<td>291.1 (3.05)</td>
</tr>
<tr>
<td>V.D.</td>
<td>142.1 (1.46)</td>
<td>4.46 (0.29)</td>
<td>105.1 (0.84)</td>
<td>30.8 (3.14)</td>
<td>7.28 (0.310)</td>
<td>292.9 (4.16)</td>
</tr>
<tr>
<td>Total</td>
<td>140.8 (2.07)</td>
<td>4.88 (0.46)</td>
<td>104.7 (1.41)</td>
<td>30.4 (3.66)</td>
<td>7.47 (0.264)</td>
<td>292.0 (4.47)</td>
</tr>
</tbody>
</table>
close maintenance of these levels by the individual subjects.

To carry this investigation further, an analysis of variance technique has been employed. The variances separated have been 1. the between-subject variance; and 2. the within subject variance. The results of this analysis are seen in Table 3.3.

**Interpretation:**

It is clear that the maintenance of a constant level of sodium, potassium, chloride and total protein is within extremely fine limits in these normal subjects. This is particularly true of sodium and potassium. The maintenance of urea and plasma osmolarity is less exact.

**Comment:**

Much work on normal biological variation has been done, especially in the field of diurnal variation affecting renal excretion of electrolytes (122). Urinary electrolyte output is in general matched by urinary water output, hence no distinct diurnal variation of plasma electrolyte levels has been demonstrated (139). To parallel the conditions of
## Table 5.3.

**Analysis of Variance**

**Four Normal Adults**

<table>
<thead>
<tr>
<th></th>
<th>Sodium</th>
<th>Potassium</th>
<th>Chloride</th>
<th>Urea</th>
<th>Protein</th>
<th>Osmolarity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$s^2$</td>
<td>$F$</td>
<td>$p$</td>
<td>$s^2$</td>
<td>$F$</td>
<td>$p$</td>
</tr>
<tr>
<td>Between Subjects</td>
<td>14.6</td>
<td>0.39</td>
<td>5.33</td>
<td>30.0</td>
<td>0.20</td>
<td>24.0</td>
</tr>
<tr>
<td></td>
<td>5.30 &lt; 0.01</td>
<td>7.35 &lt; 0.01</td>
<td>3.21 &lt; 0.05</td>
<td>2.59</td>
<td>H.S.</td>
<td>3.82 &lt; 0.05</td>
</tr>
<tr>
<td>Within Subjects</td>
<td>2.99</td>
<td>0.13</td>
<td>1.71</td>
<td>11.6</td>
<td>0.056</td>
<td>10.5</td>
</tr>
</tbody>
</table>

H.S. = Not Significant ($p > 0.05$.)
investigation of the nephritic group, blood samples were obtained at random times of day during an eight hour span. The results have clearly indicated the fine limits within which four of these quantities are regulated. The fact that urea is less closely maintained is not particularly surprising because it seems likely that such factors as food ingestion will have an unstaabilizing influence. One would however expect plasma osmolarity to be maintained within closer limits than has been demonstrated in this experiment. It will be remembered that the "within-subject" variance derived represents the sum of biological and methodological variation. It has been assumed that the methodological variation must affect the test and control groups equally; this factor was not therefore specifically isolated. However in view of the relatively greater within-subject variances affecting urea and osmolarity, the reproducibility of the method was checked. The results suggest that the major part of the variation of plasma urea is biological, but of the osmolarity, methodological. This fact will be borne in mind when analysing the results of the nephritic group.

Two further important differences between the groups remain to be discussed. Firstly, the
fact that the controls are adults and the nephritics children. It was considered ethically unjustifiable to subject normal children to such an experiment. Comparable figures for childhood are not to the author's knowledge available and it is not therefore possible to comment on this distinction.

The second difference is of greater importance; namely, the fact that the control group were on normal diet, whereas the nephritics were throughout maintained on a low-sodium diet. The response of normal adults to a low-sodium diet is however well documented in the literature; for example the results of Luntscher et al. (124) showed that, following 6 days on an intake of 11 meq. of sodium daily, the plasma sodium levels decreased by an average of only 2.9 meq./litre, due of course to progressive renal conservation of salt. The main object of the present study has however been simply to define normal biological variation; using this as a background, the variations in acute nephritis will be indicated in due course.

**Total plasma osmolarity**

There is a frequently quoted formula for calculating the total plasma osmolarity (180). It
is as follows:

\[ T.P.O. (\text{mosm}/\text{l}) = 2(\text{Na} + \text{K}) + \text{Urea} - 8 \]

Sodium and potassium are expressed in meq./l and urea in m.Mols./l i.e., \(\text{mg./100ml.}\).

It is clear that such a formula must give a rough approximation to the osmolarity only. Whether or not it can be relied upon even to this extent has been the subject of a small study.

**Results and Comment**

The results are in two parts; firstly, from twenty-one normal children; secondly, utilising the results from the nephritic group, many of which are outwith the normal range.

The results from 21 normal children for observed osmolarity against osmolarity derived from the above equation are illustrated in Figure 5.1. The arithmetical means are almost identical - 284.5 (observed) as compared with 284.7 (calculated). Nevertheless the variances are different, respectively 21.25 and 67.80. A variance ratio test indicates that this difference is highly significant \((F = 3.191, P < 0.01)\). In other words, while the means are the same, the scatter around the means is
FIG. 5.1.

PLASMA OSMOLARITY

21 Normal Children

\[ r = -0.1581 \]

\[ P > 0.1 \]
sufficiently different to suggest significantly differing parent populations. Hence it is not surprising that there is no correlation between the results \( (r = -0.1581, P > 0.1) \).

The results for the nephritics include a fair number of abnormally high readings. They comprise 64 observations from nine children. The values have been plotted in Figure 5.2 where division into two groups is seen, namely, above and below the observed value of 290 mosm/l.

For the 38 values of 290 and less, a similar picture to that of the normal group is seen; the observed mean is 283.2 against a calculated mean of 281.1. Once again the variances are significantly different \( (F = 4.74, P < 0.01) \) and there is no correlation \( (r = 0.1054, P > 0.1) \).

The higher group (observed osmolality 290) had means of 307.0 (observed) and 297.2 (calculated). These means are significantly different \( (t = 2.336, P < 0.01) \), and again there is no significant correlation although a slight positive trend is now evident \( (r = 0.2670, P > 0.1) \).

It is only when the whole group of 64 readings is considered that any relationship begins to emerge.
FIG. 5.2.

PLASMA OSMOLARITY

64 Observations in 9 Subjects

\[ r = 0.569 \]

\[ P < 0.001 \]
However, although the correlation is now highly significant ($p < 0.001$) it is in fact so poor ($r = 0.5690$) that it is difficult to regard this equation for calculating osmolarity as being of any value whatsoever.

**Conclusion**

It was originally anticipated that the calculation of osmolarity using the above equation would be sufficiently accurate to obviate the necessity to make direct observations, which require 1 ml. of plasma. The above results, combined with the impression that values for this parameter might well prove to be of real importance in the investigation of acute glomerulonephritis, have prompted the author to carry out direct estimations throughout.
CHAPTER 6

ACUTE GLOMERULONEPHRITIS:

A Short Review of Existing Theories Concerning
the Oedema of Acute Glomerulonephritis, and a Discussion
on their Shortcomings

Definition

Acute glomerulonephritis was first described
following scarlet fever by William Volks in 1810 (227).
John Blackall in 1813 was well familiar with
breathlessness as an unusual symptom of the disease (17)
but it is Richard Bright to whom the main credit is
justly given for the painstaking clinical descriptions
in 1827 of the several conditions collectively
designated for more than a century 'Bright's Disease'.
(21). The realisation that this overall term
included a number of widely differing clinical and
pathological entities resulted in a problem of
semantics of a complexity which it is hoped will never
have an equal. We are indebted to Ellis (60) for
first devising a classification of Bright's Disease
which was simple and had the supreme merit of being
based on a study of the natural history of the various
diseases included under the general title. A later generation has modified his classification in detail only, and paediatricians in particular have tended to select certain of the earlier nomenclature. Thus Ellis' 'Type I nephritis' is now generally referred to as acute (post-streptococcal) haemorrhagic glomerulonephritis (or as acute glomerulonephritis), and 'Type II nephritis' as idiopathic nephrosis.

Introduction

Acute glomerulonephritis is a disease with which clinicians are well familiar. Approximately 50-60 cases are admitted yearly to the Royal Hospital for Sick Children, Glasgow, the incidence being greatest during the winter months. The association of the disease with infection by the -haemolytic streptococcus has long been recognised, and the more puzzling aspects of its epidemiology have been clarified by the demonstration that only certain strains of this organism are nephritogenic (171). While in adults the disease carries a significant mortality, in children it is essentially benign. In the past this fact has tended to be under-emphasised (101).
It was Brod in 1949 (22) who first drew particular attention in print to what had previously been clinically obvious. This is the striking tendency of the disease to fall into two phases. The first, which in Brod's adult cases lasted up to a month he described as the acute or oedematous phase. This is characterised by oedema, hypertension, the presence of blood, albumin and casts in the urine, raised erythrocyte sedimentation rate, and blood urca, a lowered glomerular filtration rate and the secretion of reduced amounts of dilute urine. The second stage, referred to as the post-diuretic or recovery phase, is notable for the disappearance of extra-renal manifestations leaving only urinary abnormalities and a raised E.S.R., both of which steadily recover in the uncomplicated case. At the same time he recognised that some patients may have oedema with or without transient hypertension and little or no urinary abnormality, while others may have quite considerable evidence of renal impairment but a complete absence of extra-renal signs. Brod also felt that the prognosis tended to vary with the duration of the first stage. Hence his recommendation that any form of therapy should be directed towards
the encouragement of earlier diuresis (22). It is my own view that any investigation which takes no account of these stages is likely to be of little value.

The Oedema of Acute Glomerulonephritis

The nature and source of oedema fluid occurring in acute glomerulonephritis have given rise to considerable controversy. A number of conflicting theories have consequently been advanced.

In the view of many (1, 39, 62, 192) AGN is a generalised disease affecting the entire capillary bed. This stems from Beckman’s original observation in 1921 (12) that the protein content of the oedema fluid was increased and could be as high as 2.5 Gm./100 ml. Thus the moderate reduction of plasma protein concentration found during the oedematous phase of the disease could be readily explained. Reduced plasma colloid osmotic pressure would favour further transfer of protein plus fluid from the intravascular to the interstitial space. The result would clearly be a reduction of the plasma volume, but all experimental evidence indicates that this volume
is increased during the oedematous phase (125, 179, 57). To account for this discrepancy a reduction in the 'effective' plasma volume has been suggested (39), the mechanism being similar to that described in heart failure (207). To date no direct evidence exists to support such a contention.

More recent estimates using more delicate methods to estimate the protein content of the oedema fluid have shown levels of 0.4 - 0.6 Gm./100 ml. (224). These values are the same as those noted by the same investigators in many undoubted transudates (202). If the figures are accepted, the theory of generalised increase of capillary permeability becomes untenable. At the same time it is worth making the observation that, given normal protein concentration and an increase in the volume of interstitial fluid, it at once follows that the total amount of protein in this compartment must be increased. This point will be further discussed in Chapter Nine.

The occurrence of 'heart failure' in AGN was first described by Goodhart in 1879 (79). Further descriptions were furnished during the
nineteen thirties (119, 59, 231), when it became clear that oedema is one of a group of symptoms and signs including orthopnoea, the presence of various transudates, cardiomegaly and typical radiological appearances of the chest. These are generally designated, with questionable accuracy, the congestive changes of AGN.

In 1944 LaDue (114) proposed that the congestive changes were truly cardiac in origin and represented a true form of congestive failure. That author emphasised the causative importance of hypertension. Further evidence favouring a primarily cardiac nature of the phenomenon came from the work of Gore and Saphir (81) who demonstrated histological changes in the myocardium suggesting a form of myocarditis. Peters in 1953 (162) and Murphy and Murphy a year later (147) while agreeing with the general theme were unable to supply a further explanation of why the heart in AGN should sometimes fail. It was immediately obvious that the duration of hypertension in this disease is exceedingly brief. This would make unlikely heart failure due solely to this factor. Further, the degree of histological damage mentioned above is in fact rather mild and
non-specific. As Kassirer and Schwartz have pointed out (108), the myocardium is unremarkable in appearance in many patients who die as a result apparently of circulatory congestion.

When true congestive heart failure and that of AGN are analysed in renal and haemodynamic terms, some striking differences appear. Thus in congestive heart failure due to hypertension or to coronary artery disease the heart output is reduced, the circulation time prolonged, and the arteriovenous oxygen saturation difference is increased. In the kidney the renal plasma flow is reduced to a greater extent than the glomerular filtration rate resulting in an increase of the filtration fraction. The congestive picture of AGN typically shows a normal or sometimes increased cardiac output, normal circulation time and a normal arteriovenous oxygen difference. The glomerular filtration rate shows a marked degree of reduction, but the renal plasma flow is generally well maintained and the filtration fraction is consequently much below normal (47, 51, 56, 91, 177, 204). In view of the number and constancy of these basic differences it becomes unrealistic to regard the congestive changes of AGN as being due
It was Proger in 1941 (169) who first suggested that the oedema and other congestive changes in AGN might be explained on a basis of retention of salt and water. The first experimental evidence to support such a contention came from the work of MacArthur (125) who demonstrated a 20% increase in the blood volume to be present during the oedematous phase of the disease. He further showed that this increase affected the plasma alone, and that on diuresis it returned to normal values. This work has been amply confirmed using various techniques (57). It later became clear that the entire syndrome of circulatory congestion including peripheral oedema may be produced in normal subjects either by the rapid infusion of saline solution or by inducing the kidneys to retain salt and water by the prolonged administration of cortisone or corticotrophin (2). It has also been shown that both the pulmonary and systemic venous pressures increase on expanding the blood volume of healthy dogs (95). The slight reduction of the hematocrit and of the plasma protein concentration are thus due to a simple dilution phenomenon (179), and both the increased intravascular pressure and the
reduction of the plasma colloid osmotic pressure facilitate passage of fluid to the interstitial space.

