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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C O N T E N T S

Page

SECTION TWO - THE PROBLEM

CHAPTER SIX — Acute glomerulonephritis: A short review of existing theories concerning oedema. — 135

CHAPTER SEVEN — Clarifying the problem: A study of the case records from 170 children with acute glomerulonephritis. — 147

CHAPTER EIGHT — Body water distribution in acute glomerulonephritis. — 168

CHAPTER NINE — The diuresis of acute glomerulonephritis — 195

CHAPTER TEN — Other aspects: I. Electrophoretic patterns. — 234

CHAPTER ELEVEN — Other aspects: II. The chest X-ray picture and changes in the radiological heart volume. — 249

CHAPTER TWELVE — The synthesis. — 264

SPECULATIVE EPILOGUE — 283

REFERENCES — 288

ii

ABBREVIATIONS

Where applicable, units of measurement used are bracketted.

AGN	Acute glomerulonephritis.
BV	Total blood volume (ml.)
B.Wt.	Body weight (kg.)
CV.	Total red cell mass (ml.)
	Delta = Change of.
ECW	Extracellular water volume (litres).
ICW	Intracellular water volume (litres).
ISW	Interstitial water volume (litres).
K_e	Total exchangeable potassium (meq.)
Na_e	Total exchangeable sodium (meq.)
Na_{ec}	Total extracellular sodium. (meq.)
Na_{is}	Total interstitial sodium (meq.)
(Na_{is})	Concentration of sodium in interstitial water (meq./l)
Na_s	Serum or plasma sodium concentration (meq./l.)
PV	Plasma volume (ml.)
PW	Plasma water volume (ml.)

S.D. Standard deviation $= \sqrt{\dfrac{\sum(x - \bar{x})^2}{n - 1}}$

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

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

TBW Total body water (litres).

PROLOGUE

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

FIG. 1.1

(a)
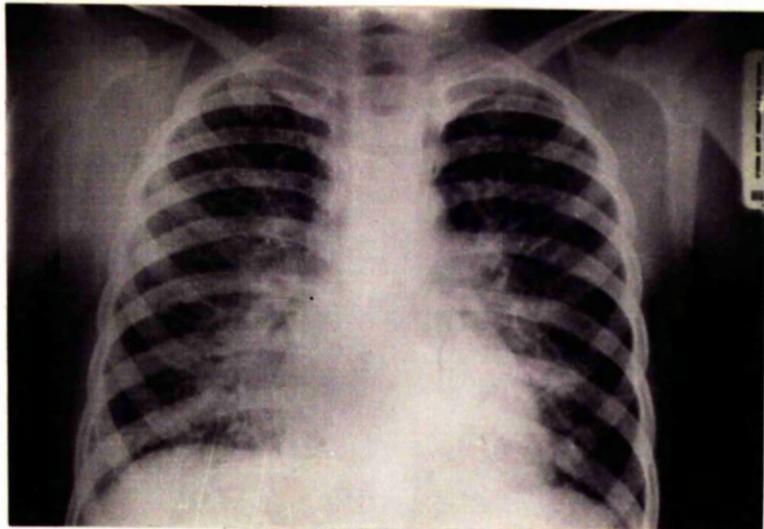
(b)
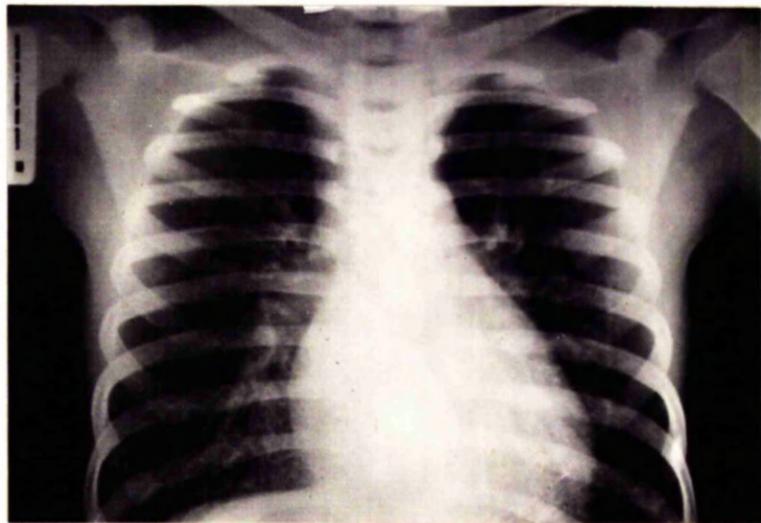
(c)
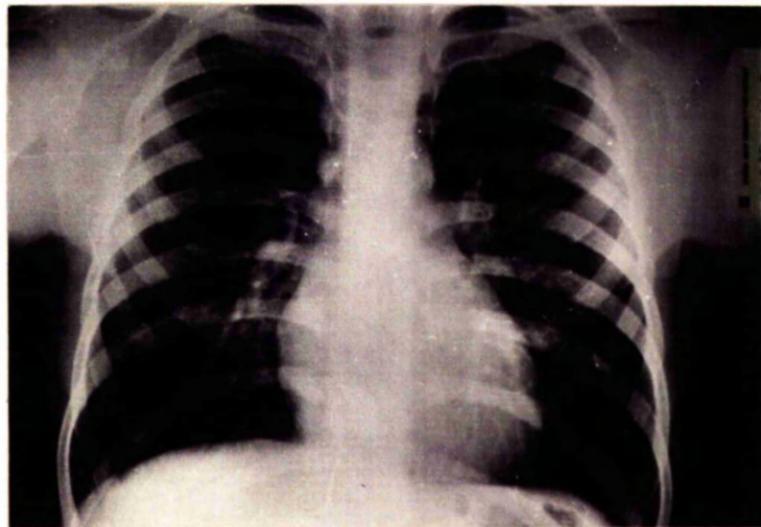
Patient A.G. See text
for details

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

"Kriegsoedeme" 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.   These 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 Bezold 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.

and 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 fall 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 one patient using

exceedingly small amounts of $_{24}Na$. (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-amino 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^I (77), and red cells labelled with either
92^P (7) or 51^{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 interstititial 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 $_{131}$I-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 $_{131}$I-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-1824 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 Wintrobe 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 venopuncture. 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, always 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.

Indicator	Required plasma concn. mg/100ml.	Dose mg/kg.	Solution available
T-1824	0.4	0.2	Aqueous 0.1%(G.T.Gurr)
Thiocyanate	7	15	Aqueous 6% (T & H Smith)
Antipyrine	5	25	Aqueous 20% (B.D.H.)

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 $\pm$ 1% giving an error in practice of $\pm$ 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 ($\pm$ S.D.): 0.290 ($\pm$ 0.003) Standard (triplicated): 6 ml. pipetted into same flask: 0.301

$\therefore$ Actual dose administered $= 6 \times \dfrac{0.290}{0.301}$ mg. $= 5.79$ mg.

The full table of corrected doses used in the series
is set out below:

TABLE 2.2

Syringe calibrations (all quantities in mg.)

	T-1824				
Syringe dose	6	4	3	2	1
Corrected	5.79	3.95	2.86	1.97	0.98
	Sodium thiocyanate				
Syringe dose	720	480	360	240	120
Corrected	729	482	358	244	122
	Antipyrine				
Syringe dose	1200	800	600	400	200
Corrected	1190	798	621	408	197

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.

2. Post-injection, 10 mins.: 5 ml., for
estimation of T-1824.

3. Post-injection, 2 hours: 2 ml., for
estimation of thiocyanate and antipyrine.

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 Bedwell 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 $50^{o}C$.

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 $52^{o}C$.   When three such columns are used
simultaneously, as in Figure 2.1, the temperature of the

FIG. 2.1.   34
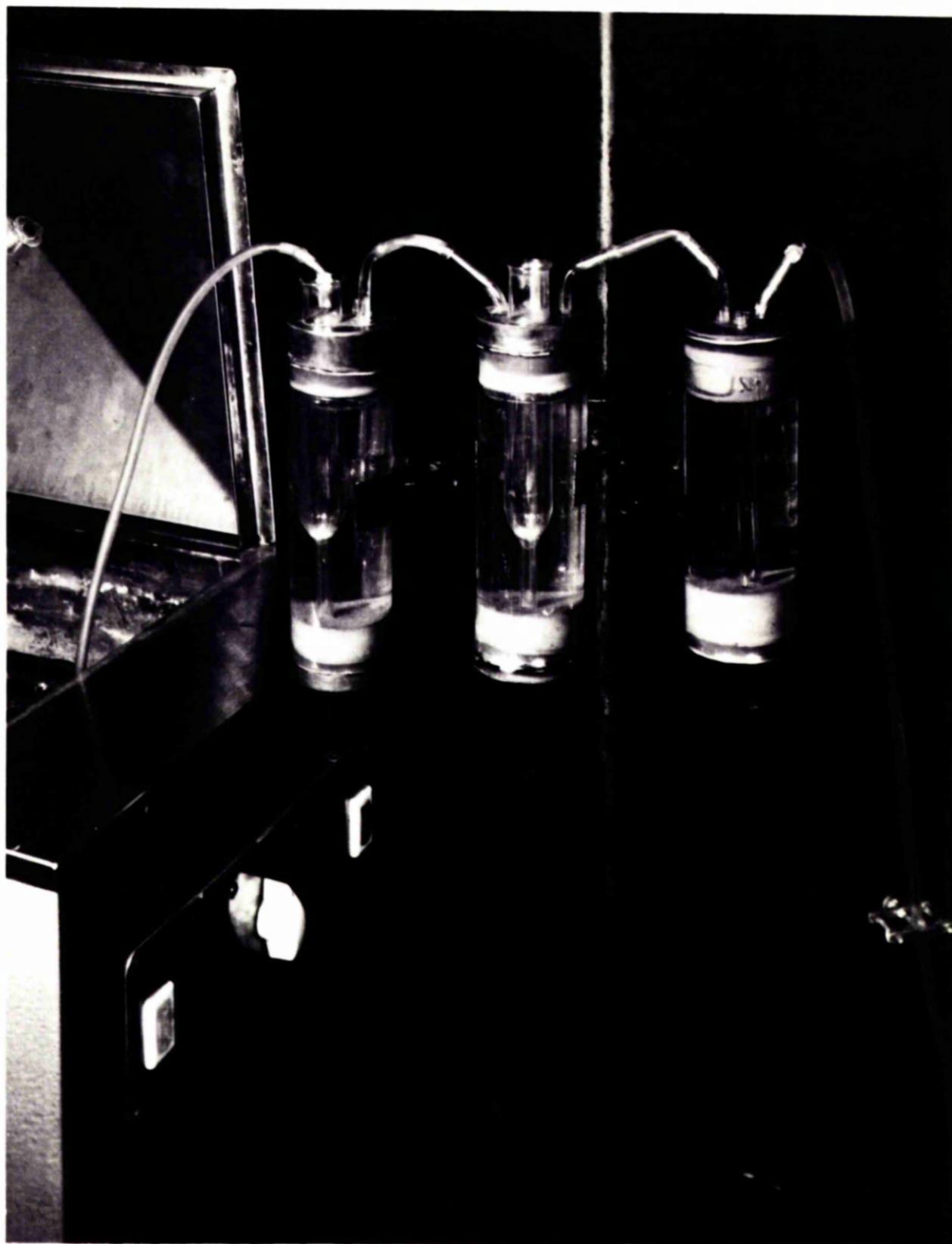
Columns for extracting T-1824 from plasma. The small-bore column for semi-micro quantities is on the right. Water at 50°C syphons from the water-bath (left) through the jackets surrounding each column

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.3E) 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.