Reduction of the glomerular filtrate, recognised for many years, provides the most likely source of retained fluid. It should however be pointed out that diuresis has been known to occur without demonstrable change of the GFR, and conversely that an occasional case with massive oedema has been found to have a GFR within the accepted normal range (51). At the same time the relatively crude methods available for carrying out this measurement may well be insufficiently sensitive to detect slight changes which could still account for large alterations in the excretion of sodium and water.

While some degree of tubular impairment is frequently found, this is in general considerably less severe than the functional abnormality affecting the glomeruli (51). A reduced amount of glomerular filtrate perfusing tubules whose function is relatively intact might be expected to favour more complete reabsorption of salt and water (108) - a state generally referred to as "glomerulo-tubular imbalance".
It may be reasonably stated that most authorities at present accept the view that the oedema of acute glomerulonephritis, together with the other congestive phenomena associated with the disease are accountable on the above basis (32, 223, 16, 52).

What remains to be pointed out? Is there any respect in which the above theory fails to explain the observed facts? In the author's view there are two major objections to the acceptance of this view in toto. Firstly, the rate at which oedema collects is in AGN remarkable. In most patients it is indeed the first symptom of the disease. It is well established that an increase of body fluid to the tune of 10-15% of body weight must be present for oedema to be clinically demonstrable. For example, in the studies of Albert et al., referred to above (2) fluid retention was induced in non-cardiac subjects by prolonged administration of A.C.T.H. and cortisone. Detectable oedema appeared only in subjects who retained 16 lbs. of fluid or more. The authors state the surface areas of their subjects but not unfortunately their body weights. However unless all their patients were under five feet tall, this amount of fluid represents an accumulation of at
least 15% of body weight. With an average daily urinary output of around 700 ml, a 20 kg child must take quite a number of days to retain the two to three litres of water necessary. This hardly fits with the appearance of oedema at the apparent outset of the illness, unless one postulates a prior period of asymptomatic salt and water retention. It is clearly almost impossible to obtain direct evidence on this point, and in the author's experience the patients' histories tend to be in this respect uninformative.

The second objection is based on a comparison of AGN with acute renal failure due to lower nephron nephrosis. While oedema may occur in the latter condition, it usually follows the administration of excessive fluid. Cases initially presenting may be profoundly oliguric, but, as Swann and Morrill have pointed out in a classical study of eighty-five such cases, "absence of periorbital oedema is strongly in favour of acute renal failure."(213). As will be seen in the chapters to follow, many patients with AGN are only moderately oliguric and yet develop oedema early.
To the author it therefore seems that while many of the tenets of present opinion rest squarely on the rock of experimental evidence, a factor is still missing from the puzzle. Addition of fluid by reduction of the renal output cannot be regarded as the final answer.
CHAPTER 7

CLARIFYING THE PROBLEM

A Study of the Case-records from 170 Children with Acute Glomerulonephritis

The basic purpose of this study has been to abstract from case records every available clinical fact, to separate the cases into those with and those without oedema and to carry out a statistical analysis to identify any gross differences between the groups.

Material and Methods

The records have been analysed from all children diagnosed as suffering from acute glomerulonephritis and admitted to the Royal Hospital for Sick Children, Glasgow between 1st January 1962 and 31st December 1964. These have totalled 170, 104 boys and 66 girls. The age-range was 1.6-12.8 years. Four cases retrospectively diagnosed as haemolytic-uraemic syndrome have been excluded, together with three children who had albuminuria and haematuria but a predominantly nephrotic serum electrophoretic pattern together with a normal A.S.O. titre.
The cases have been looked after in two medical units employing basically the same therapeutic regime. Penicillin, oral or intramuscular has been given to all patients but for varying periods of time (see below). Diet: all subjects have been maintained for the first week or until diuresis was complete on a low sodium, low calorie, restricted fluid diet consisting of one litre of half-strength milk daily, with boiled sweets *ad lib.* Mean daily intake was therefore approximately 600 calories and 14 meq. of sodium. The diet was thereafter gradually increased. Four children who were severely orthopnoeic on admission together with two others who had marked oliguria were put on a modified Bull regime for a few days. The combination of semi-starvation with the rapid onset of diuresis has resulted in early loss of weight in all save five patients, these being in the oedema-free group. Daily weighing was carried out as a routine. The weights recorded in this study have been (1) on admission; (2) the lowest weight attained, usually after a period of 6 - 9 days; (3) on dismissal. These weights have been expressed as a percentage of the mean expected weight for age obtained from the table in standard
use at the hospital (190). In calculating this percentage, due allowance has been made for the period elapsing between the three observations. Patients have been allowed up when the E.S.R. returned to normal and were dismissed home on an average of two weeks after albuminuria and haematuria have cleared. A small number of children in whom these signs were unduly prolonged were discharged when no further improvement was observed.

Details of how the various observations were made and recorded are set out below. Frequency tables were drawn up on a KDF/9 Electronic Computer operated by the Computer Department of the University of Glasgow.

**Results and Comments**

**Oedema.**

The presence or absence of oedema during the first few days has been recorded in all patients. It was observed in 104 (61.2%). All the features which are analysed below have been separated into two groups on this basis.

**First Symptom**

In the vast majority of cases, the first
symptom of ACN is either discolouration of the urine or puffiness of the face. A clear statement of which came first was obtained from 159 case records. Puffiness of the face occurred first in 86 of them (54%). Of the 94 cases with oedema, 76 (81%) developed facial swelling first, whereas in the 65 non-oedematous children 55 (85%) complained first of discolouration of the urine. It is concluded that if oedema is going to occur it is generally the earliest evidence of the disease.

**Unusual presenting feature.**

Four children, all oedematous, presented with orthopnoea. Two cases, one from each group, exhibited hypertensive encephalopathic fits.

**Weight.**

Histograms showing the distributions of percentage expected weights at the three selected times are shown in Figure 7.1. This includes all children, with or without oedema and it is clear that the weights both on admission and dismissal centre around 100%. The arithmetical means for the three observations are:
FIG. 7.1. ACUTE NEPHRITIS

Changes in Body Weight. 168 Patients

PATIENTS

ON ADMISSION

FOLLOWING DIURESESIS & LOW CALORY DIET

ON DISCHARGE

PERCENTAGE
Expected Weight

80 100 120
1. On admission.  2. Lowest weight.  3. On dismissal

100.93%  92.73%  100.31%

At first sight it seemed surprising that the weights on admission should not average above 100%. Since the great majority had recently suffered from a streptococcal infection, it is possible that weight-loss on this account could be balanced by fluid retention. This was checked by division into the oedematous and the oedema-free groups. The results are as follows (mean ± S.E.):

**TABLE 7.1.**

<table>
<thead>
<tr>
<th></th>
<th>Weight 1</th>
<th>Weight 2</th>
<th>Weight 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema Present</td>
<td>104.0(1.63)</td>
<td>93.2(1.06)</td>
<td>101.2(1.27)</td>
</tr>
<tr>
<td>Oedema absent</td>
<td>96.2(1.62)</td>
<td>92.0(1.53)</td>
<td>100.0(1.42)</td>
</tr>
</tbody>
</table>

The mean values for weight 1 are highly significantly different ($t = 3.825, P < 0.0005$). The differences between the mean values for the other two groups are not significantly different ($t = 0.689, 0.5 > P > 0.4$, and $t = 0.645, 0.6 > P > 0.5$ respectively). Thus it is clear that the oedematous group are initially the
heavier, as would be expected. The magnitude of this difference is nevertheless of considerable interest. If for the sake of argument the oedema-free group have no diuresis, the loss of weight recorded in these patients amounting to 4.2% must represent loss of body solids only. Since the dietary intake has been uniform throughout, it would be reasonable to expect the same degree of solid weight loss to affect also the oedematous group. It follows that the loss of weight as fluid from the oedematous group would then be only 6.6%. If the non-oedematous children do in fact lose some fluid, the fluid weight loss in the oedematous group can still be no more than 104.0 - 93.2 = 10.8%.

Even this maximum figure is significantly less than, for example the figures quoted in Chapter 6, in which detectable oedema only appeared in subjects gaining 16% or more of body weight as accumulated fluid.

A point of further interest lies in the fact that out of the 10½ children with oedema, 34 (33.7%) had a percentage expected weight which was greater on admission than on dismissal. In eleven of these the oedema was generalised while in the remaining
twenty three it was detectable in the face only.
The average (with S.D.) of the percentage weights
for this group is as follows:

<table>
<thead>
<tr>
<th>Weight 1</th>
<th>Weight 2</th>
<th>Weight 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>98.1 (2.31)</td>
<td>91.5 (1.96)</td>
<td>102.6 (2.19)</td>
</tr>
</tbody>
</table>

None of these values differ significantly from
those of the non-oedematous group (1. t = 0.697,
0.25 > P > 0.2; 2. t = 0.181, 0.45 > P > 0.40;
3. t = 0.992, 0.2 > P > 0.15.) It would not appear
possible that this group could simply have lost
excessive 'solid' weight prior to the onset of
nephritis, since one would then have expected weight
No.2 to be lower than in the other groups. It is
clear that this group in particular must have retained
remarkably little fluid and some other explanation
for the genesis of the oedema must be sought.

Age:
The distribution of the children's ages was
as follows (see also Figure 7.2.a):
Oedematous cases (hatched blocks) compared with non-oedematous (solid lines).
TABLE 7.2.

<table>
<thead>
<tr>
<th></th>
<th>3</th>
<th>3-4</th>
<th>5-6</th>
<th>7-8</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema present</td>
<td>10</td>
<td>17</td>
<td>30</td>
<td>21</td>
<td>26</td>
</tr>
<tr>
<td>Oedema absent</td>
<td>5</td>
<td>22</td>
<td>20</td>
<td>14</td>
<td>5</td>
</tr>
</tbody>
</table>

These distributions are significantly different
\( (\chi^2 = 12.034, \; 0.025 > P > 0.01) \). Oedema thus tends to be present in older children with AGN.

Sex

The sex-distribution was almost identical between the two groups.

History of Previous Infection.

A clear history of the presence or absence of an infection within the three weeks prior to the onset of nephritis was available in 163 cases. 117 (71.8%) gave such a history, either of throat, ear, or non-specific upper respiratory infection (U.R.I.). The distribution was as follows:
TABLE 7.3.

<table>
<thead>
<tr>
<th></th>
<th>Throat</th>
<th>U.R.I.</th>
<th>Ear</th>
<th>Nil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0edema present</td>
<td>54</td>
<td>10</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>0edema absent</td>
<td>29</td>
<td>8</td>
<td>7</td>
<td>19</td>
</tr>
</tbody>
</table>

These distributions are almost identical

\[ \chi^2 = 1.095, 0.8 > P > 0.7. \]

A.S.O. Titre

The values for A.S.O. titre done on admission were available in 163 patients. These were divided into normal and abnormal groups (abnormal 200 units/ml.) The distribution between oedema-free and oedematous groups was also almost identical

\[ \chi^2 = 0.077, 0.8 > P > 0.7. \]

Day of Disease when Admitted to Hospital

There was an obvious possibility that cases free of oedema on admission might already have had a diuresis. A clear-cut history of the date of onset of symptoms was available in 164 cases.
The distributions are illustrated in Figure 7.2.c. and were as follows:

<table>
<thead>
<tr>
<th>Days after onset of symptoms</th>
<th>1-3</th>
<th>4-6</th>
<th>7-9</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema present</td>
<td>44</td>
<td>30</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Oedema absent</td>
<td>38</td>
<td>14</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

There is a trend in these results which fails to reach significant level ($\chi^2 = 7.09, 0.1 > P > 0.05$). It is interesting that the trend should be towards earlier admission of the non-oedematous cases, which effectively disposes of the possibility that these children were admitted to hospital in the post-diuretic phase of the disease.

**Blood Urea.**

It appeared reasonable at first sight to postulate that the oedematous group represented the more severely affected end of the clinical spectrum of AGN. If oedema is entirely due to diminished
GFR one would expect the blood urea to reach significantly higher levels in this group. The estimation was carried out on admission in 159 cases and it was repeated in 51 children at variable times during the recovery phase.

The arithmetical means for the initial estimation were:

- Oedema present (n = 98): 55.8 mg./100ml.
- Oedema absent (n = 61): 51.0 mg./100ml.

These values must be interpreted with caution since the distribution of the results is in each case Poisson and not Normal. For the same reason a 't'-test is not valid. A $\chi^2$-analysis is more informative:

**TABLE 7.5.**

<table>
<thead>
<tr>
<th>Blood urea (mg./100ml.)</th>
<th>40 and less</th>
<th>41-60</th>
<th>61-100</th>
<th>101-200</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema present</td>
<td>41</td>
<td>25</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>Oedema absent</td>
<td>25</td>
<td>16</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

N.B. This analysis excludes the value of 326 mg./100ml. occurring in one oedematous child, the only fatality of the series.
These results (illustrated in Figure 7.2.d.) reveal a trend which is just significant ($X^2 = 7.88, P = 0.025$), indicating that the levels do tend to be higher in the oedematous cases. Two features seem to the author to be of interest. Firstly, the rather marginal difference separating the groups, when a somewhat larger one might have been expected. Secondly, the occurrence of a small number of oedema-free patients with early evidence of severe nitrogen retention (blood urea 100–200 mg./100ml.) One such case, an eight year old boy who had a blood urea of 200 mg./100ml. made an uncomplicated recovery, albumin being absent from his urine after six weeks. He was discharged home, remaining clinically well for six months, during which time his urine was examined on three occasions, being normal each time. After this period he underwent a second episode of AGN, slighter milder than the first but in other respects identical. His blood urea on this occasion was 137 mg./100ml. and again no oedema could be detected. The condition rapidly cleared and eighteen months later he remains well. It is possible that such cases of brisk severity but from oedema could exhibit a different pattern.
of renal pathology, certainly in the functional sense and possibly also from a histological viewpoint.