6.   Celite.   Celite 545, powdered filter medium.

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 95% 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 ¼" 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 0.25 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 13,000 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

$\therefore$ 1 ml. Plasma $= \dfrac{R}{V} = T$

30 μgm. standard (colorimeter) = S

∴ concentration in 1 ml. plasma $\frac{T}{S}$ X 30 = C

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

Total body haematocrit = (Observed het.) x 0.91

= H

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

Blood volume = (P + Co) 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
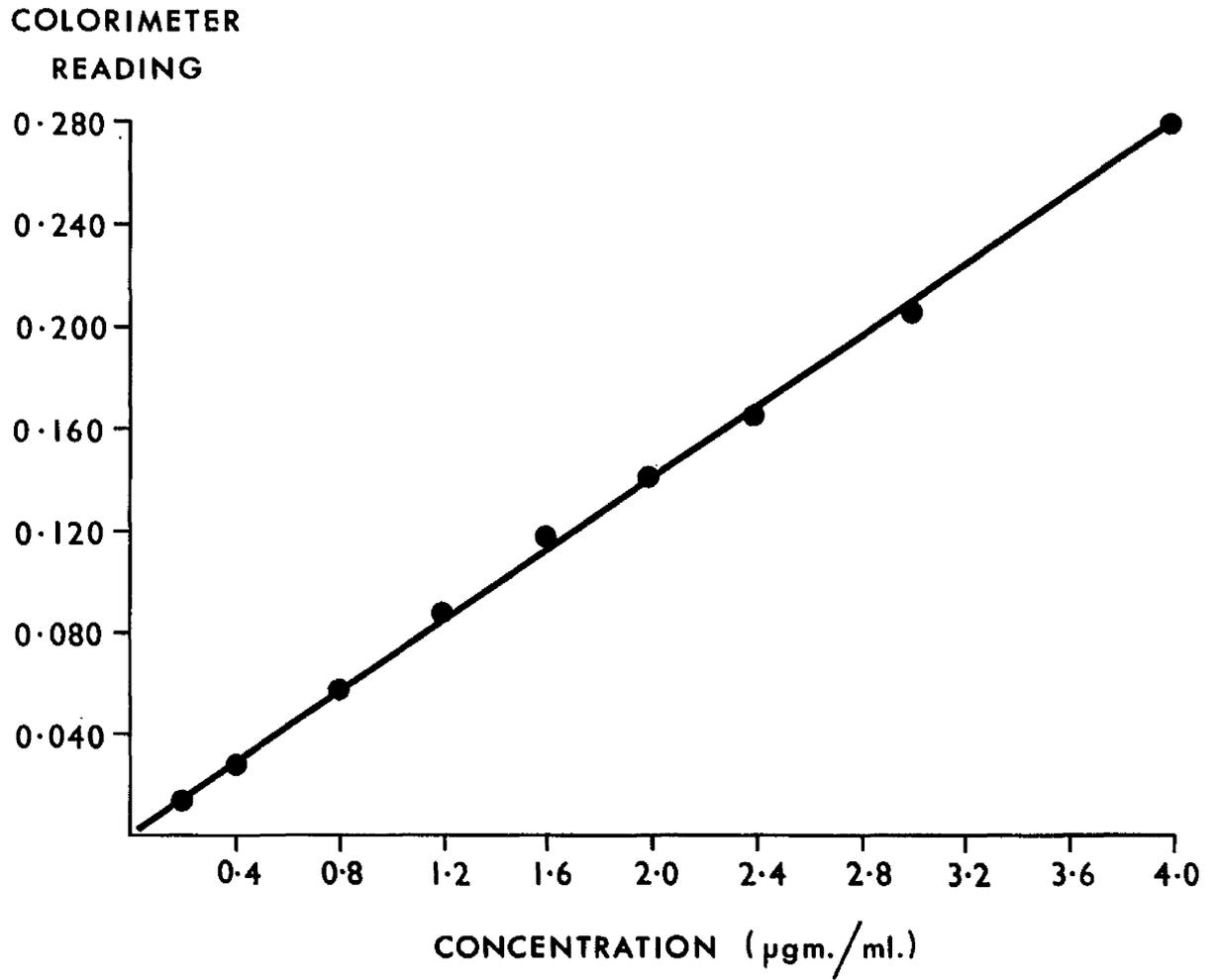
Test: Mean of 10 such estimations

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

This represents a mean recovery of 99.3% $\pm$ (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%.

3.   Recoveries – semi-micro method.

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% $\pm$ (S.D.) 2.0%.

4. <u>Coexistence of T-1824 with sodium thiocyanate and antipyrine in plasma.</u>

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

Doses administered:

T-1824	3 ml.	=	3 mg.
Sod. thiocyanate	1 ml.	=	60 mg.
Antipyrine	0.4 ml.	=	80 mg.

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

<u>Results:</u>   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).

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

5. <u>Evaporation of 35% acetone at 50°C</u>

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 $Fe(NO_3)_3$. $9H_2O$ 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

$\therefore$ Plasma concentration $\frac{(P-B)}{S} \times 3 = C$ mg./100 ml.

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.%.

$$\text{Thiocyanate space} = \frac{D}{C_w \times 10} \text{ litres.}$$

Control Experiments

1. **Linearity**

   Experiment 8. Within wide limits (0-20 mg./100 ml.) the colour produced from thiocyanate by this method obeyed the do Beer-Lambert Law.

2. **Recoveries.**

   Experiment 9. 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: (Uvispek readings.)

Standards: Mean of three = 0.1603

Test: Mean of twelve estimations:

= 0.1698 ± (S.D.) 0.0032.

Plasma blank (duplicated) = 0.012

Test minus plasma blank = 0.1578.

This represents a mean recovery rate of 98.4% ± (S.D.) 1.9%.

The total error of estimating thiocyanate space in this way is thus approximately ± 5%

3.   Presence of citrate in samples - 1.

Experiment 9.   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.

4.   Presence of citrate in samples - 2.

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 antipyrine 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 $(3CdSO_4.8H_2O)$ 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.

49

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 $80^{\circ}C$ 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:
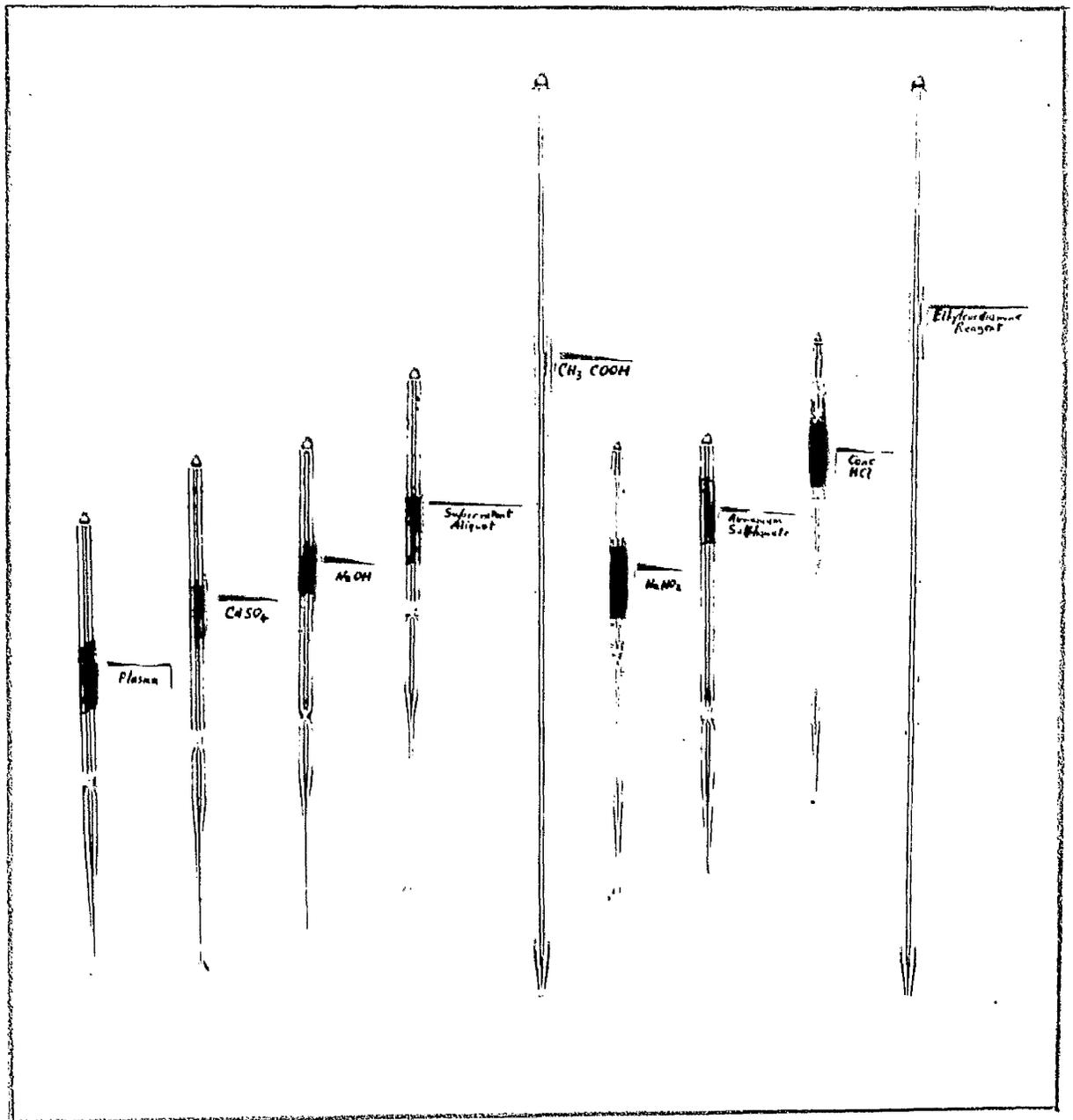
Set of micro-pipettes used
throughout for estimation
of antipyrine

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.(concn.)   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.

$\therefore$ plasma concentration $C_p = \dfrac{T}{S}$ x 4mg./100 ml.

Plasma water concentration $C_w = C_p$ x $\dfrac{100}{100-Pr}$

Antipyrine space $= \dfrac{100}{C_w \times 10}$ litres

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

Lean body mass = Antipyrine space x $\dfrac{100}{73}$ kg.

Control Experiments.

1.   Linearity

Experiment 11.   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

$$= 0.417 \pm (S.D.) \ 0.005$$

This represents a mean recovery of 100% $\pm$ (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.

i.e. where C = plasma concentration

T = time after injection

then $\text{Log } C \propto T$

or $\text{Log } C = \kappa.T$,

where κ 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] I-labelled I.H.S.A. (82 , 71). The disappearance rate in newborn

FIG.2.4.

T-1824 ELIMINATION
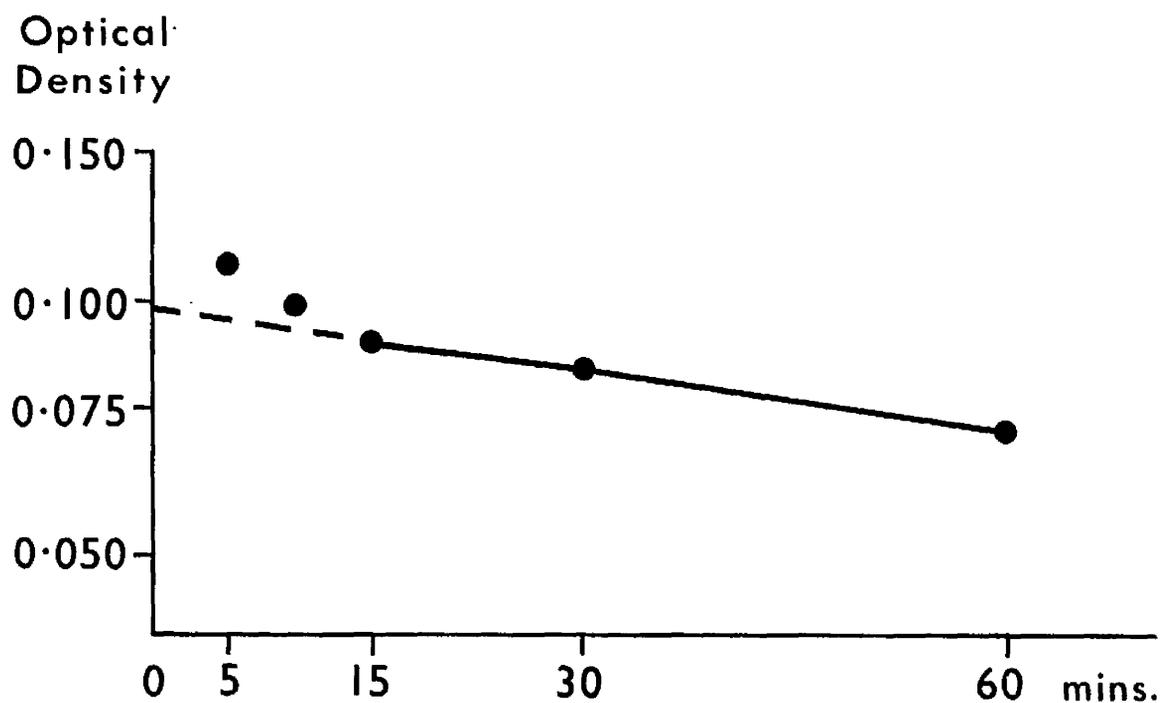
A typical plasma elimination
curve for T-1824, becoming
exponential after 15 mins

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

FIG.2.5.

ACUTE NEPHRITIS
THIOCYANATE ELIMINATION
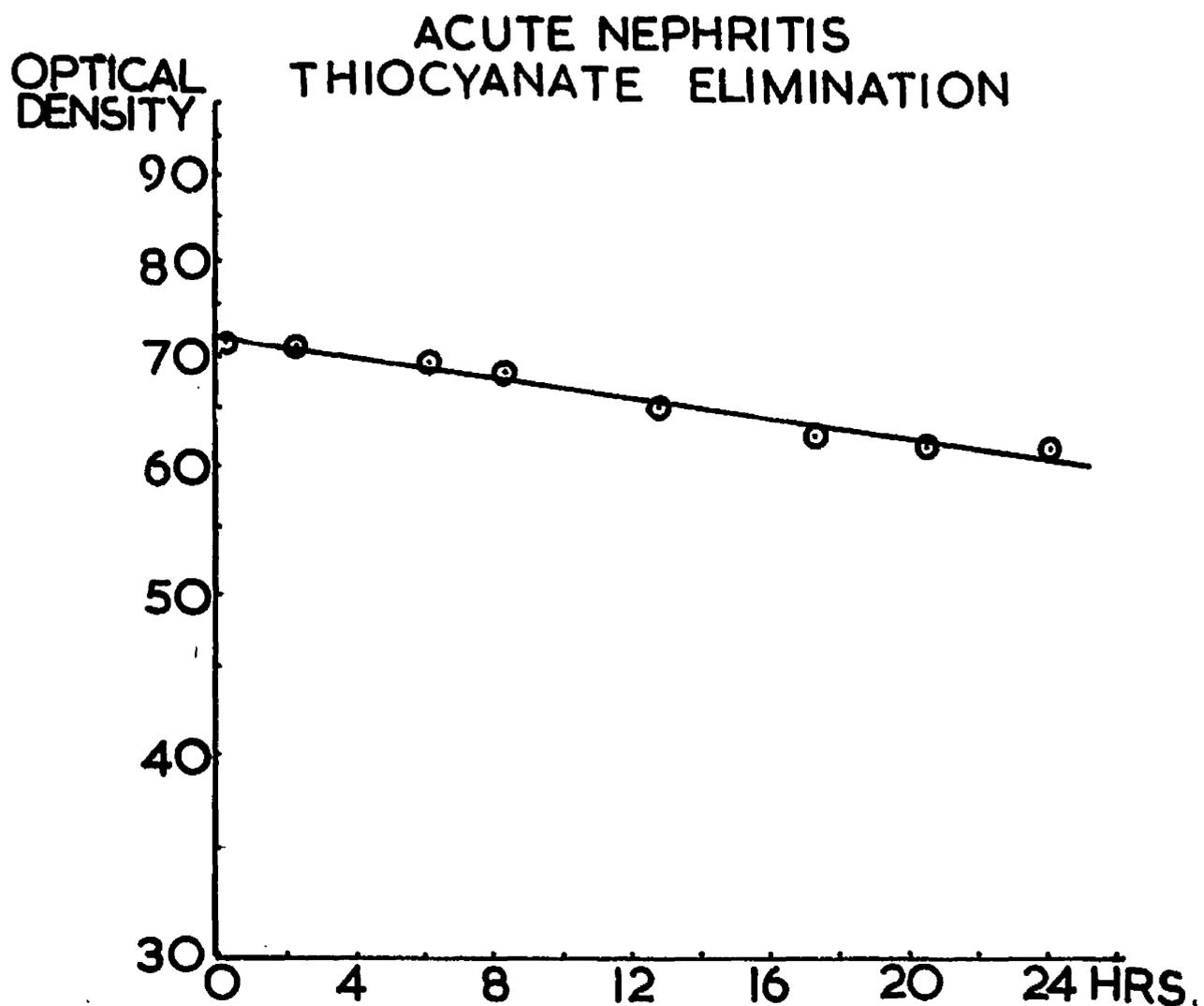
Elimination of thiocyanate
from an oedematous subject
with AGN

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

FIG 2.6.