The results of 51 estimations of blood urea during the recovery phase show similar significant trend. The values from all fifteen of the non-œdematous group were normal (40 mg./100ml.), whereas of the 36 children originally œdematous, 22 (61%) of the results were less than 40 mg./100ml., nine were between 40 and 50, and the remaining five above 50. This trend is also just significant ($x^2 = 6.73, 0.05 > P > 0.025$).

**Albuminuria and Erythrocyturia**

An attempt was made to analyse the degree of albuminuria and erythrocyturia, while recognising that the many variables and approximations inherent in such a procedure must render the results of doubtful value.

**Albuminuria.** The routine method for testing for albuminuria in this hospital is the boiling method, carried out by house physicians. Grading into three categories of severity is subjective. A mean of the estimates for the first four days following admission to hospital was recorded for the purpose of the present
study.

**Erythrocyturia.** A mixed up specimen of urine was examined and recorded as 'cells per high power field'. Once again the mean of the first four days has been used here. Grade 1 - <10; 2 - 10 - 20; 3 - 30.

Analysis of the results shows no significant difference for either measurement. In the case of the red cells, the distributions between the oedematous and non-oedematous groups are almost identical ($\chi^2 = 0.24, 0.9 > P > 0.8$). With albumuria, a clear trend ($\chi^2 = 4.35, 0.2 > P > 0.1$) suggests that this tends to be heavier in the children with oedema.

**Blood Pressure**

Far and away the most striking differences associated with the presence or absence of oedema are those affecting the blood pressure, both systolic and diastolic. The value used in this analysis has been in each case the highest individual value quoted from a daily blood pressure chart. No consideration has been given to the duration of hypertension.
The results, which speak for themselves, are illustrated in Figure 7.2.e, and are as follows:

**TABLE 7.6**

<table>
<thead>
<tr>
<th>Systolic B.P. (mm Hg)</th>
<th>85-104</th>
<th>105-124</th>
<th>125-144</th>
<th>145-174</th>
<th>174+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema present</td>
<td>6</td>
<td>17</td>
<td>39</td>
<td>29</td>
<td>11</td>
</tr>
<tr>
<td>Oedema absent</td>
<td>6</td>
<td>43</td>
<td>11</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ x^2 = 47.28, \ p < 0.0005 \]

**TABLE 7.7.**

<table>
<thead>
<tr>
<th>Diastolic B.P. (mm Hg)</th>
<th>65</th>
<th>65-84</th>
<th>85-104</th>
<th>105-114</th>
<th>114+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema present</td>
<td>11</td>
<td>19</td>
<td>47</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>Oedema absent</td>
<td>9</td>
<td>47</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

\[ x^2 = 56.5, \ p < 0.0005 \]

Pyrexia.

The highest temperature recorded in all patients during the first week has been analysed, and
it was found that on the whole the non-oedematous patients tended to have the highest temperatures. This result is just significant (\( \chi^2 = 9.23, 0.05 > P > 0.025 \)).

Treatment with Penicillin

Comparability of treatment of the two groups was tested by dividing the cases primarily into those receiving penicillin for periods longer or shorter than fourteen days, and secondly, into those in whom treatment had been started within or after two days following admission to hospital. No significant differences were found (\( \chi^2 = 4.04 \), \( 0.3 > P > 0.2 \)).

Outcome

A comprehensive follow-up survey has not been attempted, largely since the period is not yet long enough for this to be possible. No significant difference was found between the groups with regard to the duration of hospitalization (\( \chi^2 = 6.94 \), \( 0.3 > P > 0.2 \)). The results are illustrated in Figure 7.2.b. and reveal a slight trend towards longer periods on the part of the oedematous group.
There have to date been ten recurrences, five from each group. Where this has occurred, the "duration of albuminuria" (see below) refers only to the first episode.

Only one death has so far occurred in the series, a mortality of 0.6%. This was in a six year old boy who was from the outset the case most severely affected. He was initially grossly oedematous but normotensive, had a blood urea of 326./100 ml, and was oliguric for the unusually long period of fourteen days. While this was followed by diuresis, a considerable degree of albuminuria and haematuria persisted. He developed a progressive sub-acute form of the disease and died after nine months.

On the other hand the outcome in a further fourteen is uncertain. Ten of these defaulted from out-patient follow-up, mostly soon after discharge from hospital. When last seen proteinuria was present in all, but it seems likely that a number would later return to normal. Four patients (2.4%), two of which were from each group have persistent albuminuria after more than a year.
The vast majority of the cases had a favourable outcome, the urine becoming free from albumin and red cells at variable times as follows:

**TABLE 7.8.**

<table>
<thead>
<tr>
<th>Categories</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oedema present</td>
<td>78</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td>Oedema absent</td>
<td>49</td>
<td>7</td>
<td>4</td>
</tr>
</tbody>
</table>

\[ \chi^2 = 0.94, \quad 0.7 > p > 0.6 \]

Categories:

1. Urine clear before dismissal from hospital.
2. Urine clear 1-6 months after dismissal.
3. Urine clear 7-12 months after dismissal.

The close agreement in Category 1 (urine clear before dismissal), namely, 49 (73%) of the oedema-free group and 78 (80%) of the oedematous group is typical of these results and strongly suggests that the presence of oedema can hardly be an important factor in the prognosis of AGN. At the same time it is appreciated that the disappearance of protein and erythrocytes
from the urine cannot necessarily be taken as a final criterion of recovery from the disease. Minor degrees of residual renal dysfunction not sought in this study could ultimately lead to renal failure, a possibility which must obviously be checked by long-term follow-up.

**Summary.**

The records from 170 children with AGN have been divided into two groups depending on the presence or absence of oedema at the outset.

The oedematous child tends to be older, and loses less weight on diuresis than would be expected if the entire source of oedema fluid was derived from renal retention. He is strikingly prone to hypertension and has a slightly higher initial blood urea. On the other hand he is less pyrexial, while the amounts of protein and erythrocytes in his urine together with his rate of recovery and probably his ultimate prognosis do not differ significantly from those of his non-oedematous counterpart.
CHAPTER 8
BODY WATER DISTRIBUTION IN ACUTE GLOMERULONEPHRITIS

The demonstration of intracellular dehydration

As stated in Part One of this Thesis, the studies on body water distribution described have been leading up to the application of these methods to acute glomerulonephritis. It was made clear that whereas case-to-case variation of the values obtained was relatively great, subjects appear to maintain their individual body water distribution within relatively fine limits. Hence, as Doxiadis and Gairdner pointed out some years ago (49), an experiment purporting to demonstrate changes in body water distribution must utilise results obtained from serial estimations carried out in the same subject.

Material and Methods.

The investigation has been carried out on ten children, six boys and four girls of average age 6.4 years. The clinical data are tabulated in Table 8.1 and indicate that in each case the
### Table 8.1

**Clinical Data**

<table>
<thead>
<tr>
<th>Series Case No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>F</td>
<td>F</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td><strong>Age (Yrs,Mths)</strong></td>
<td>6.4</td>
<td>3.10</td>
<td>9.7</td>
<td>6.2</td>
<td>4.10</td>
<td>6.1</td>
<td>7.10</td>
<td>6.0</td>
<td>3.8</td>
<td>9.1</td>
</tr>
<tr>
<td><strong>Prev.infection</strong></td>
<td>Throat</td>
<td>Throat</td>
<td>Throat</td>
<td>Throat</td>
<td>Throat</td>
<td>Throat</td>
<td>Both ears</td>
<td>Throat</td>
<td>L.ear</td>
<td>Throat</td>
</tr>
<tr>
<td>% Exp. Wt. 1.</td>
<td>96</td>
<td>101</td>
<td>110</td>
<td>128</td>
<td>104</td>
<td>110</td>
<td>104</td>
<td>93</td>
<td>99</td>
<td>171</td>
</tr>
<tr>
<td>% Exp. Wt. 2.</td>
<td>92</td>
<td>91</td>
<td>97</td>
<td>105</td>
<td>101</td>
<td>99</td>
<td>92</td>
<td>82</td>
<td>85</td>
<td>155</td>
</tr>
<tr>
<td>% Exp. Wt. 3.</td>
<td>99</td>
<td>100</td>
<td>104</td>
<td>113</td>
<td>106</td>
<td>109</td>
<td>102</td>
<td>90</td>
<td>92</td>
<td>166</td>
</tr>
<tr>
<td>Days after onset</td>
<td>1</td>
<td>?</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>6</td>
<td>?</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Oedema</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Highest B.P.</td>
<td><strong>110</strong></td>
<td><strong>100</strong></td>
<td><strong>170</strong></td>
<td><strong>190</strong></td>
<td><strong>120</strong></td>
<td><strong>130</strong></td>
<td><strong>170</strong></td>
<td><strong>170</strong></td>
<td><strong>135</strong></td>
<td><strong>150</strong></td>
</tr>
<tr>
<td></td>
<td>65</td>
<td>70</td>
<td>110</td>
<td>120</td>
<td>80</td>
<td>100</td>
<td>120</td>
<td>120</td>
<td>100</td>
<td>110</td>
</tr>
<tr>
<td>Urine albumin</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Urine-R.B.C.</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Urine-Casts.</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Blood urea(mg.%)</td>
<td>33</td>
<td>27</td>
<td>40</td>
<td>73</td>
<td>40</td>
<td>53</td>
<td>33</td>
<td>67</td>
<td>40</td>
<td>37</td>
</tr>
<tr>
<td>A.S.O.(u/ml)</td>
<td>2500</td>
<td>2500</td>
<td>2500</td>
<td>333</td>
<td>833</td>
<td>500</td>
<td>2500</td>
<td>125</td>
<td>333</td>
<td>200</td>
</tr>
</tbody>
</table>
correctness of the diagnosis can hardly be doubted. Eight of the children had oedema clinically detectable on admission. The remaining two gave a clear history of onset of the disease within the previous 48 hours and are thus unlikely to have been in the post-diuretic phase.

Body water distribution was estimated in each patient twice: 1. Within two hours of admission. 2. When diuresis was judged to be complete. The time intervals between estimations varied from six to twelve days. No obvious diuresis was observed to occur with the two non-oedematous children and the second estimations were carried out after an arbitrary period of nine days. The measurements carried out have been total body water, extracellular water and plasma volume, using the same indicators and precisely the same methodology as has already been described. Repeat estimations have in all cases been carried out using the same administering syringes and doses as for the initial determination. Plasma water concentrations of antipyrine and of thiocyanate have been calculated using measured concentrations of plasma proteins in each case. Rates of elimination of T-182 and of thiocyanate
Block diagrams representing mean body water distribution of ten children with AGN before and after diuresis. Comparison is made in each case with water distribution in an average normal child of appropriate size. The compartments, in order downwards are: red cell mass, plasma volume, interstitial water, intracellular water, and body solids.
were determined by using five hour samples as well as the ten minute or two hour sample used for the standard estimation. This was done in the case of T-1824 in six subjects and with thiocyanate in five.

In case No.10 total exchangeable sodium was estimated with 20 uc. of $^{24}\text{Na}$ using the method described by Veall and Vetter (218). This was carried out at the same time as the two determinations of body water distribution.

Results.

The results of the two estimations of body water distribution on ten subjects are indicated in Table 8.2 together with the arithmetical means for the groups. These means are illustrated in Figure 8.1 where comparison is made with the body water distribution of normal children of like size. The normal values are taken from the study of twenty normal children described in Chapter 3, and illustrated in the similar block diagram of Figure 3.1. The values for the nephritics have been expressed in absolute terms rather than as ml./kg. body weight, since the marked change in weight (mean 2.00 kg.)
<table>
<thead>
<tr>
<th>Weight (lbs)</th>
<th>6.67</th>
<th>6.90</th>
<th>7.75</th>
<th>6.90</th>
<th>7.95</th>
<th>7.25</th>
<th>6.75</th>
<th>7.00</th>
<th>8.56</th>
<th>7.25</th>
<th>7.00</th>
<th>4.00</th>
<th>5.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (in)</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
<td>172.4</td>
</tr>
</tbody>
</table>

**Body Water Distribution in kg**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracellular</td>
<td>7.0</td>
</tr>
<tr>
<td>Intracellular</td>
<td>6.8</td>
</tr>
<tr>
<td>Intracellular</td>
<td>6.6</td>
</tr>
<tr>
<td>Intracellular</td>
<td>6.4</td>
</tr>
<tr>
<td>Intracellular</td>
<td>6.2</td>
</tr>
<tr>
<td>Intracellular</td>
<td>6.0</td>
</tr>
</tbody>
</table>

**Sample 8.5**

1.73
renders the use of such a proportional expression meaningless.

It is clear that, whereas the post-diuresis results are substantially normal, the initial or pre-diuretic values show a characteristic pattern. The expected increases in plasma and total extracellular volumes are present, but there is a marked decrease in the intracellular volume. The values for total body water show a slight increase above normal.

The consistency of these changes are indicated in Figure 8.2 which shows the percentage alterations in individual subjects of the components of body water distribution from the first to the second estimation. Nine out of the ten subjects lose weight and total water. All ten show very considerable loss of extracellular water, but in nine it is clear that this occurs while the intracellular volume expands. The tenth (case no. 3) shows a very slight contraction of his intracellular space.

In short, the average child of around 20 kg. in the first week of hospitalization loses 2 kg. weight, 1 kg. of which is water. The loss of 1 kg. solid weight following a week's semi-starvation seems not unreasonable.
Individual changes in body water distribution occurring with diuresis. Small figures refer to case-numbers.
At the same time, his extracellular space decreases by approximately 2 litres, 1 litre of which appears to replenish a depleted intracellular space while the other 1 litre is presumably excreted in the form of a diuresis.

It is clear that these changes are considerably more marked in the oedematous children than in those free of this sign. Patients nos. 1 and 5 exhibit alterations in volumes corresponding to about one half of the mean values for the whole series. Nevertheless it is of interest that the same phenomena, namely decrease of the E.C.W. and increase of the I.C.W. should be present in these cases also.

The slight increase of total body water occurring in subject no.8 is difficult to comprehend at first sight. However it seems possible that the factor of starvation may result in excessive production of endogenous water by breakdown of body fat. Such water when first released would certainly be intracellular and may account for the relatively large increase of the compartment seen in this child following diuresis.
Patient no. 10, who was mildly oedematous and by far the biggest child in the series, showed a moderate decrease in his E.G.V. but a comparatively slight rise in his intracellular space. It may be of significance, and will be demonstrated in Chapter 9, that at the time of his first estimation of B.W.D. his diuresis had already commenced. A few hours after completion of the investigation (24 hours after admission), oedema was no longer appreciable.

The alterations in blood volume confirm the results of previous investigators. The decrease with diuresis affects mainly the plasma volume, the two non-oedematous children showing the smallest decreases. There is unexpectedly a significant decrease in the red cell mass, but inspection of the results shows that this is almost entirely due to the excessive losses in cases nos. 2 and 10 (165 and 166 ml. respectively). Between the estimations, both children suffered from copious epistaxes which presumably account for the inordinate losses.

The plasma protein concentrations, used in the calculation of antipyrine and thiocyanate spaces, are also shown in Table 8.2 and show a slight mean rise following diuresis. The variations and
behaviour of this quantity will however be dealt with in detail in Chapter 9.

**Statistical Analysis**

In analysing these results two approaches have been made:

1. Demonstration that the changes occurring within the subjects are statistically significant.
2. Comparison of the results before and after diuresis with B.W.D. in the normal child.

**Changes within the subject.** Serial alteration in subjects can usually be analysed by the 'paired t-test' technique. Its application to the present series requires care. If the volumes are expressed in ml./kg. body weight, the alteration of weight renders the test invalid. When the results are expressed in absolute terms, the large range of total body weight must be included in the calculation. For example, a 2 litre alteration of, say E.C.V. occurring in a child weighing 10 kg. clearly has a different physiological significance from the same change in volume occurring in a child of 40 kg. For this reason the use of ratios is indicated, and if logarithms
are taken the agreement with normal distribution is more satisfactory i.e. Log (Reading 1) - Log (Reading 2)
The value for 't' has then been calculated by dividing the mean of these values by their standard error. With nine degrees of freedom, the probabilities are:

- **Weight.** \( t = 5.109, \ P < 0.001 \)
- **T.B.W.** \( t = 3.834, \ P < 0.005 \)
- **E.C.W.** \( t = 7.118, \ P < 0.001 \)
- **I.C.W.** \( t = 3.757, \ P < 0.005 \)

These results are all highly significant.

- **Plasma vol.** \( t = 3.265, \ P < 0.01 \)
- **Cell vol.** \( t = 1.925, \ 0.1 > P > 0.05 \)
- **Blood vol.** \( t = 3.219, \ P < 0.02 \)

Changes in the plasma and total blood volumes are statistically significant whereas those affecting cell volume on this number of subjects are not.

**Comparison with normal B.V.D.** The mean weights of the nephritic group (23.66 kg. before diuresis and 21.66 kg. after diuresis) compare satisfactorily with that of the twenty normal subjects (23.57 kg.). To eliminate the variable arising from the large range of body weight, an analysis of co-variance technique
has been used. The results are seen in Table 8.3, where the comparisons which are significant are underlined. Thus in the pre-diuretic phase extracellular water is significantly increased and intracellular water significantly reduced. Following diuresis neither of these spaces differ significantly from normal. Total body water is not significantly abnormal in either comparison. It is probable that this underlines the weakness of analysing such results in this way. Evidence has already been presented that total body water decreases significantly with diuresis. Since initial increase inferred thus is presumably responsible for an increase in body weight, the expression of water as a percentage results in an apparently smaller increase than is occurring in absolute terms. The same may be said for the failure to demonstrate significant increase of the plasma volume. This factor could also to some extent account for the significantly low values for red cell volumes in the pre-diuretic phase, since their proportion of body weight must decrease when total water rises. In addition, the decrease of the total intracellular volume presumably affects also the red cells.
TABLE 8.3.

CHANGES IN BODY WATER DISTRIBUTION

ANALYSIS OF COVARIANCE

<table>
<thead>
<tr>
<th></th>
<th>T.B.W.</th>
<th>E.C.W.</th>
<th>I.C.W.</th>
<th>P.V.</th>
<th>C.V.</th>
<th>B.V.</th>
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<td>Nephritics 1.</td>
<td>607.6</td>
<td>342.6</td>
<td>265.0</td>
<td>52.1</td>
<td>21.9</td>
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<td>269.8</td>
<td>328.9</td>
<td>47.4</td>
<td>25.0</td>
<td>72.4</td>
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</tbody>
</table>

1. F. | 0.237 | \underline{41.675} | \underline{4.461} | 3.108 | \underline{5.475} | 0.053 |
      | P.   | N.S.   | \textless0.01 | \textless0.05 | N.S. | \textless0.05 | N.S. |

2. F. | 0.223 | 0.049  | 0.370  | 0.022 | \underline{4.876} | 0.017 |
      | P.   | N.S.   | N.S.   | N.S. | \textless0.05 | N.S. |

Notes on Table 8.3. overleaf
Notes on Table 8.3.

1. Nephritics 1: Mean of results obtained in pre-diuretic phase.

2. Nephritics 2: Mean of results obtained in post-diuretic phase.

Normal: Mean of results from series analysed in Chapter 3.

All values expressed in ml./kg. body weight.

2. 'F' represents throughout the ratio:

\[
\frac{\text{"Adjusted mean" variance}}{\text{Variance common to compared groups}}
\]

3. Comparable regression coefficients have in all cases been compared by a separate variance ratio test.

No significant differences have been found.
Following diuresis the red cell volume is again significantly low. The excessive loss of blood by two of the subjects has already been commented upon, and when these results are omitted the mean of the remaining eight cases (22.4 ml./kg.) no longer differs significantly from normal (t=1.874, 0.1 > P > 0.05).

The Possibility of Artefact

It is tempting to assume immediately that the interpretation of the above results is that the oedema fluid, represented by an additional mean volume of two litres of extracellular water, has been derived by renal retention of one litre and transfer of the second litre from the intracellular space. Since oedema formed in this manner represents a concept new to the literature, it is imperative here and now to consider carefully the validity of the results.

The close correlation of the values obtained after diuresis with the normal pattern is a fairly strong argument in favour of these results being accurate. It is clearly the earlier figures which must be called in question.
The pattern of the volumes could be caused by:

1. An artificially low antipyrine volume of dilution.
2. An artificially high thiocyanate volume of dilution.

Antipyrine penetrates rather slowly into oedema fluid, and its use for the determination of total body water in the grossly oedematous adult has been called in question (100). Incomplete mixing with its ultimate volume of dilution results inevitably in a curved disappearance slope when the concentrations in plasma are plotted against time on semi-logarithmic paper i.e. when the elimination is not exponential. It is obvious why this should be so; once complete mixing has taken place, the substance is metabolised and excreted at a rate at any given moment directly proportionally to the concentration, thus giving an exponential slope. The addition of the mixing factor must therefore cause an increased rate of disappearance as long as equilibration is occurring, and this in its turn results in an excessively high level of indicator in the plasma and an artificially low apparent volume of dilution. It follows that when the elimination
curve is exponential, mixing is already complete and the result obtained is likely to be accurate. It may be necessary to study the elimination slope over a considerable period to ensure that this condition is operative. The disappearance of antipyrine was determined over a period of twenty hours in two subjects in the present series (nos. 7 and 10). The exponential nature of the slopes in both was undoubted, that from no.10 being illustrated in Figure 2.5, P.56.

The possible entry of thiocyanate into cells is the next point for consideration. Such a phenomenon has been described in pyrexial states by Overman (154). When the ratio of the first to the second estimation of thiocyanate space is plotted against the observed body temperature at the time of the first estimation (Figure 8.3) no correlation is demonstrable. It is also worth stating that the ratio of first to second interstitial volume correlates roughly with the clinical assessment of the amount of oedema present, (Figure 8.4).

If the behaviour of any of these indicator dilution substances differed basically from one estimation to the second, it is possible that this
Changes in thiocyanate volume of dilution showing no correlation with body temperature.
ACUTE NEPHRITIS

0  no oedema
1  oedema of face only
2  mod. gen. oedema

Rough correlation demonstrated between changes in observed interstitial space and degree of clinical oedema.
could be reflected in a consistently different rate of elimination; e.g. if albumin, to which T-1824 is attached were being lost to the interstitial space at an increased rate, this might well cause an increase in the rate of elimination. Similarly if thiocyanate were penetrating cells to a significant extent a similar effect might be shown. The elimination curves for T-1824 (6 cases) and thiocyanate (5 subjects) are shown in Figures 8.5 and 8.6. No consistent trend is evident. Mathematically, the rate of elimination represented by the constant \( \lambda \), is given by the expression:

\[
\lambda = \frac{\log C_t - \log C_{t'}}{t' - t}
\]

where \( C_t \) and \( C_{t'} \) are the concentrations of the indicator in plasma at times \( t \) and \( t' \) respectively.

These values have been calculated for each slope. When the mean of the differences between the values for relating to the first and second determinations are divided by their standard error, it is clear that the variations in slope are quite insignificant.
Individual T-1824 elimination curves before diuresis (solid lines) and after (broken lines)
Individual thiocyanate elimination curves before diuresis (solid lines) and after (broken lines)
A similar result has been obtained for antipyrine 
($t = 0.1666$, $P > 0.8$.)

The final piece of evidence to be presented 
in this context has been obtained from measuring the 
total exchangeable sodium in Patient No. 10 using 
20 c. of $^{24}$Na. This was done simultaneously with 
both the estimates of body water determination.

Results: 1. $Na^+_o = 2015$ meq. ($44.7$ meq./kg.)

2. $Na^+_o = 1550$ meq. ($37.0$ meq./kg.),
a difference of 465 meq.

Since the plasma sodium concentrations at the 
relevant times are known, it is immediately possible 
to calculate the total extracellular sodium ($Na_{ec}$) 
using this and the thiocyanate space.

Plasma sodium 1. 146 meq./litre.

2. 130 meq./litre.

First estimation:

Total plasma sodium $Na^+_s = 146 \times 2.2$ (P.V. in litres)

= 327.0 meq.

The concentration of sodium in interstitial 
fluid is given by:
\[(Na_{is}) = Na_9 \times \frac{95}{93.28}\]

where 95 = Donnan equilibrium constant for cations (228).

and 93.28 = Plasma water concentration = 100 - plasma protein concentration

= 148.7 meq./L.

In a similar way the interstitial space may be calculated after modifying the observed thiocyanate space using the Donnan factor of 105 for anions (228). The total interstitial sodium thus derived is 1356 meq., giving a total extracellular sodium of 1688 meq.

Second estimation:

Using a similar process the second estimate of extracellular sodium is 1285 meq., a difference of 398 meq. This compares with the difference between the two estimates of total exchangeable sodium of 465 meq. If significant amounts of thiocyanate were entering cells, the difference between the two values for extracellular sodium would be expected to be much greater. It is interesting that the percentage ratios of extracellular to exchangeable sodium for the two estimations should be almost identical (83.5% and 82.9% respectively). Thus this slight difference
between 465 meq. and 398 meq. may represent the body's natural conservation of the intra- and extracellular sodium ratio.

An alternative possibility which must be considered is that of abnormal sodium entry into cells at the time of the first estimation. If sodium enters cells in abnormal amounts, under most circumstances an osmotically equal amount of potassium will leave the cells to preserve equilibrium. Since practically all body potassium is intracellular, one would expect to find a reduction of the total exchangeable potassium ($K_e$) demonstrable. The values for $K_e$ in the same patient have been calculated using the Edelman equation (53):

$$\frac{(Na_s) = K \times Na_e + K_e}{T.B.W.} \quad ............(1)$$

The value for the constant $K$ has been obtained from the regression equation derived by Muldowney and Williams (146). This equation is:

$$y = 2(0.88x) + 39 \quad ............(2)$$

where $y = \frac{Na_e + K_e}{T.B.W.}$

and $x = (Na_s)$

By substituting in equation (1) above,
the values for $K_0$ can be readily calculated and have been found equal to:

1. 2160 meq. (48.1 meq./kg.)
2. 2015 meq. (48.1 meq./kg.)

On this reasoning it seems unlikely that significant intracellular migration of sodium can be occurring, and it is of great interest that the body would appear to maintain its potassium in this way. Further important applications of this principle will be considered in Chapter 9.

**Summary.**

Body water distribution has been estimated in ten children with acute glomerulonephritis before and after diuresis. The results suggest that the oedema fluid is derived at least in part by transfer of water from the intracellular to the extracellular phase. The work has been carefully scrutinised to exclude artefact, and it is concluded that the weight of evidence strongly suggests that the results are valid.
CHAPTER 9

THE DIURESIS OF ACUTE GLOMERULEONEPHRITIS

A three-dimensional study of the retention and excretion of water and electrolytes

Introduction.

The presence of oedema coexisting with a degree of intracellular dehydration has been demonstrated in the preceding Chapter. This combination was to the author an unexpected and surprising discovery. Intracellular dehydration is a comparatively rare phenomenon in clinical medicine. In childhood, the most completely authenticated example is probably that of hypertonic dehydration due to water deprivation, a condition which is not uncommon during the first two years of life (63). In this instance deprivation of water leads to extracellular hypertonicity and compensatory withdrawal of water from cells. The extracellular volume is by this means relatively well maintained but only at the expense of the intracellular. The condition is associated with a substantial mortality and a common sequel is damage to the central nervous system. The total body water falls below normal and this would appear to differentiate it radically from the
situation occurring in AGN. Indeed the only other circumstance known to the author paralleling this pattern is the progressive intracellular dehydration inferred from the results described in chapter 4 of the present Thesis. The reader will recall that in newborn infants deprived of fluid, progressive expansion of the extracellular fluid compartments was demonstrated. When discussing these results it was suggested that a fluid shift would almost certainly be due to alterations of extracellular osmolarity. It is now fully accepted that under all circumstances the total intracellular osmolarity equals precisely the osmolarity of the fluid immediately outwith the cells.