58

ACUTE NEPHRITIS
ANTI-PYRINE ELIMINATION

CASE J.B.
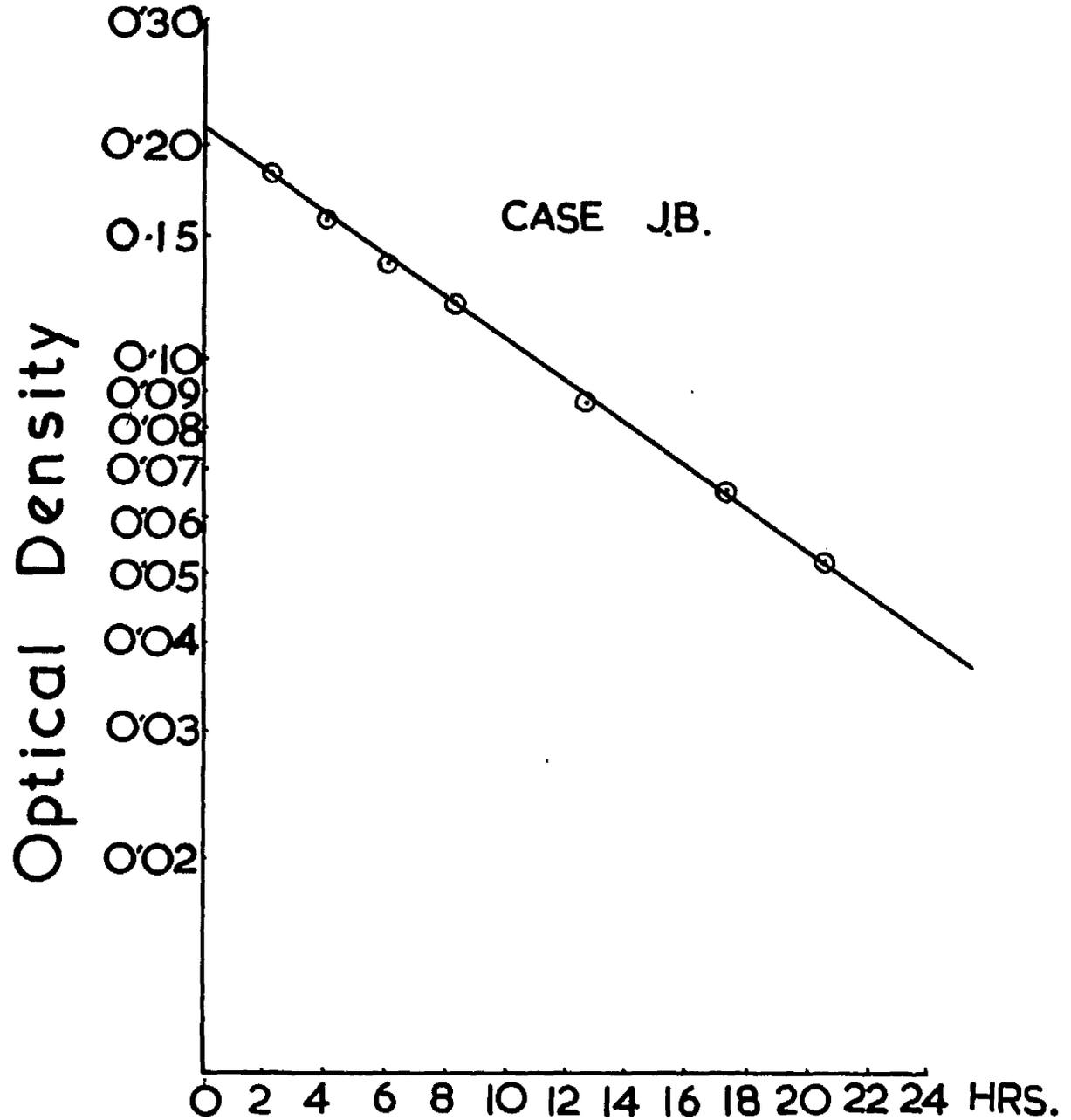
Exponential elimination over
24 hrs. of antipyrine from
oedematous subject with AGN

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:

Plasma water = T-1824 space x 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. = Thiocyanate space - 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

I. THE AGE GROUP $1\frac{1}{2}$ - 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 x $\frac{100}{73}$, 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 bdoy 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

Subject No.	Age Years	Weight kg.	T.B.W. litres	E.C.W. litres	P.V. ml	B.V. ml
1	11.34	33.0	20.90	8.06	1203	1965
2	7.84	23.6	14.54	6.35	1292	2010
3	8.97	20.4	12.33	5.12	933	1377
4	2.03	10.5	5.96	2.79	485	692
5	1.50	11.2	6.30	3.49	498	764
6	2.56	13.6	7.19	3.16	688	1044
7	11.97	23.8	17.36	6.46	1074	1739
8	2.08	11.5	9.06	3.64	425	648
9	3.58	11.2	5.92	3.34	507	745
10	7.97	26.6	13.80	5.89	1136	1817
11	6.55	22.6	14.76	5.54	1019	1623
12	10.68	26.7	13.70	6.29	1202	2063
13	11.97	45.7	23.90	11.35	2250	3490
14	11.29	36.5	17.43	9.36	1750	2640
15	7.92	27.2	18.64	8.21	1280	1912
16	7.41	25.6	16.60	7.89	1430	2111
17	12.17	39.5	22.67	9.82	1693	2474
18	11.04	25.6	17.58	7.67	1735	2750
19	5.75	16.5	9.35	4.73	796	1180
20	8.50	20.1	11.37	5.59	1060	1586
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

FIG.3.1.

Body Water Distribution Average of 20 Normal Children 2-11 Years
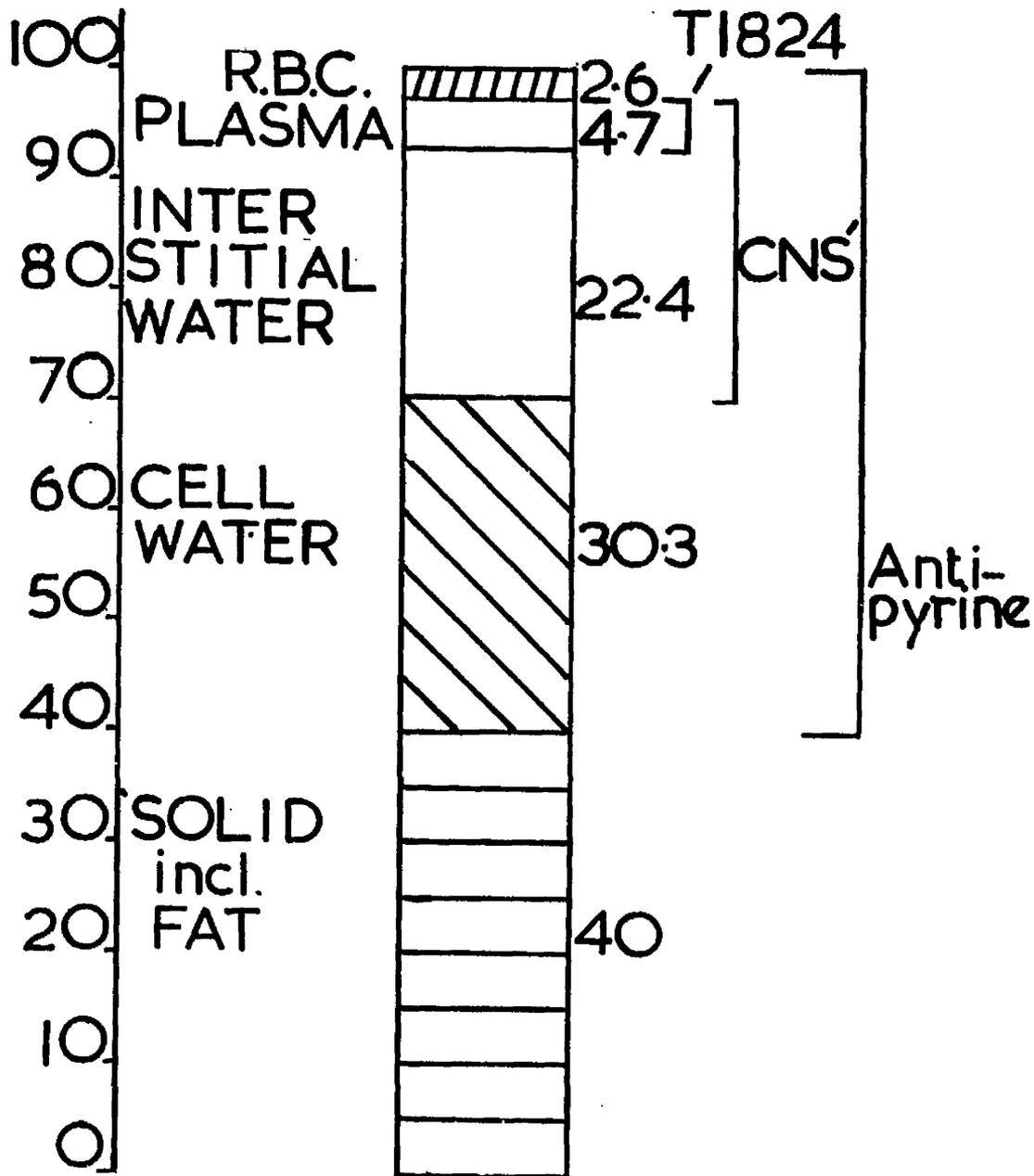
Block diagram representing mean percentage values for body water and solids. The volumes of distribution of the three indicators utilised are shown

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 those equations as
a general guide.

FIG.3.2.

68
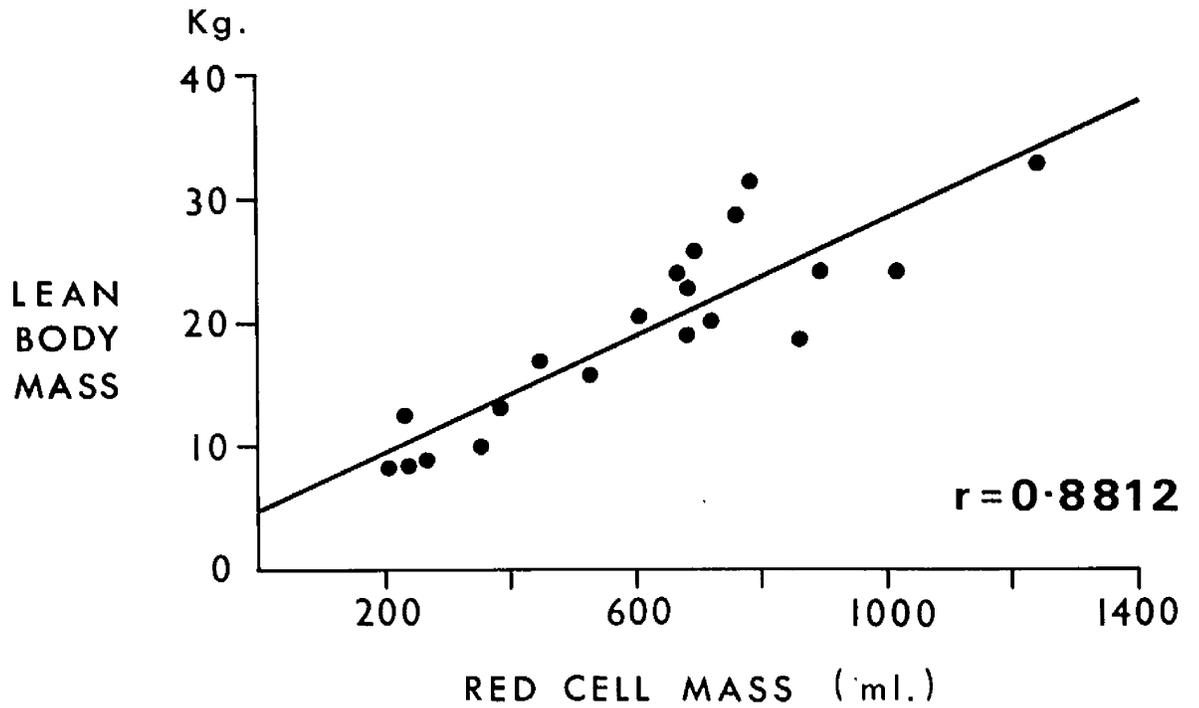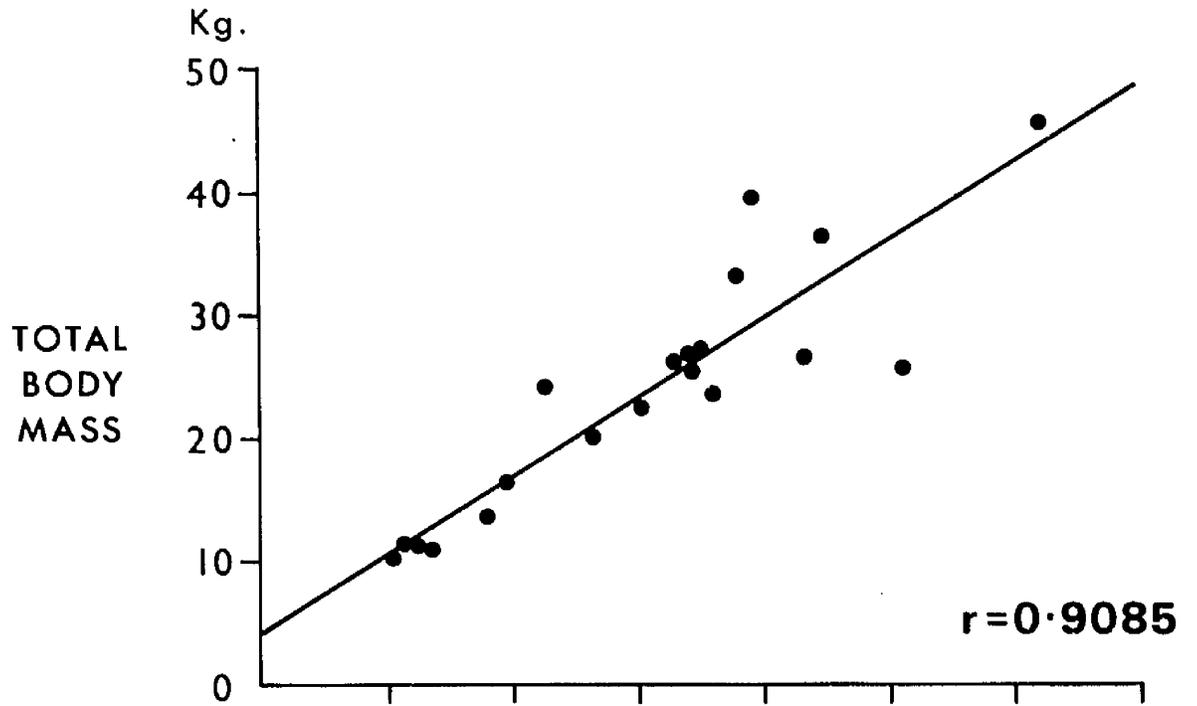
20 NORMAL CHILDREN

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 3.2

*y	*x	r	Regression equation	
I.C.W.	T.B.W.	0.9775	y = - 0.419	+ (0.583) x
E.C.W.	B.W.	0.9696	y = 0.654	+ (0.237) x
I.C.W.-C.V.	T.B.W.	0.9683	y = - 0.402	+ (0.538) x
I.S.W.	B.W.	0.9644	y = 0.637	+ (0.194) x
I.S.W.	T.B.W.	0.9599	y = 0.400	+ (0.344) x
E.C.W.	T.B.W.	0.9572	y = 0.419	+ (0.417) x
P.V.	E.C.W.	0.9516	y = - 87.29	+ (193.48)x
B.V.	E.C.W.	0.9444	y = - 139.9	+ (299.9) x
T.B.W.	B.W.	0.9421	y = 1.53	+ (0.528) x
P.V.	B.W.	0.9339	y = 27.37	+ (46.36) x
B.V.	B.W.	0.9335	y = 25.55	+ (7238) x
P.V.	I.S.W.	0.9274	y = - 72.43	+ (229.31)x
B.V.	I.S.W.	0.9198	y = - 115.8	+ (355.25)x
C.V.	B.W.	0.9085	y = - 1.61	+ (26.02) x
C.V.	E.C.W.	0.9072	y = - 52.4	+ (106.43)x

y	x	r	y	x	r
B.V.	T.B.W.	0.8944	I.C.W.-C.V.	I.S.W.	0.8646
P.V.	T.B.W.	0.8885	I.C.W.-C.V.	B.W.	0.8519
C.V.	T.B.W.	0.8813	C.V.	I.C.W.	0.8151
I.S.W.	I.C.W.	0.8809	B.V.	I.C.W.	0.8099
E.C.W.	I.C.W.	0.8745	P.V.	I.C.W.	0.7949
I.C.W.	B.W.	0.8714	C.V.	I.C.W.-C.V.	0.7829

* 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 B.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.

FIG.3.3.

20 NORMAL CHILDREN
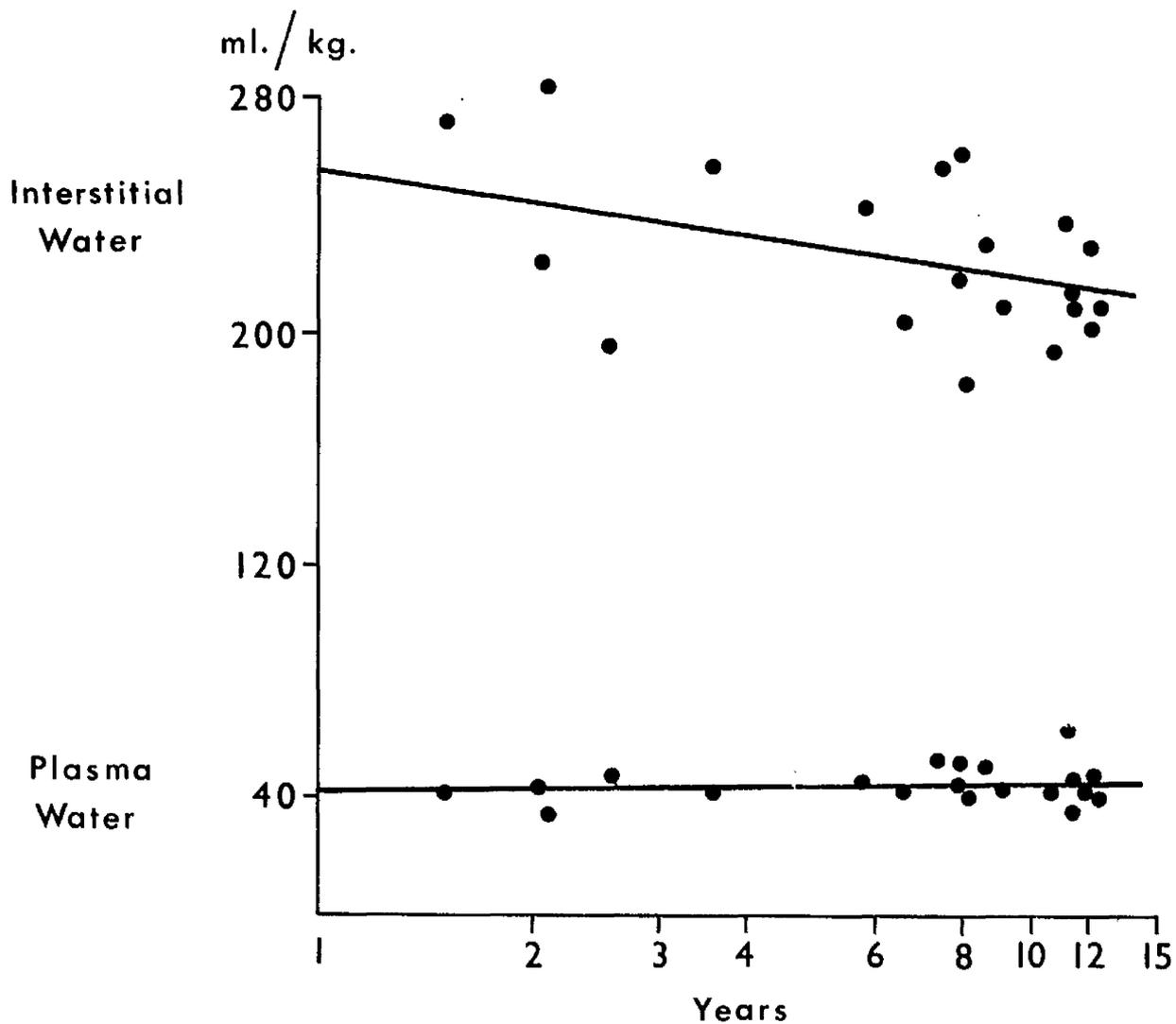
Log-linear regression of
interstitial water and
plasma water on age. The
slope of the upper
regression line is
significant.

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-Rathbun 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-Rathbun 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:

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

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

FIG. 3.4.