Bearing these facts in mind it was clear to the author that the line of investigation most likely to provide an explanation of the observed facts would be in the realms of conservation and excretion of water and electrolytes. This chapter is an account of the experiment which was designed to fulfill these needs.

Material and Methods

Thirteen children with acute glomerulonephritis have provided the case material for this study. Two (Case Nos. 6 and 10) formed a part of the study of the
previous Chapter. The clinical details of the remainder are indicated in Table 9.1. With the exception of No. 17 (P. McC.) the children in this group are on average older than the other group. There were two reasons for this: firstly, the procedure to be described involved venepuncture on each day for a period of nine days. Only children not objecting to this were utilised, and this factor in several instances resulted in rejection of cases after four or five days. Secondly, the collection of total urinary output is clearly facilitated by older and more co-operative children. In consequence of these facts, the collection of this small series took almost a year.

Twelve of the series were initially oedematous, Nos. 12 and 21 grossly so. The solitary non-oedematous child (No. 16) is the boy who was described in some detail in Chapter 7 (p. 160). He nevertheless had a small but recognisable diuresis and his results have therefore been included. P. McC. (No. 17) was severely orthopnoeic on admission and was too ill to be weighed. His youth precluded repeated venepuncture and his contribution in common with some others is that of urinary data alone.
# TABLE 9.1

## CLINICAL DATA

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<td></td>
</tr>
<tr>
<td>Urine-Casts</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood urea (mg.%)</td>
<td>107</td>
<td>130</td>
<td>79</td>
<td>70</td>
<td>77</td>
<td>202</td>
<td>96</td>
<td>83</td>
<td>43</td>
<td>50</td>
<td>74</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A.S.O.(u./ml.)</td>
<td>1250</td>
<td>200</td>
<td>1250</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>500</td>
<td>50</td>
<td>2500</td>
<td>500</td>
<td>50</td>
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</tr>
</tbody>
</table>

Notes on Table 9.1. overleaf
Notes to Table 9.1

1. Case Nos. 11-17 (plus No. 6) constitute the 'early' diuretic group.

2. Case Nos. 18-21 (plus no. 10) constitute the 'late diuretic' group.

3. The facts here have been categorized as in Table 8.1.
The procedure was a simple one. From the time of admission all urine was collected with the utmost care by a nursing staff co-operating to the fullest capacity. Collections were divided into 24 hour periods and maintained for a period of eight to ten days, by which time all cases had lost their oedema and diuresis was considered complete. The total volumes together with the concentrations of sodium, potassium and chloride were measured. Checks on the completeness of the 24 hour collections, such as the endogenous creatinine output, were not used. On the slightest suspicion that a collection might be incomplete that sample was discarded.

On each day throughout the same period a 4 ml. venous blood sample was withdrawn with minimal stasis. After estimating the haematocrit the plasma was separated, all the samples being stored at 4°C, until the period of collection was complete. The estimations were then carried out in one batch by the same observer. Sodium, potassium, chloride, calcium, magnesium, urea, total protein and total plasma osmolarity were determined on each sample. The methodology has already been described.
The clinical management of all patients was described in Chapter 8. The dietetic part of the therapeutic regime is in this study of extreme importance. The daily intakes are repeated here:

<table>
<thead>
<tr>
<th>Fluid</th>
<th>1 litre (Half-strength milk).</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>600</td>
</tr>
<tr>
<td>Sodium</td>
<td>14 meq.</td>
</tr>
<tr>
<td>Potassium</td>
<td>4 meq.</td>
</tr>
<tr>
<td>Chloride</td>
<td>20 meq.</td>
</tr>
</tbody>
</table>

(These values have been calculated by determining the electrolyte-concentrations in full-strength milk and also in the glucose sweets given to the children:

- **Milk:**
  - Sodium: 25 meq./l
  - Potassium: 3.3 meq./l
  - Chloride: 36 meq./l

- **Sweets (average of three):**
  - Weight: 5.77 Gm.
  - Sodium: 0.13 meq.
  - Potassium: 0.03 meq.
  - Chloride: 0.30 meq.)

A complete balance technique has not been attempted, but with the intake and urinary output accurately known it is felt that this ideal is not far
from being realised, provided it is valid to assume that the loss of faecal and 'insensible' water and electrolytes remains constant. None of the patients developed diarrhoea, and in the relatively stable environment represented by bed-rest in a hospital ward maintained constantly at $36^\circ C$ ($68^\circ F$) it seems reasonable to assume that variability of insensible loss can hardly have been an important factor.

**Results and Comment.**

At the outset, a recognisable pattern was sought in order to render comparison between subjects possible. Since the patients were about to lose their oedema, a volume of urine increasing day by day was confidently expected. In fact this pattern was found in only eight (Nos. 6, and 11-17 inclusive) of the total series of thirteen patients. In the remainder, the average urine output was greater and showed no recognisable pattern. It was immediately apparent that there must be some basic difference between the subjects making up the two groups. The simplest explanation appeared to be the possibility that the group showing a distinct pattern were in the very early phase of diuresis, while the second group
were in a somewhat later phase, their diureses having presumably commenced before admission to hospital. These groups are accordingly designated "early diuretic group" and "late diuretic group" and all the results to follow have been presented on this basis. While evidence bearing on the validity of this assumption will be presented at several points of this Chapter, it would be as well to point out immediately that according to the case-histories the 'early' group were admitted to hospital sooner following the onset of the disease than the 'late' groups:

- **Early group:** Mean = 2.5 days admitted after onset
- **Late group:** Mean = 5.2 days admitted after onset.

These means are significantly different ($t = 2.25$, $P < 0.05$).

**Early Diuretic Phase.**

This group comprises eight subjects. Results from the urinary part of the investigation are available from all, and daily blood determinations have been carried out on five of the children. All the graphs illustrating this investigation have been drawn to a standard pattern, which is the arithmetical mean for
the group - one S.D. plotted against time.

To find a pattern for these results it has been necessary to eliminate one variable. This is inherent in the fact that the day of onset of diuresis need bear no relation to the day of admission to hospital, which has necessarily been the first day of study. Thus some patients may start to have their diuresis within a few hours of admission, while in others the onset has been delayed for several days. To deal with this, an attempt has been made to 'fit' the results from the different subjects together by adjusting the commencing day of study with this in mind. The adjustments resulting have been remarkably slight, since no child in this group had an onset of diuresis later than three days after admission to hospital.

While the increasing urinary volumes gave a sound basis for comparison, that afforded by the patterns of sodium excretion is much better, and accordingly the entire study has been based on the demonstrated 'fit' of the total amounts of sodium excreted daily.

The pattern of total sodium excretion is shown in the lowest graph of Figure 9.1. It is clear that
FIG. 9.1
**ACUTE NEPHRITIS**
**EARLY DIURETIC PHASE**

Urine: 8 Cases

- **Na CONCN. meq/l.**
- **VOLUME ml.**
- **TOTAL Na meq.**

N.B. The graphs throughout this chapter represent daily mean ± S.D.
during the first two days of the study there is a profound degree of sodium retention, and that the sodium diuresis which follows is intense, the daily amounts rising from a mean of 8 meq. to a mean of 83 meq. in four days - a more than ten-fold increase. It is significant that this pattern occurs despite the low sodium intake, which in the normal subject would be manifested within this period by a reduction of the sodium output.

The pattern of water excretion also depicted in Figure 9.1 reveals a picture of much smaller change. In the oliguric phase the volume is only moderately reduced to a mean of 480 ml., rising steadily to a mean of 947 ml. after four days. The relationship of these findings is clearly shown when sodium concentration is considered, as shown in the upper graph of Figure 9.1. The increase here is from 21 to 92 meq./litre after four days.

A very similar pattern typifies the excretion of chloride (Figure 9.2).

It is immediately clear that the oliguric phase in these patients must be characterised by hypertonic retention of salt, and that the reverse holds true with
ACUTE NEPHRITIS
EARLY DIURETIC PHASE

Urinary Chloride: 8 Cases

CONCN.
meq/1.

TOTAL
meq.

1 2 3 4 5 6 7 8 DAYS
the onset of diuresis. It would further appear clear that diuresis was not complete at the end of the study period in this group.

An entirely different picture emerges when urinary potassium is considered (Figure 9.3). It has not been possible to separate the potassium derived from erythrocyturia from that secreted by the kidney. Nevertheless, the total output of potassium remains strikingly constant in spite of the immense changes affecting the excretion of sodium, chloride and water. The concentration of potassium does show a slight mean drop during the first five days (from 58 to 29 meq./litre), and when these values are correlated with time the reduction is found to be significant, \( r = -0.4684, P < 0.01 \).

When the concentrations of sodium in serum and urine are considered together in the five patients in whom this is possible, a fact of great importance becomes clear (Figure 9.4). With the subjects on a constant intake of salt and water, it might be anticipated that a rising concentration of urinary sodium would be associated with a fall in the concentration of this ion in the blood. No such fall occurs. The author feels that the demonstration of
ACUTE NEPHRITIS
EARLY DIURETIC PHASE

Urinary Potassium: 8 Cases

Note relative constancy of potassium excretion as compared with sodium and chloride.
ACUTE NEPHRITIS
EARLY DIURETIC PHASE

Sodium Conc. : 5 Cases

SERUM
meq/l.

160
140
120

URINE
meq/l.

140
120
100
80
60
40
20
0

Marked increase in urinary sodium concentration while the plasma sodium remains constant
this fact is of crucial significance. There are in his view only three possible interpretations of this phenomenon:

1. Coincidental transfer of water from the extracellular space (of which the plasma is a part) to the intracellular.

2. Transfer of sodium from the intracellular to the extracellular phase, and hence directly excreted in the urine.

3. A combination of 1. and 2., above.

The evidence suggesting which of these interpretations is most likely to be correct will be discussed in Chapter 12. The constancy of the values for serum sodium has been verified by applying the Analysis of Variance technique described in Chapter 5. In this series, the 'within subject' variance is considerably greater than in the controls indicating that the sodium levels are less stable. When the 'between days' variance is taken out, its value is found to be quite insignificant ($F = 0.527$), confirming the lack of any overall day-to-day trend.

The same pattern is seen with urinary and serum chloride concentrations (Figure 9.5). The reduction of urinary potassium concentrations is
ACUTE NEPHRITIS
EARLY DIURETIC PHASE

Chloride Concn.: 5 Cases

**SERUM**
meq/l.

**URINE**
meq/l.

Fig. 9.5.
associated with a slight lowering of the serum potassium from 6.2 to 5.3 meq./litre. When correlated against time, this is just significant ($r = -0.3802, P < 0.05$). The initial mean of 6.2 meq./litre is in any event above the accepted normal range (Figure 9.6).

No particular trend is evident in the results for serum calcium and magnesium, the values remaining within the normal range throughout the test period (Figure 9.7).

As would be expected, concentrations of plasma urea show a steady reduction towards normal (Figure 9.8) which in this group is not quite attained during the period of the study. The mean value on the final day was 49 mg./100ml.

The values obtained for total plasma osmolarity are shown in the upper graph of Figure 9.9. A clear drop occurs from 312 mOsm./litre on the first day to 265 mOsm./litre on the eighth. When correlated with time this reduction is highly significant ($r = -0.4815, P < 0.01$).

If transfer of water or sodium is occurring at cellular level, one would expect this to be indicated by demonstrable changes of extracellular osmolarity. The plasma osmolarity cannot truly reflect such changes,
ACUTE NEPHRITIS
EARLY DIURETIC PHASE

Potassium Conc. : 5 Cases

SERUM
meq/l.

URINE
meq/l.

8
6
4
2
0

1 2 3 4 5 6 7 8 DAYS
ACUTE NEPHRITIS
EARLY DIURETIC PHASE

Serum Calcium: 5 Cases

Serum Magnesium

Fig. 9.7.
FIG. 9.8.

ACUTE NEPHRITIS
EARLY DIURETIC PHASE

PLASMA UREA
mg./100 ml.

5 Cases

0 40 80 120 160 200
1 2 3 4 5 6 7 8 DAYS
FIG. 9.9

ACUTE NEPHRITIS
EARLY DIURETIC PHASE

Total Plasma Osmolarity

5 Cases

mOsm./l.

330
310
300
290
280
270
260
250

1 2 3 4 5 6 7 8 DAYS

Osmolarity minus (Urea + Proteins)

mOsm./l.

330
310
300
290
280
270
260
250

For explanation see text
since most of the undissociated particles contributing to the observed readings are distributed equally inside and outside the cells. In the present series the high blood urea must make a particularly notable contribution. Furthermore, the concentration of protein in interstitial fluid is extremely low compared to that of plasma. The calculated milliosmolar values for urea and protein have accordingly been subtracted from the observed values for osmolarity to derive the lower graph in Figure 9.9. The slight decrease of osmolarity is no longer statistically significant \( (r = -0.2324, P > 0.1) \).

The final results to be presented are the readings of total body haematocrit and plasma protein concentrations (Figure 9.10). If alterations of both are due largely to changes of the plasma volume, one would expect the values to show parallel variation. Further, if reduction of the plasma volume is associated with diuresis, both should progressively rise. The graphs indicate that the protein concentrations tend to rise from the third day, but increase of the haematocrit is delayed until the seventh day. There is indeed a total lack of correlation between the results \( (r = -0.0660, P > 0.1) \). It is clear either
Plasma protein concentrations (solid) compared with haematocrit determinations (broken). There is no correlation ($r = -0.066$). Cf. Figure 9.18
that additional factors must be present at this phase of diuresis, or that increase of the plasma volume is only commencing towards the end of the study period in this group.

**Late Diuretic Phase.**

When we turn to the results obtained from the second group, a rather different picture is presented. Other than the fact that the excretion of sodium, chloride and water is maintained at high levels, no progressive pattern is discernible (Figures 9.11 and 9.12).

The total urinary potassium excretion again remains virtually constant until the last three days, when a slight rise is seen. A very slight decrease of urinary potassium concentration from a mean of 46 meq./litre to 27 meq./litre occurs during the first four days (Figure 9.13), but the change is sufficiently consistent to be statistically significant when correlated with time ($r = -0.4783, P < 0.05$).

One of the most striking features occurring in this group is the steady decrease of serum sodium illustrated in Figure 9.14. The mean change is from 143 to 132 meq./litre and is highly significant.
As compared with the early diuretic phase, no overall pattern is here evident.
FIG. 9.12

ACUTE NEPHRITIS
LATE DIURETIC PHASE

Serum Chloride Concn. : 5 Cases

Urine Chloride Concn.

Urine Total Chloride

Note progressive fall in serum chloride level
FIG. 9.13

ACUTE NEPHRITIS
LATE DIURETIC PHASE

Serum Potassium Concn.: 5 Cases

Urine Potassium Concn.

Urine Total Potassium

1  2  3  4  5  6  7  8  9 DAYS
FIG. 9.14

ACUTE NEPHRITIS
LATE DIURETIC PHASE

Serum Sodium Concn. : 5 Cases

Urine Sodium Concn.

The progressive fall in serum sodium concentration shown here is highly significant.
Further, using the same Analysis of Variance technique employed in Chapter 5, it has been shown that in this group a significant 'between days' variance exists ($F = 2.83, P < 0.05$) which affords further confirmation that the progressive decrease of serum sodium is significant. The greatest individual decrease was seen in Case No.19 (P.M.), in whom a level of 145 meq./litre on days one and two became one of 120 meq./litre by day seven. This overall finding contrasts markedly with those of the early diuretic group, and a similar trend is seen to affect the levels of serum chloride (Figure 9.12).

No particular trend is evident when the serum potassium (Figure 9.13), calcium or magnesium (Figure 9.15) concentrations are considered.

The initial plasma urea levels (Figure 9.16) are much lower in this group than in the early diuretic group, the mean here being 55 mg./100 ml. The pattern exhibited in the first three days is not unlike that of the last three days of the early group.

The values for plasma osmolarity are shown in Figure 9.17. Modification of the observed values by subtracting from them the calculated milliosmolar values for urea plus proteins makes much less difference than
FIG. 9.15

ACUTE NEPHRITIS
LATE DIURETIC PHASE

Serum Calcium: 4 Cases

Serum Magnesium
The much lower values shown here should be compared with those in Fig. 9.8
Insufficient observations were obtained for day 4 to render meaningful the use of S.D.
in the early diuretic group since the urea levels are much lower. A slight decrease occurs in the first three days, but there is no statistically significant trend.

The final point of considerable interest arising from the results of this group lies in the closely parallel trend of the values for total body haematocrit and plasma protein concentration (Figure 9.18). The correlation is now highly significant ($r = 0.7523$, $P < 0.001$). It seems that by the time this phase is reached the factors causing changes to occur in both measurements have become unified. Diminution of the plasma volume represents of the plasma volume represents the obvious explanation, and it is of interest that such a change should appear to be maximal in the late, rather than the early, phase.

Two further points of difference between the early and late groups remain to be presented.

The pattern of weight change occurring (Figure 9.19) indicates that loss of weight occurs earlier in the late group than in the early, although the ultimate total losses of weight are similar. This is presumably accounted for by the greater initial urinary volumes secreted by the later group.
Plasma protein concentrations (solid) compared with haematocrit determinations (broken).
The correlation is highly significant ($r = 0.752$).
Cf. Figure 9.10.
While the ultimate total weight loss of the early and late diuretic groups is similar, the timing of the loss is seen here to be markedly different.
It might be reasonable to suppose that the features shown by the late group would in general be milder than those of the earlier group if for no more profound reason than that the markedly lower plasma urea levels in the late group suggest that the children are already further advanced into the recovery phase. It is therefore extremely interesting that the blood pressure readings in the late group should be no lower than in the early group. Indeed, while the highest mean systolic pressures show little difference (141 mm. Hg for the early group as against 149 for the later group), the mean diastolic pressure recorded in the later group is markedly higher (104 mm. Hg as compared with 88 mm. Hg.) With those small numbers the difference between these means just fails to reach significance ($t = 1.827, 0.1 > P > 0.05$), but it seems possible that it could be related to the generally higher values for serum sodium found initially in the later group. The implications of these findings will be discussed in Chapter 12.

Summary.

In a study of the day-to-day alterations in plasma and urinary electrolytes in thirteen children
with AGN, certain basic differences were found necessitating division of the series into two groups. It is probable that these groups represent the early and late phases of diuresis in AGN.

In the 'early' group, hypertonic retention of salt relative to water was demonstrated in the oliguric phase. In the ensuing diuresis the urinary concentrations of salt rose while the plasma concentrations remained constant, indicating either shift of water into, or sodium out of, body cells during this phase. The total potassium excretion remained almost precisely constant throughout the test period.

In the late phase no similar relative changes of concentration of salt were demonstrated. A significant drop of serum sodium did however occur. The haematocrit and plasma protein concentrations rose uniformly in this phase suggesting that the reduction of plasma volume is a late phenomenon.

The values for plasma urea were initially much lower in the late diuretic group, whereas the readings of blood pressure tended to be higher than in the patients in the early diuretic phase of AGN.
CHAPTER 10
OTHER ASPECTS OF ACUTE GLOMERULONEPHRITIS

I. Electrophoretic patterns

Introduction

It is strange that the electrophoretic pattern of serum proteins occurring in AGN should have received little attention. Most clinical review articles on paper electrophoresis do not mention the condition. Prinz in 1961 (168) drew attention to the typical pattern of moderate reduction of albumin and elevation of gamma-globulin and demonstrated gradual return to normal over periods which were often prolonged. Chiesura (277) and Imperato (102) in the Italian literature have shown similar findings, but the changes occurring with diuresis, and the correlation of patterns with the presence or absence of oedema do not appear to have been previously investigated.

Material and Methods

This investigation is in two parts:

1. Protein electrophoretic patterns were carried out in 39 of the 170 case records analysed in Chapter 7. In each case the estimation was carried out within
24 hours of the child's admission to hospital. This series has been utilised to determine the overall pattern to be expected in AGN and for comparing the results between the oedematous and non-oedematous cases. These estimations and those of twenty normal children were carried out by the Biochemistry Department, Royal Hospital for Sick Children, Glasgow.

2. Electrophoretic patterns have been determined before and after diuresis in fourteen children with AGN. Eleven are from the series of patients described in the last two Chapters (Nos 1, 3, 10, 12-16, 18-21). The remaining three are from further typical cases of AGN on whom no other investigations were carried out and whose clinical data are not presented. The time intervals between estimations varied from seven to twelve days.

The methodology of paper electrophoresis employed was described in Chapter 5. The method used by the routine laboratory and by the author has been throughout the same.

Results and Comment.

Figure 10.1 is a block diagram illustrating the mean electrophoretic pattern obtained from twenty normal children of age-range two to twelve years. The mean values ± one S.D. are indicated for each fraction and
ELECTROPHORETIC PATTERN
20 Normal Children

T. P. = 6.82 ± 0.50

Gm/100 ml. %

Y-Glob 1.10 (± 0.24) 16.1

β- 1.01 (± 0.26) 14.8

α- 1.22 (± 0.36) 18.0

Albumin 3.49 (± 0.40) 51.1

Block diagram representing mean normal electrophoretogram.
compare well with the results of published series.

Where the degree of separation was sufficient to allow separate scanning, the mean $\alpha_1$-globulin was found to be 0.33 Gm./100 ml. and the mean $\alpha_2$ 0.99 Gm./100 ml., giving a mean $\frac{\alpha_2}{\alpha_1}$ ratio of 4.3/1.

The mean pattern in 39 children with AGN is shown in Figure 10.2. The total protein level is moderately reduced as are those of albumin and $\alpha$-globulin, the $\beta$-globulin is normal and the $\gamma$-globulin moderately increased. This pattern has a similarity to that described in a number of acute infections (168) except that in these conditions the reductions of total protein and albumin are less than those observed here in AGN.

When analysed statistically by t-test the results are:

Total protein: Highly significantly low

$$(t = 4.729, P < 0.001).$$

Albumin: Highly significantly low

$$(t = 7.220, P < 0.001).$$

$\alpha$-globulin: No significant difference

$$(t = 0.086, P > 0.90).$$
Mean electrophoretogram for 39 children at various stages of AGN. Fractions depicted in same order as in Figure 10.1 Normal pattern is indicated on left.
**β-globulin:** Significantly low

\[ t = 2.201, \ p < 0.05 \].

**γ-globulin:** Highly significantly raised

\[ t = 3.119, \ p < 0.005 \].

The raised levels of γ-globulin are presumably associated with infection. Since estimation of A.S.O. titre was carried out as a routine measure, a direct correlation has been possible and is shown in Figure 10.3. Calculation of linear correlation indicates a highly significant positive relationship \( r = 0.602, \ p < 0.001 \). Owing to the different ways of expressing the results of these two estimations, one would expect a correlation if present to be parabolic instead of linear, and the appearance of the scattergram does rather suggest this to be the case. It is therefore probable that the true correlation is higher than suggested by the above value.

**Presence of Oedema.** The results from these 39 children have been separated according to the presence or absence of oedema. The grouping is seen in Figure 10.4, contrasted with the normal pattern. It is clear that the results from the non-oedematous group are considerably
ACUTE NEPHRITIS

A.S.O. & Y-Globulin

U/mL

2000

1000

500

200

50

0 0.5 1.0 1.5 2.0 2.5 Gm.%

r = 0.602

P < 0.001
FIG. 10.4

ACUTE NEPHRITIS

Association with oedema. Normal pattern is shown on left-hand scale.
closer to the normal pattern than those of the oedematous group. Statistical analysis indicates the following difference from the normal pattern (values for 'P' bracketed):

**TABLE 10.1**

GROUP COMPARISONS WITH NORMAL (t - tests)

<table>
<thead>
<tr>
<th>Protein</th>
<th>Oedema present</th>
<th>Oedema absent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein</td>
<td>H.S. Low(&lt; 0.001)</td>
<td>N.S. (&gt; 0.2)</td>
</tr>
<tr>
<td>Albumin</td>
<td>H.S. Low(&lt; 0.001)</td>
<td>H.S. Low(&lt; 0.001)</td>
</tr>
<tr>
<td>α-globulin</td>
<td>N.S. (&gt; 0.7)</td>
<td>N.S. (&gt; 0.6)</td>
</tr>
<tr>
<td>β-globulin</td>
<td>S. Low(&gt; 0.02)</td>
<td>N.S. (&gt; 0.4)</td>
</tr>
<tr>
<td>γ-globulin</td>
<td>H.S. High(&lt; 0.005)</td>
<td>H.S. High(&lt; 0.001)</td>
</tr>
</tbody>
</table>

In thirteen of the total of 39 estimations adequate separation of the α-globulin into α₁ and α₂ fractions was achieved. These showed a reduction of the ratio to a mean value of 2.6 compared to the normal value equal to 4.4, and this change appears to be due to a combination of slight increase of the α₁ fraction with reduction of the α₂. The numbers of cases is too
small for adequate analysis, but with this data the use of t-tests has given the following results:

**TABLE 10.2**

<table>
<thead>
<tr>
<th>CHANGES IN $\alpha_2/\alpha_1$ RATIO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ($\pm$ S.E.)</td>
</tr>
<tr>
<td>AGN (13 cases)</td>
</tr>
<tr>
<td>Normals (10 cases)</td>
</tr>
<tr>
<td>AGN - Oedema present (8 cases)</td>
</tr>
<tr>
<td>AGN - Oedema absent (5 cases)</td>
</tr>
</tbody>
</table>

**Alterations with diuresis.** In the fourteen sets of results estimated by the author, the patterns before and after diuresis are indicated in Figure 10.5. In general the patterns resemble those of the oedematous and non-oedematous groups, as would be expected. With diuresis, the total protein, albumin, $\beta$- and $\gamma$-globulins all increase, while the $\alpha$-globulin decreases slightly. The percentage increases of the $\beta$- and $\gamma$-globulins are very similar being 7% and 4% respectively. The increase of albumin is somewhat greater - 27%.
ACUTE NEPHRITIS

Changes with Diuresis

Before

Normal

After

Y

5.94

6.82

1.53

1.10

1.57

1.78

1.01

0.85

1.17

1.22

0.98

2.46

3.49

3.11

Alb.
It may be presumed that a number of factors are likely to be concerned in these changes. For example the alteration of plasma volume taking place with diuresis is certainly of importance. A reduction of the water dilution factor would be expected to cause a uniform rise of all the protein fractions. In the context of the present work it is possible to form an estimate of the magnitude of this factor using the mean decrease of plasma volume shown in ten subjects in Chapter 8. Since only three of the present series have had their plasma volumes estimated, this application of one set of results to another must necessarily be somewhat speculative.