E.C. WATER
(Litres)

WEIGHT (Kg.)
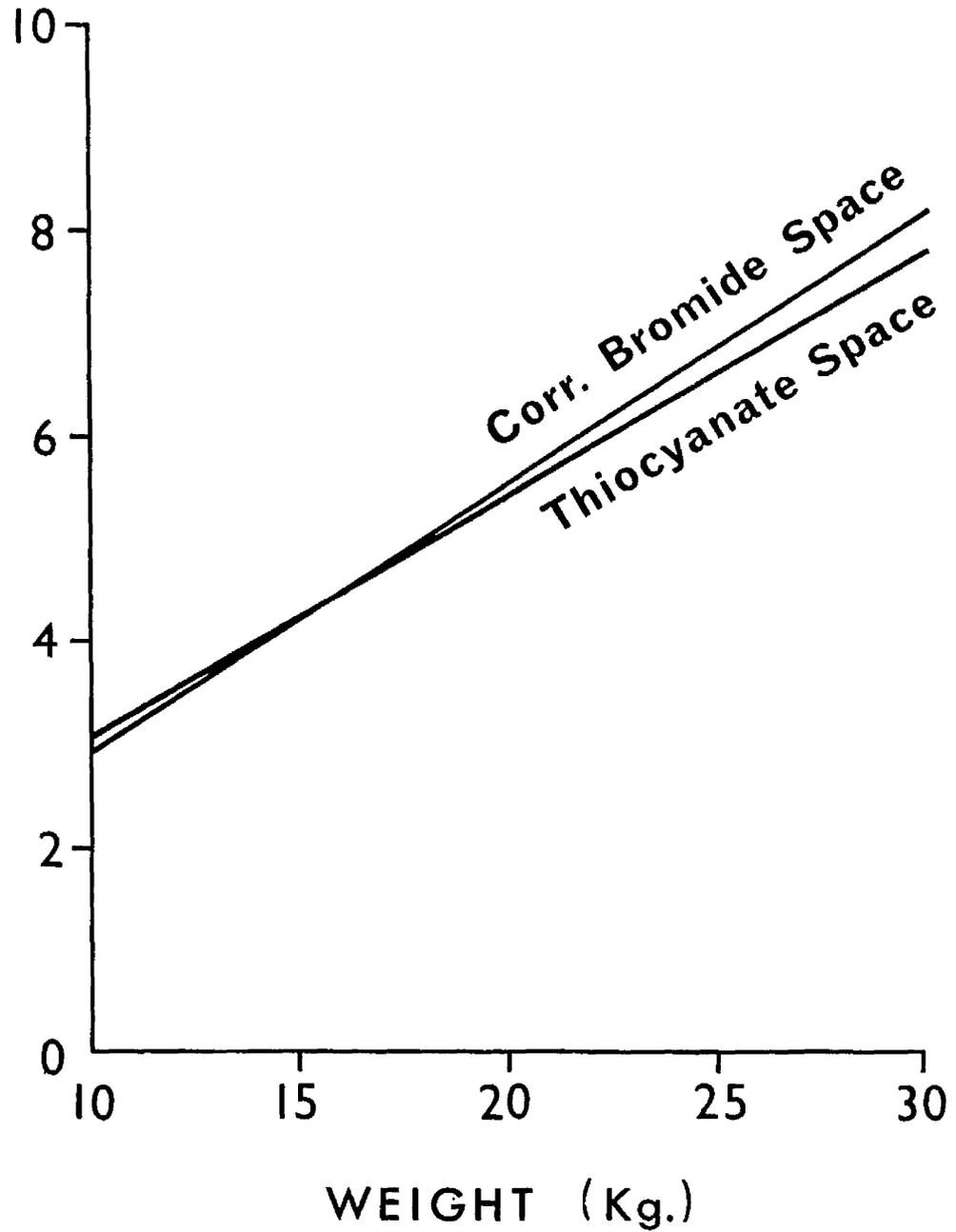
Regression lines illustrating
estimation of extracellular
water from the 'corrected'
bromide space - from results
of Cheek (26), and from the
thiocyanate space (present
series.)

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 30 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:

	Antipyrine	Thiocyanate	T-1824
Infants under 2.00 kg.	50 mg.	30 mg.	0.4 mg.
2.01 - 3.00 kg	60 mg.	40 mg.	0.5 mg.
over 3.00 kg.	80 mg.	60 mg.	0.7 mg.

A venous sample (5 ml.) was withdrawn 10 minutes post-injection for T-1824, haematocrit and, in 34 subjects, hawmoglobin 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 cynmethaemoglobin 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 4.1

TWIN DATA

Case No	Age	Weight (kg.)	T.B.W. (ml./kg.)	E.C.W. (ml./kg.)	I.C.W. (ml./kg.)	Plasma Vol (ml./kg.)	Total Body Hct %	Hb G%
1	10 hrs	2.?8	-	362	-	44.0	50.3	17.4
2	5 hr.	3.25	-	307	-	34.5	55.7	17.9
3	7 hr.	3.29	678	355	323	50.7	31.6	13.2
4	5 hr.	3.06	-	339	-	43.0	47.4	16.0
5	7 hr.	2.0?	753	347	406	32.6	59.6	20.1
6	8 hr.	3.23	629	347	282	41.0	44.4	15.9
7 +	5 hr.	2.00	702	299	403	34.8	49.9	-
8 +	4½ hr.	2.49	684	325	359	33.0	49.9	-
9	1 d	1.99	-	308	-	48.7	56.1	19.3
10	1 d	1.91	-	403	-	38.5	48.3	16.2
11	1 d	2.25	-	312	-	46.0	52.5	17.8
12	1 d	2.13	666	388	278	54.5	44.3	14.6
13	1 d	2.31	741	363	378	47.0	41.8	-
14	1 d	2.04	762	395	367	45.1	42.6	-
15	2 d	2.09	737	339	-	50.4	48.1	17.3
16	2 d	2.19	741	340	401	44.3	48.8	17.5
17	2 d	2.51	-	363	-	46.4	56.8	20.3
18	2 d	2.29	-	419	-	50.0	50.0	16.6
19	2 d	1.72	737	379	358	44.2	43.1	-
20	3 d	2.20	-	584	-	53.6	47.2	17.2
21	3 d	2.68	-	426	-	43.2	45.2	15.0
22 +	3 d	2.22	761	389	372	52.8	43.1	16.1
23	3 d	2.15	251	397	354	51.0	43.5	15.8
24	3 d	1.76	229	352	378	37.0	37.0	15.1
25	3 d	3.17	689	334	350	51.4	39.0	-
26	3 d	2.32	709	381	328	54.3	35.6	-
Mean ± S.D.		2.46	715. ± 10.3	359.8 ± 7.0	355.8 ± 10.1	45.1 ± 1.32	46.6 ± 1.31	16.8 ± 0.42

+ Denotes pairs of twins

TABLE 4.1. (contd.)

THE DATA

Case No	Age	Weight (kg.)	T.B.W. (ml./kg.)	E.C.W. (ml./kg.)	I.C.W. (ml./kg.)	Plasma Vol (ml./kg.)	Total Body Hct %	Hb G%
27	4 d	1.58	-	423	-	58.1	52.6	17.9
28	4 d	2.04	754	369	385	48.6	52.9	17.3
29	4 d	2.31	741	359	382	39.9	50.0	18.8
30	4 d	2.01	774	372	402	43.8	49.9	18.9
+31	4 d	1.62	749	357	392	55.0	45.2	-
32	4 d	1.46	644	376	268	46.6	50.5	-
33	5 d	2.14	-	359	-	51.6	46.5	16.6
34	5 d	2.01	-	348	-	57.9	41.8	15.2
35	5 d	2.20	814	341	473	46.3	44.8	16.2
36	5 d	2.17	693	348	345	44.2	53.7	18.9
37	5 d	1.97	642	322	320	41.4	49.1	17.5
38	5 d	2.07	617	314	303	46.2	47.4	17.4
39	5 d	2.45	761	371	390	51.5	48.0	-
Mean = S.E.		2.00	718.9 ÷ 20.8	358.4 ÷ 7.5	366.0 ÷ 18.5	48.5 ÷ 1.64	48.7 ÷ 0.97	17.5 ÷ 1.17
+40	6 d	1.87	-	381	-	55.6	51.8	18.2
+41	6 d	1.87	-	368	-	54.0	56.9	19.5
42	8 d	3.57	669	348	321	41.6	42.6	16.0
43	9 d	2.18	831	345	486	56.8	40.7	14.2
+44	12 d	2.05	606	372	234	61.0	42.6	15.1
45	12 d	2.75	714	401	313	43.3	47.4	17.4
46	13 d	1.99	640	298	342	53.3	48.0	-
Mean		2.33	692.0	359.0	339.2	52.2	47.1	16.7
Total Mean(±S.D)		2.31	712.5 ÷ 10.4	359.3 ÷ 4.8	356.4 ÷ 10.1	47.15 ÷ 1.03	47.3 ÷ 0.85	16.98 ÷ 0.28

+Denotes pairs of twins

FIG.4.1.