The average decrease of plasma volume has been shown to be of the order of 18%. If this figure is applied to the protein fractions measured in the pre-diuretic phase, they may each be expected to increase by the amounts illustrated in Figure 10.6. The actual rise with diuresis shown as the right-hand line for each fraction indicates that the β- and γ-globulin fractions rise less than would be expected, albumin rises rather more, but that the behaviour of the α-fraction is quite different in that it decreases below the level of the pre-diuretic value.
ACUTE NEPHRITIS

Plasma Protein Changes
14 Children

For each protein fraction, the left-hand vertical line represents the mean pre-diuretic level; the right-hand lines represent the levels following diuresis. The broken horizontal lines represent the levels to be expected after diuresis assuming an average plasma volume decrease of 18%.
It could be argued that the parallel increase of β- and γ-globulins suggests that these fractions are affected by one factor alone, that of plasma dilution. If that were the case the increase in albumin above the expected level becomes even more striking. Why should this be? One possible explanation is that of return of albumin from the interstitial space with diuresis. It was stated in Chapter 6 that the protein concentration in oedema fluid in AGN is normal. An increased volume of this space must mean that the total interstitial protein is above normal, and it is not necessary to postulate increased capillary permeability for this to occur. An increase in the volume of interstitial water derived either from the intravascular compartment or from trans-cellular shift is bound to be associated with a certain amount of shift of protein from the plasma to maintain colloid osmotic equilibrium. It would further seem reasonable that albumin should be involved preferentially owing to the comparatively small size of its molecules.

The relative disappearance of α-globulin cannot be explained on the present evidence. Urinary elimination appears to be the most likely cause, but
without turnover studies and separation of urinary proteins further comment is not possible.

Summary.

The typical electrophoretic pattern occurring in AGN has been demonstrated. This consists of a moderate reduction of total protein, albumin and \( \alpha \)-globulin together with a raised \( \gamma \)-globulin, the latter correlating positively with the level of A.S.O. titre. These changes decrease in magnitude with diuresis, but it has been shown that reduction of the plasma volume cannot be the only factor responsible.
CHAPTER 11

OTHER ASPECTS OF ACUTE GLOMERULONEPHRITIS

II. The chest x-ray picture and changes occurring in the radiological heart-volume.

Introduction

Changes in the appearances of the chest X-ray in AGN are common and sufficiently typical to be of considerable practical diagnostic value, but have excited a surprisingly small literature. Holzel and Fawcitt in 1960 (98) described changes in 25 out of 40 cases, noting cardiomegaly, pleural effusions, 'intrapulmonary oedema' and segmental or lobar collapse and consolidation. All these signs tended to clear with the onset of diuresis. Substantially the same appearances have been reported more recently in a larger series from America (112). While it is generally agreed that most of the changes are due to rapid expansion of the blood volume, alternative mechanisms have been suggested, such as allergy to the haemolytic streptococcus (234).

It has not been the author's intention to add to the qualitative information already available. Rather has an attempt been made to link the changes
quantitatively with the presence or absence of oedema. Further, the well-recognised alteration of heart-size suggests itself as a possible tool with which to throw further light on the fluid changes of AGN.

**Material and Methods**

Similar to the subject matter of the preceding Chapter, this study is also in two parts.

1. Chest X-ray examination was carried out in 89 of the 170 children whose case records have been analysed in Chapter 7. These were done in all cases within 24 hours of admitting the child to hospital, and in addition the examination was repeated at varying times thereafter in 36 patients. This time-interval has been in most instances more than one week and the second X-ray has therefore been assumed to have been taken in the post-diuretic phase. The facts noted have in all cases been abstracted from the radiological report-sheet.

2. Changes in heart volume. The case material for this part of the study has comprised 20 patients. Eighteen have been listed previously (Case Nos. 2-10, 12-16 and 18-21). The remaining two patients were once again suffering from AGN, the appearance of which
was quite typical and in both subjects associated with oedema. Since determination of heart volume was the only investigative procedure carried out the complete clinical details of these two patients have not been specifically recorded.

The method used for determination of the heart volume has been that of Lind (120), modified later by Domenet et al. (48).

Postero-anterior and lateral films of the chest are taken at focus-film distance using a stationary grid. Three measurements are made:

1. From the P.A film, (a) the long axis (l) from the point of junction of the aorta or superior vena cava to the left lower pole of the heart. (b) the short axis (s) from the right cardio-phrenic angle to the junction of the pulmonary conus and the left ventricle or the left atrial appendage.

2. From the lateral film, the longest diameter (d) of the cardiac shadow in the horizontal plane.

Calculation: where l, s and d are measured in cms.,

\[ V \text{ (heart volume in ml.)} = l \times s \times d \times K \]

K is a factor which includes the ellipsoidal conversion factor (since the heart is not an ellipsoid) and also a correction for the effect of radiological
magnification. The distances used in the present study have been the same as in the series of Domenet et al. (48), whose value for 'K' of 0.410 has accordingly been used here. Lind in his extensive and careful study quotes the reproducibility of the method to be ± 2.5%.

Determinations were carried out immediately on admission of the child to hospital and following diuresis, the range being 5-14 days. Where body water distribution was determined (nine subjects), heart volume was measured in each case on the same day. The names and dates on the films were obscured and the series was then presented in random order to a consultant radiologist who made all the measurements in one session. For comparative purposes, cardio-thoracic ratio was measured at the same time.

RESULTS

1. Chest X-ray appearances in AGN.

X-ray was carried out on 32 patients who were free from oedema. The appearances were considered normal in 25 (78%). In 57 oedematous children, only 13 (23%) showed normal appearances. Statistical analysis is not necessary to confirm the significance
(a) Oedematous phase: cardiomegaly and pleural transudates are clearly visible.

(b) Six days later: normal chest x-ray (Case G.R.)
of these figures.

The abnormalities recorded were mainly the following:

1. Generalised cardiomegaly.
2. Increase of pulmonary vascular markings.
3. The presence of pleural transudates.

An excellent, if relatively mild example of these changes, and their disappearance with diuresis, is shown in Figure 11.1 (Case No. 4, G.R.)

The distribution of abnormalities between oedematous and non-oedematous children is seen below:

**TABLE 11.1.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Oedema absent</th>
<th>Oedema present</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>25</td>
<td>13</td>
</tr>
<tr>
<td>Heart +; Lungs 0.</td>
<td>4</td>
<td>7</td>
</tr>
<tr>
<td>Heart 0; Lungs: V+</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Heart 0; Lungs: PF+</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Heart 0; V+, PF+</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Heart +; Lungs: V+</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Heart +; Lungs: PF+</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Heart +; V+ PF+</td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>32</strong></td>
<td><strong>57</strong></td>
</tr>
</tbody>
</table>

0: Normal. +: Abnormal. V: Pulmonary vasculature

PF: Pleural fluid.
From this table it is clear that when oedema is present, cardiomegaly is the commonest single abnormal finding (37 out of 44 abnormal films), and increase of the pulmonary vascular markings and pleural transudates appear with approximately equal frequency. In the non-oedematous group, only seven films were abnormal, four of these showing cardiomegaly alone.

Repeat chest X-rays were done on nine children from the non-oedematous group and 35 of the group who had been originally oedematous. Only one of the first group was reported abnormal, showing slight cardiomegaly only. Of the second group, 29 (83%) were normal, five showed cardiomegaly as an isolated finding, and a solitary case showed evidence of a small pleural transudate eight days after admission.

Radiological abnormalities of the chest in AGN must obviously be regarded as a transient phenomenon, disappearing during diuresis.

2. Changes in measured heart volume.

In 19 of the cases in the series, a marked decrease of heart volume was recorded between the first and second determinations. In the twentieth, a child (D.C., Case No.16) without either oedema or hypertension,
a very small (3.1%) increase was found, which is probably within the experimental error of the method. The mean changes were as follows:

TABLE 11.2

CHANGES IN HEART VOLUME (MEAN AND RANGE)

<table>
<thead>
<tr>
<th>Heart Volume I (ml.)</th>
<th>Heart Volume II (ml.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>352 (209-352)</td>
<td>385 (192-452)</td>
</tr>
</tbody>
</table>

Mean difference = 67 ml. (19.1%)

\[ t = 6.921 \quad P < 0.001 \]

The individual changes are illustrated in Figure 11.2. The alterations of cardio-thoracic ratio (Figure 11.3) show a similar if less consistent picture. It has not been the object in this study to make a formal comparison between the relative merits of measurements of C-T ratio as against heart volume. This has been already done with efficiency by Rosendal (182) who showed heart volume to be considerably the more reliable method. The greater consistency shown
ACUTE NEPHRITIS

Changes in Heart Volume

The open circles represent three non-oedematous subjects. The 45° line represents perfect agreement between the two determinations.
ACUTE NEPHRITIS
Changes in C-T Ratio

Details as in Figure 11.2
here would tend to confirm this finding.

Having shown the occurrence of large changes of heart volume to occur in AGN, the results have been employed in an attempt to identify the factor which is most important in causing cardiomegaly. In the early literature hypertension was the cause most often quoted, while in the past ten years changes in blood volume have been considered to be of first importance.

It is in fact difficult to separate these factors, since as has been shown both hypertension and hypervolaemia tend to occur in the same patient.

Changes of heart volume have been correlated with the highest diastolic pressure recorded in 20 children (Figure 11.4). The results fall into two groups, with diastolic blood pressures above and below 90 mm. Hg. In the hypertensive group, there is no suggestion of correlation. It must be stated immediately that the weakness of such an argument lies in the failure to take account of the duration of hypertension.

Eighteen estimates of blood volume from nine of the patients described in Chapter 8 are correlated with heart volume in Figure 11.5. Since both measurements are of volumes, any correlation may be
In general, larger changes in heart volume occur in the hypertensive patients. Nevertheless within this group no correlation is seen.
Determinations carried out in pre-diuretic phase (closed circles) linked with those done following diuresis (open circles). The mean changes are highly comparable.
expected to be linear, and it is extremely high
\( r = 0.9136, P < 0.001 \). The lines indicating the
changes in seven of the series lie almost parallel
to the regression line. In the remaining two (Case
Nos. 8 and 9) the decrease of heart volume is
proportionately greater than that of blood volume.
These patients both had a marked degree of hypertension,
but not more so than others showing almost perfect
correlations between changes of heart and blood volume.
There are presumably other factors concerned, but it
is felt that the results of this study strongly confirm
the importance of changes of the blood volume in this
respect.

The corollary to this work is surely the
possibility that this technique could be utilised to
determine at precisely what stage contraction of the
blood volume is maximal. The radiation hazard bars
frequent serial observations, but some interim readings
were taken in this series, and the significance of
these will be discussed in Chapter 12.

**Summary**

Abnormalities of the chest X-ray occur frequently
in AGN, particularly in cases with oedema. They nearly
always disappear with diuresis.

Using a method for radiological measurement of the heart volume, a marked decrease has been shown to occur during diuresis. The changes correlate well with observed changes in blood volume but not with diastolic hypertension.
CHAPTER 12
THE SYNTHESIS

A discussion concerning the significance of salient points presented in the preceding Chapters

The experimental work described has been designed with a view to throwing light on a number of different aspects of acute glomerulonephritis. It remains to be seen if it is possible to construct a coherent picture from it. It will have been immediately obvious to the reader that certain aspects of the results given in Chapters eight and nine are complementary. In Chapter Eight it was shown that body water distribution in the oedematous phase of AGN takes the form of a combination of expansion of the extracellular space with contraction of the intracellular. Following diuresis these relationships returned to a pattern approximating normality. During the oliguric phase, renal retention of water and salt was shown in Chapter Nine to be hypertonic, and it immediately follows that resulting extracellular hypertonicity must result in compensatory shift of water out of the cells. This is illustrated diagrammatically in Figure 12.1. The average daily urinary output of a
Proposed mechanism by which extracellular volume is expanded by 1.15 litres following renal retention of 600 ml. water and 160 meq. sodium. See text for explanation.
child weighing 20 kg. is something of the order of 700 ml., which contains perhaps 100 meq. of sodium chloride (190). If these amounts are decreased following the onset of AGN to, say 400 ml. and 20 meq., the effects on body water distribution will tend to be as illustrated. It can be seen that the extracellular space is expanded by 1.15 litres following a total renal retention of only 600 ml. water. This would tend to explain two of the features commented upon in Chapter Six. Firstly, the remarkably rapid rate at which oedema seems to form in AGN becomes comprehensible. Secondly, the work of Swann and Merrill (213) and later of Harrison et al. (89) have shown that in AGN the urinary sodium concentration tends to be less than 30 meq./litre, whereas in the oliguric phase of acute renal failure due to tubular necrosis a concentration greater than this figure is the rule. The results of the present series accord well with this figure. It would be reasonable to assume that the retention occurring in acute renal failure is likely therefore to be isotonic or hypotonic and not accompanied by osmotic withdrawal of intracellular fluid. The difference in the rates of accumulation of
Oedema in the two conditions could thus be easily explained.

In this connection the work of Remerchik et al. is of considerable interest (175). These authors measured body water distribution in adults, one of whom had AGN and the other seven acute renal failure due to tubular necrosis. The indicators used were anti-pyridine and radio-sulphate. In the seven cases with tubular necrosis both the ECW and the ICW were increased, the latter to a slightly greater extent and contracting more noticeably with diuresis. In the eighth case a pattern similar to that described in the present work was found. Somewhat similar findings were reported by Schwartz et al. (189) in a case report of a child with a fatal disease which was probably AGN.

It has already been pointed out that at the commencement of diuresis the increasing concentration of urinary sodium in the presence of a constant level of extracellular sodium could only be explained either by the movement of water into the cells or by the transfer of sodium from the cells to the extracellular fluid and hence directly excreted in
the urine. The arguments as to which solution is correct are largely those which were discussed in connection with the validity of the results of body water distribution in Chapter Eight. It was concluded that the evidence pointed to little if any sodium transport.

To this must be added evidence obtained from the patterns of urinary potassium in early diuresis. The remarkably constant output of this ion argues strongly against any considerable alteration of its distribution in the body throughout the period in question and this in its turn is further evidence against a large degree of sodium shift. It is however possible that a small amount of potassium leaves the cells during the oliguric phase to account for the slight elevation of serum potassium observed. Whether this is due to cellular breakdown or to transfer of the ion from the cells along with water is problematical. Elkinton and Winkler (58) showed many years ago that under conditions of experimental water depletion loss of intracellular potassium occurred which was in excess of the amount associated with protein catabolism. Such a phenomenon could account to some extent for the
observation which is perhaps the strongest single argument against the presence of intracellular dehydration in this disease. The fact to which the author refers is the presence of normal levels of serum sodium in the oliguric phase. These were indeed somewhat lower than those found in the 'late diuretic' group. This apparent discrepancy is perhaps occasioned by the higher sodium intake inherent in a normal home diet given presumably to these children for a longer, and indeed the most critical, period of the disease.

**Order of Events.**

While the order of events leading to the formation of oedema in AGN can only be surmised from the present data, a fairly precise picture of the sequence during diuresis is ready to hand.

Figures 12.2 and 12.3 represent a summary of the main findings from M.C. (Case No.13) from the early diuretic group, and J.B. (Case No.10) from the late group. In M.C. initial oliguria associated with a rising level of plasma urea is followed by profound sodium diuresis, the volume of urine rising less dramatically. At this stage cellular rehydration must be occurring. Hypertension was not marked in this case.
Summary of findings from child in early diuretic group.
Fig. 12.3.

Summary of findings from child in late diuretic group
(maximum 140/90 mm. Hg.) but the fall to normal levels seems to start at this time. The plasma protein concentration begins to rise also around the third to fourth day. It is likely that this is at first not due to diminution of the plasma volume, since the heart volume is unchanged on the 5th day, and the haematocrit (not shown) did not start to rise in this case until between the sixth and seventh day. Thus it would appear that decrease of the plasma volume is almost the last event in the sequence.

The evidence from J.B. is confirmatory, except that the drop in blood pressure is delayed. The period of cellular rehydration has presumably occurred before the start of the test, and the rise of the haematocrit occurs between the third and sixth day of this later period.

The fact that the plasma protein concentration starts to rise earlier than the haematocrit is puzzling, but probably very informative. While it could be due to prolonged loss of red cells in the urine, the very small change of total red cell mass in practically all the cases in which this was determined militates strongly against such an explanation. It was shown in Chapter 10 that the rise of serum
albumin following diuresis is probably greater than can be accounted for by diminution of the plasma volume alone. It has further been alleged that there is an increase of total albumin present in the interstitial space during the oedematous phase. During the very early phase of diuresis the shift of interstitial water to cells would tend to increase the concentration of albumin in this space, diminishing the colloid osmotic pressure gradient between the interstitial and plasma spaces and favouring early return of albumin to the plasma.

On the basis of these considerations a pattern of events, surmised and observed, is proposed and illustrated in Figure 12.4.

Sodium and Hypertension

The cause of hypertension occurring in AGN remains unknown. Hypervolaemia per se might well be a contributary cause since a mild degree may be provoked in the normal subject by acute expansion of this compartment (2). The association, however between hypertension and sodium retention is very well known and merits analysis in the present context,
FIGURE 12.4

**ACUTE PHASE**

Glomerular lesion → Glomerular filtrate ↓

A.D.H. ineffectual ← Hypertonicity of renal medulla ↓

Oliguria, Hyponatraemia

Hypertonic Sodium Retention ←

E.G. Volume ↑

E.G. Osmolarity ↑

E.G. Space ↓

I.G. Water ↓

Oedema + Hypervolaemia + Intracellular Dehydration

**DIURESIS**

Early

Urine volume ↑ = Urinary [Na] ↑ = Urinary Na ↑

Interstitial water → Cells ↓

Interstitial albumin → Plasma ↓

Plasma proteins ↑

Late

High sodium and water output maintained

Plasma volume ↓

Heart volume ↓

Haematocrit ↑
It has been known for some time that hypertension could be provoked in rats following the chronic ingestion of excessive salt (40). It has further been shown that prolonged renal retention of salt leads to hypertension, and an ingenious application of Starling's Law by Borst and Borst de Geus (18) suggests that hypertension is the inevitable cardio-dynamic response to the necessity of eliminating excessive accumulation of extracellular fluid brought about by primary sodium retention. Conversely, the hypotensive effect of the thiazide group of diuretics is thought to be due to elimination of sodium (30).

The mechanism by which sodium in excess provokes hypertension is also unknown, but in this respect the work of Tobian and Binion (215) is of interest. These workers demonstrated increased sodium and water content of the media and intima of the renal arteries of hypertensive human subjects, and suggested that the same phenomenon occurring in arterioles could cause decrease of lumenal size and an increase in the peripheral vascular resistance. Their results give no indication as to whether this extra sodium and water was intra- or extracellular. Some writers (94) have assumed the distribution to be essentially
intracellular, but this is not necessarily so.

In acute glomerulonephritis there is no doubt about the existence of abnormal sodium retention, and it has been shown here that it occurs hypertonically with respect to water. Why should so much sodium be retained in this way? One must immediately consider the nature of the renal lesion present. As has been substantiated in an extensive literature, the lesions are largely glomerular. The fact that the major functional abnormality is diminution of the glomerular filtration rate would appear to confirm the importance of this lesion. Tubular abnormalities are indeed present (225), and some impairment of tubular function is also on occasions demonstrable (51), but the two findings correlate poorly (159). Marked reduction of the glomerular filtrate must obviously impair the effectiveness of Henle's loop as a counter current multiplier. Consequently the medullary interstitial fluid is less hypertonic than normal, and following adequate sodium reabsorption the response to A.D.H. may well be inadequate (138).

Through all this it will have been clear to the reader that while information regarding many facts is
wanting, the persistent silence until now concerning one particular factor is becoming embarrassing. This factor is of course the possible influence of sodium-retaining steroids, of which aldosterone is the most powerful currently known. This important hormone, once thought only to act on the renal tubules causing sodium retention and secondary potassium excretion, is now known to have a number of other actions. It causes increased sodium transport in gut (121), and favours contraction of involuntary muscle in the small intestine (209) and in the heart (185). There is conflicting evidence concerning its action on the individual cell membrane; Helmer (94) makes it clear that it causes increased passage of sodium into, and potassium out of, cells. The work of Rapp (172, 173) on experimental adrenal regeneration hypertension, would appear to confirm this. However Conn and his group (183) recently showed that in bone the amount of sodium, which in this site is largely intracellular, was decreased by aldosterone, and Spach and Streeten (202) have demonstrated that sodium exchange in canine erythrocytes is retarded in vitro by the steroid. The results of Woodbury and Koch are of particular
interest. These investigators showed in mice given aldosterone for short periods that the total extracellular sodium of muscle was increased as was to a mild degree the intracellular potassium, while the intracellular water was slightly reduced. (236). This pattern could well be similar to that present in AGN.

It is curious that assessment of aldosterone secretion in AGN does not appear to have been carried out. The technical difficulties are of course formidable, as suggested by the variable results obtained by Cope and Pearson (31) in a study of cases suffering from renal failure due to various causes.

If angiotensin is the trophic substance for aldosterone, as now seems almost certain, the observation by Helmer (94) that renin activity is not increased in the disease may well be of significance.

Furthermore, the circumstances which lead to increased secretion of aldosterone are now well documented. The most important appear to be diminished blood volume, acting through the secretion of renin from the juxtamedullary apparatus, and excessive loss of sodium. Neither of these can apply to AGN, where the opposite circumstances are present. For these
reasons it seems to the author that hyperaldosteronism is not likely to be present in this disease. Lastly, excess aldosterone is by no means an essential ingredient in the pathogenesis of hypertension. In nephrosis, associated secondarily with the production of large amounts of aldosterone, hypertension is generally absent, and even in primary aldosteronism it is by no means a constant finding (210).

What relevance does the possible status of aldosterone have to the body water distribution occurring in AGN? If hyperaldosteronism should be present, then for the reasons stated it is at present difficult to forecast what ionic shifts could be occurring at cellular level. If on the other hand this situation is not present, there is no obvious reason why ionic shifts should take place. We are then left with the situation of intracellular dehydration demonstrated in this work. Is it possible that this could have a bearing on the genesis of hypertension, perhaps by such a mechanism as the induction of smooth muscle spasm? The less frequent occurrence of hypertension in acute tubular necrosis (213) in which intracellular dehydration is probably
not present, comes immediately to mind. It is also pertinent to inquire as to the effect of generalised intracellular dehydration on renal tubular function. Some speculative comments on this theme will be found in the Epilogue, but before closing this Chapter there are two further points worthy of mention.

Oedema and Age.

It will be recalled that in Chapter Seven it was shown that oedema in AGN occurs significantly more frequently in older children. A curious thing indeed! Is this because older children tend to have the disease in a more severe form? The results of the same study did not bring out this point. Or is oedema more easily recognised in older children? Again, an unlikely possibility. Or could it be due to the increasing length of Henle’s loop with growth making for more complete reabsorption of sodium? The matter gives food for further thought but will not be pursued at greater length here.

Therapeutic Implications

Acute glomerulonephritis is in the main a self-limiting disease. It has often been considered(22)
that the prognosis bears a relationship to the
duration of the pre-diuretic phase, and the earlier
onset of diuresis in childhood cases may be relevant
in this respect. Logically, treatment should primarily
be aimed at promoting early diuresis, the biggest
problem being the total ignorance of what really brings
this about. The main standby of treatment in the
acute phase, as all authorities are presently agreed,
is rigid salt restriction, and the present results
strongly support this viewpoint. They would indeed
suggest that the appropriate treatment for the
condition is removal of large quantities of sodium
thereby rendering the extracellular fluid more
hypotonic and encouraging cellular rehydration. The
alimentary tract provides the obvious route for removal
of salt, but ion-exchange resins available at present
are relatively inefficient and liable to introduce
additional electrolyte disturbances. In the small
proportion of cases which progress rapidly to complete
anuria and in whom the mortality is at present almost
100% (89, 22), cellular hydration could conceivably be
accomplished by the technique of peritoneal dialysis
using a solution with a hypotonic sodium and chloride content.

Since patients presenting with 'heart-failure' nearly all respond to conservative measures, one can hardly advocate more heroic treatment. The use of cardiac glycosides is time-honoured, but since the heart is not abnormal it is difficult to rationalize their use. The author has personally never been convinced that clinical improvement could be ascribed to these drugs and feels that this treatment is inappropriate. The even older measure of venesection provides a more logical approach by which to tide the patient over the acute phase.
SPECULATIVE EPILOGUE

Intracellular dehydration - an important 'missing link' or a chance finding of scant interest?

From this study of acute glomerulonephritis, let me pick out five features. Four of them are known from the work of others or have been demonstrated here, and the fifth is speculative. They are:

1. Diminished glomerular filtration.
2. Excessive sodium reabsorption.
3. Intracellular dehydration.
4. Absent or minimal increase of ionic shifts across cell membranes.
5. Normal secretion of aldosterone and by inference, of renin.

It appears to the author that AGN is probably not the only condition to exhibit all or most of these particular features. The most obvious parallel to be drawn may well be that of toxaemia of pregnancy. In this disorder diminished GFR has been demonstrated (5 ), and the existence of typical glomerular lesions (128) is now well recognised. Intracellular dehydration was shown by the studies of Plentl and Gray (164) to be present although results in this context are not unanimous (129).
Finally there is strong evidence that hyperaldosteronism (9) and increased secretion of renin are not implicated (24) and the former may indeed be present in smaller amounts than in normal pregnancy.

Oedema and hypertension have a strong association with both toxaemia and acute glomerulonephritis. Is a possible connection somewhere to be found within this pattern?

When we come to consider the enormous subject represented by essential hypertension we are faced with a problem of peculiar complexity. In a vast literature there is conflicting evidence over changes in body water distribution, but on the whole the evidence suggests changes to be minimal or absent. The theory that in primary sodium-retaining states the extracellular volume is maintained at normal levels only at the expense of a degree of systemic hypertension (18) only underlines, if true, the problems of obtaining proof from studies on subjects with established hypertension. In AGN, we have seen that we are presented with an experimental model of acute changes occurring within a very short space of time and to such a gross degree that the alterations have been readily detectable. This
contrasts keenly with the picture of extremely gradual change occurring in essential hypertension. Further, the consensus of opinion at present favours the view that hyperaldosteronism is absent (43). It is suggested that the occurrences of variables such as the gradual onset of congestive failure has hitherto clouded this problem (43). The association between increased tissue sodium and hypertension is well recognised, and it has been suggested that intracellular ionic alterations could result in sustained contraction of the smooth muscle cells of arterioles (72). Is it possible that ionic shifts are in fact minimal, and that a degree of intracellular dehydration in the absence of hyperaldosteronism could be the primary factor? This would only be likely to occur if the retention of sodium postulated to occur (18) was hypertonic rather than isotonic, but the extremely gradual nature of the changes provides a barrier to effective study.

There is a fascinating rider to this hypothesis. It has long been known that experimental animals develop hypertension on a salt-loaded diet. In the rat, this has been shown by Meneely et al. (137) to result in an
increase of total exchangeable sodium and, most interestingly in a slight rise of exchangeable potassium. These investigators found no evidence of a rise of intracellular sodium. An increase in the amount of intracellular potassium would tend to increase the volume of intracellular water. Is it possible that one of the primary mammalian homeostatic mechanisms is a defence of intracellular hydration?

One further flight of fancy remains to be aired. It is well known that the extracellular concentrations of electrolytes present in the baby at birth are identical to that of the mother. For example, maternal hyponatraemia has been shown to be associated with the electrolyte abnormality in the baby and a distinctive clinical picture (4). Should hypertonic sodium retention in the mother prove to be a factor in the genesis of toxaemia, it would follow that the foetus would be affected in the same way. This could suggest a causative factor in the high incidence of oedema occurring in infants born to eclamptics, a feature so far unexplained. It should be stated that the small number of experimental results available do not suggest the presence of a pattern of body water distribution.
consistent with such an idea (28). At the same time the range of normal values used for comparison has tended to be so wide that the matter must be considered at present to be sub index.

It is a far cry from the transient acute dyspnoea of our patient A.G. to speculation concerning the ultimate nature of a number of important diseases. "The study of patients and their symptoms" wrote Hippocrates, "is the essential basis of all medical knowledge."
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