86

NEONATE SERIES

TOTAL BODY WATER. 30 Subjects
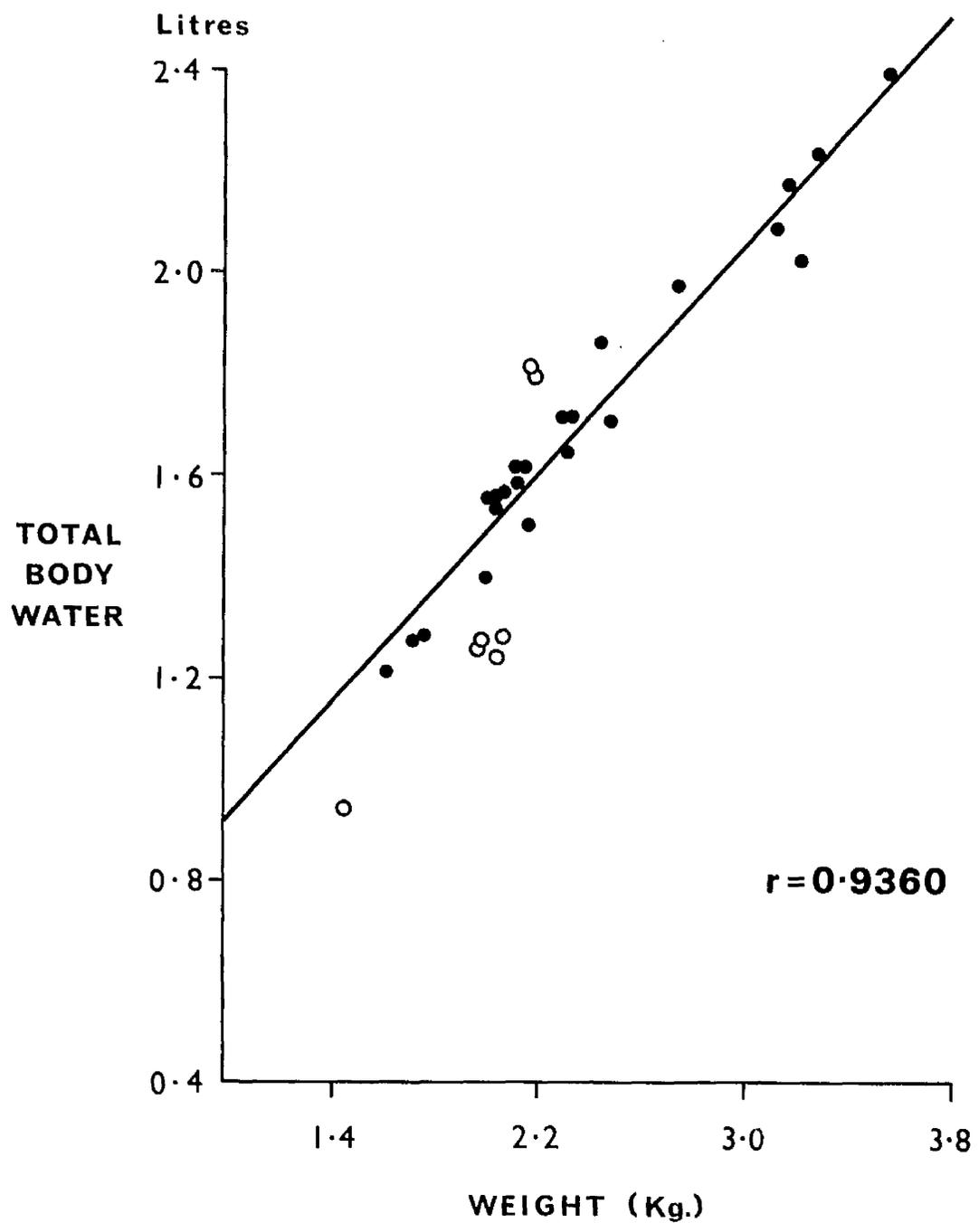
Open circles refer to seven
subjects with 'abnormal' total
body water (see text)

FIG.4.2.   87

NEONATE SERIES

INTRACELLULAR WATER. 30 Subjects
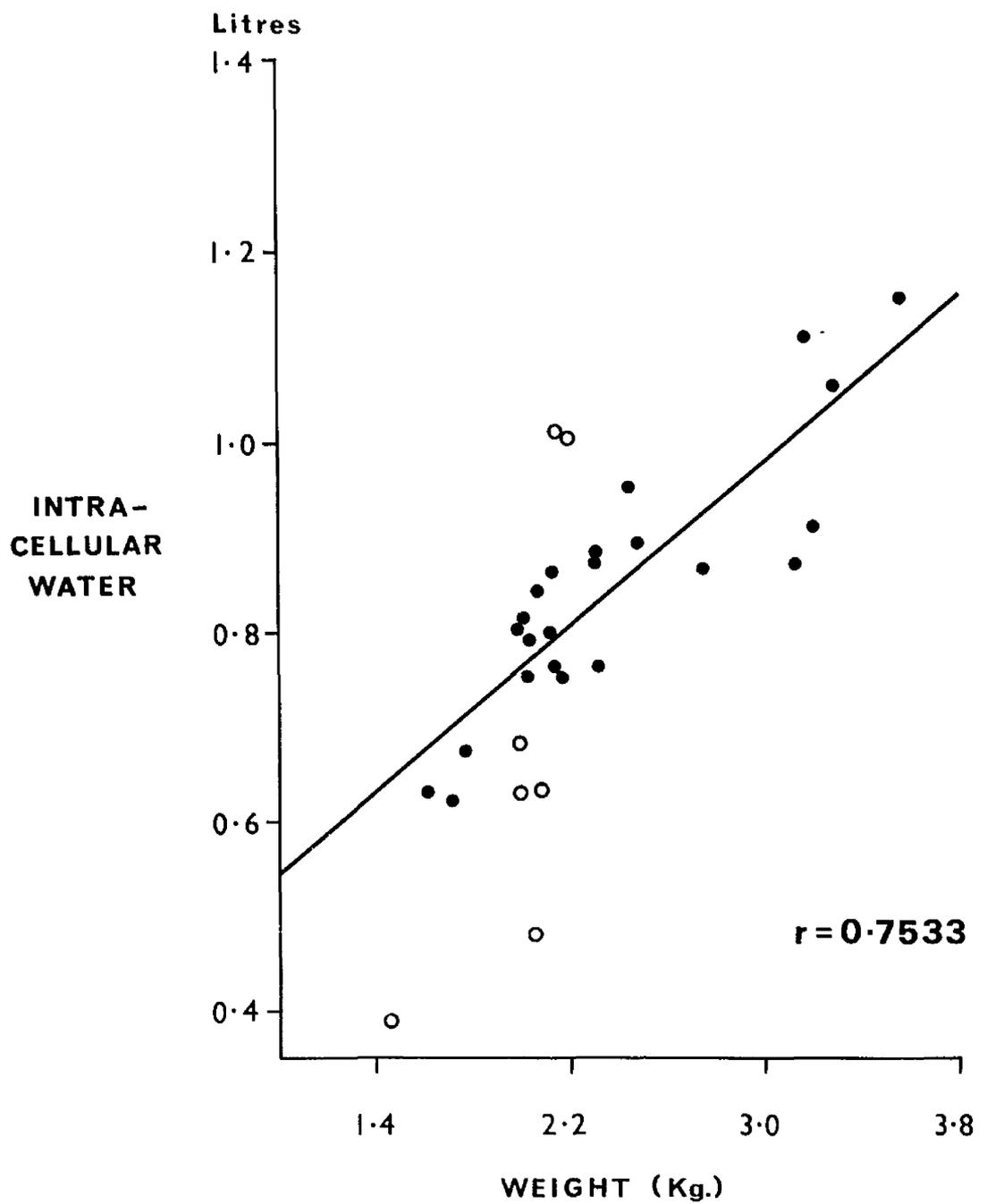
Open circles – as in
Figure 4.1.

FIG.4.3.   88

NEONATE SERIES

EXTRACELLULAR WATER.   46 Subjects

r=0·9127

EXTRA-
CELLULAR
WATER

Litres

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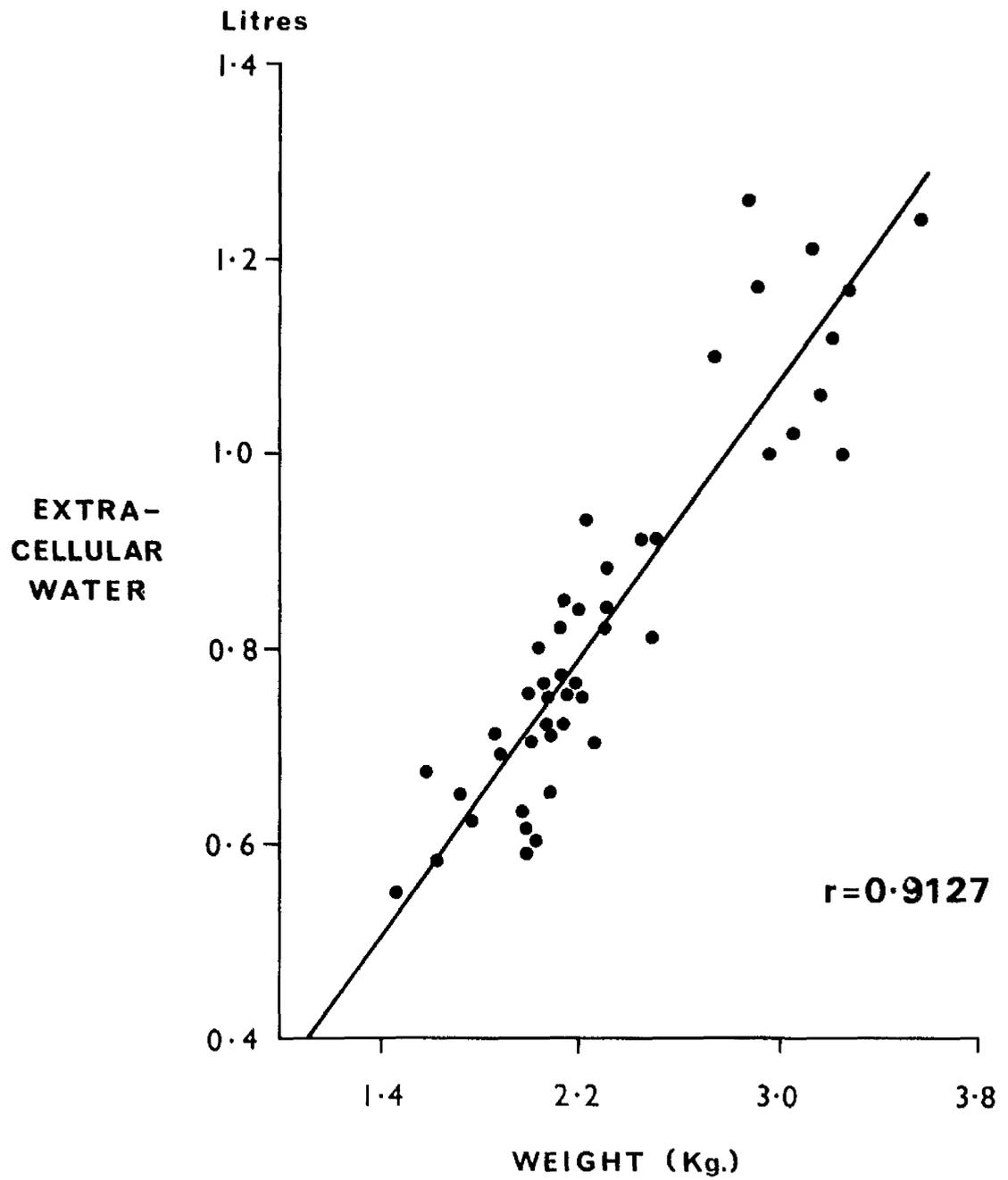
The infants have been divided into those of three days and less (closed circles) and those of more than three days old (crosses). It should be noted that the linear regression line with its 95% confidence limits refers only to the 15 infants aged three days and less. These are negatively correlated $(r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

FIG.4.4.
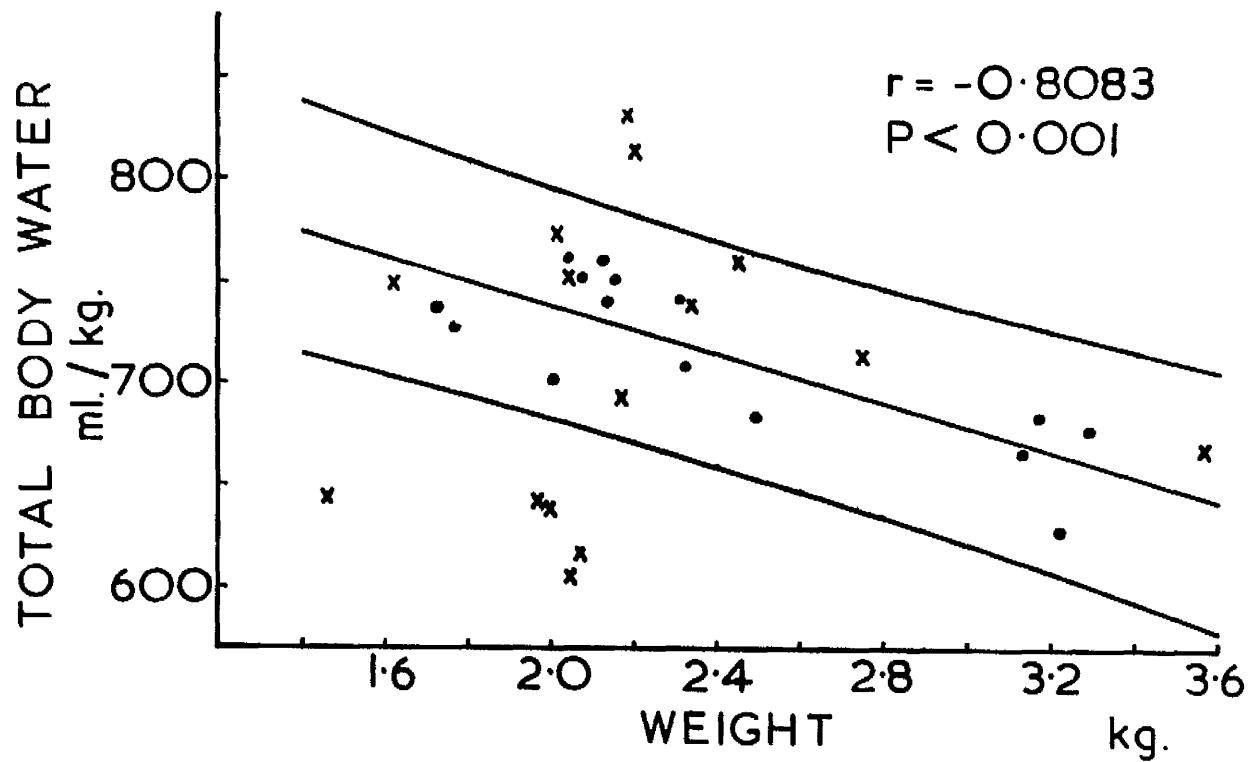
The regression line and its 95%
confidence limits refer only to
infants aged three days and
younger (closed circles.)
Crosses indicate older subjects.
Note the seven 'abnormal' values
are all from infants weighing
less than 2.2 kg.

FIG.4.5.
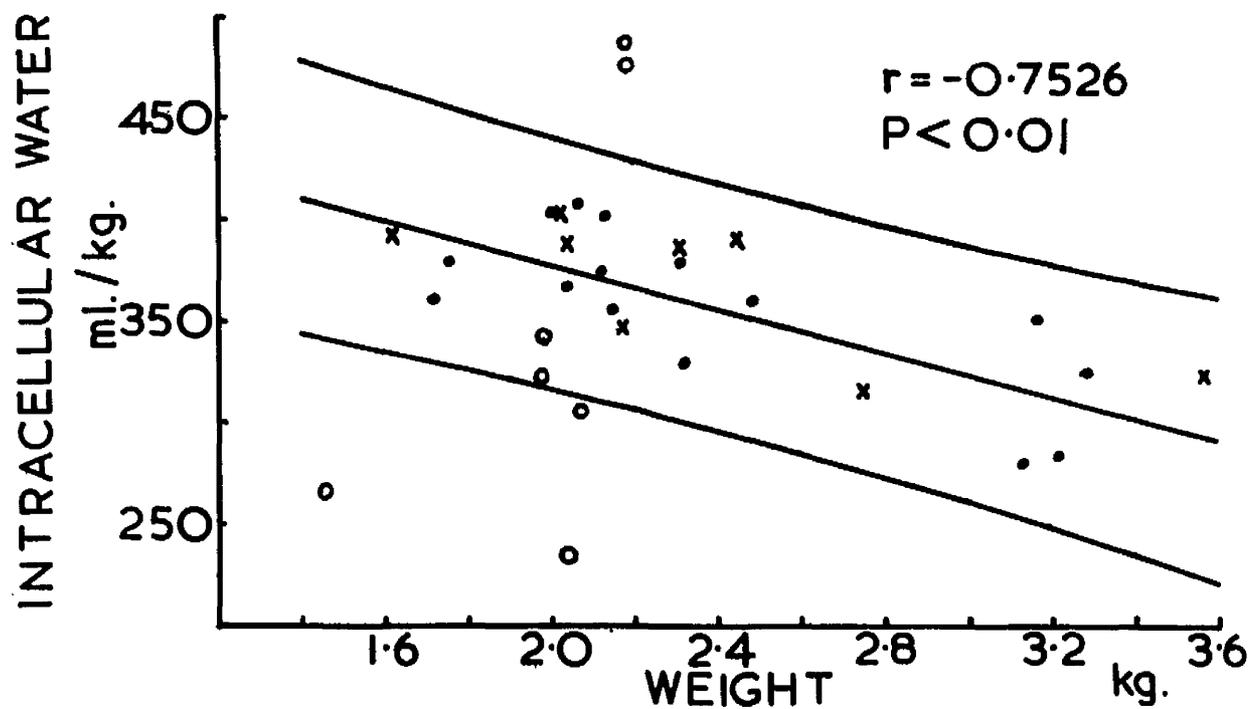
Closed circles, crosses, regression
line and 95% confidence limits as
in Figure 4.4.   The open circles
apply to the seven infants with
'abnormal' total body water.

FIG.4.6.
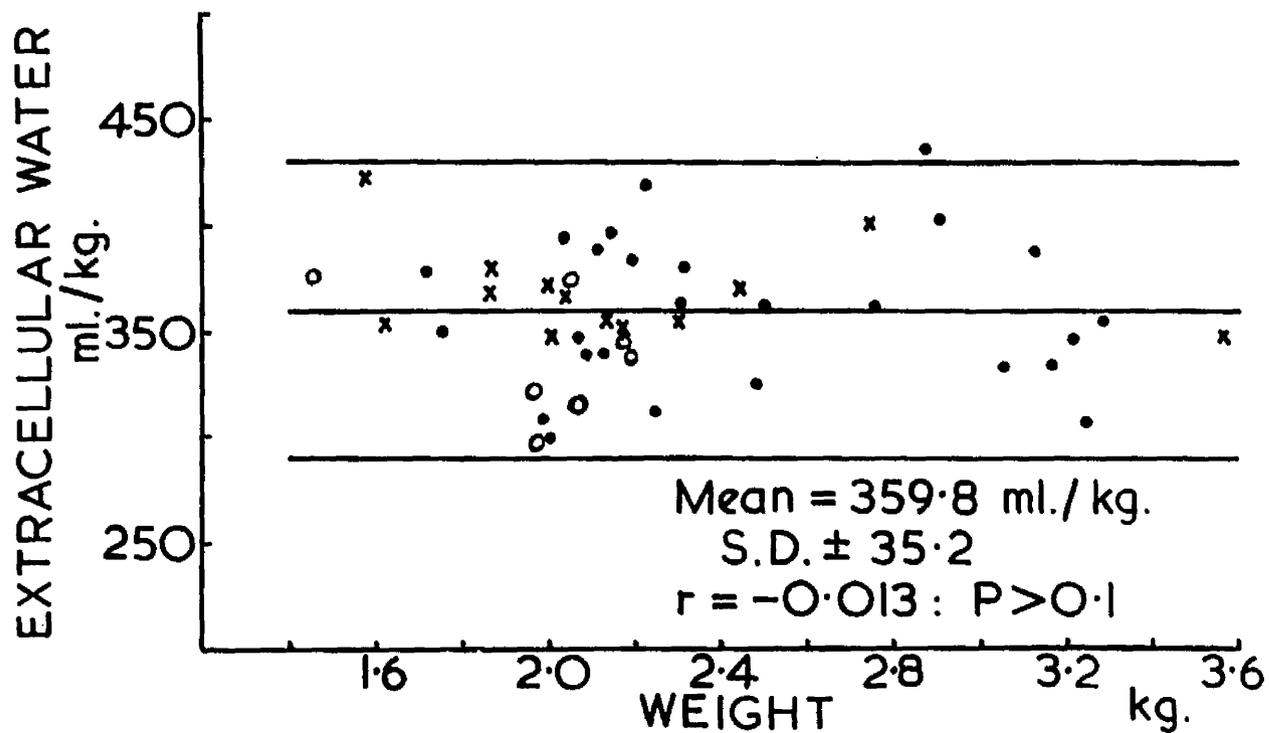
Subjects denoted as in Figure 4.5.
The ratio of extracellular water
to body weight is constant.

FIG.4.7.
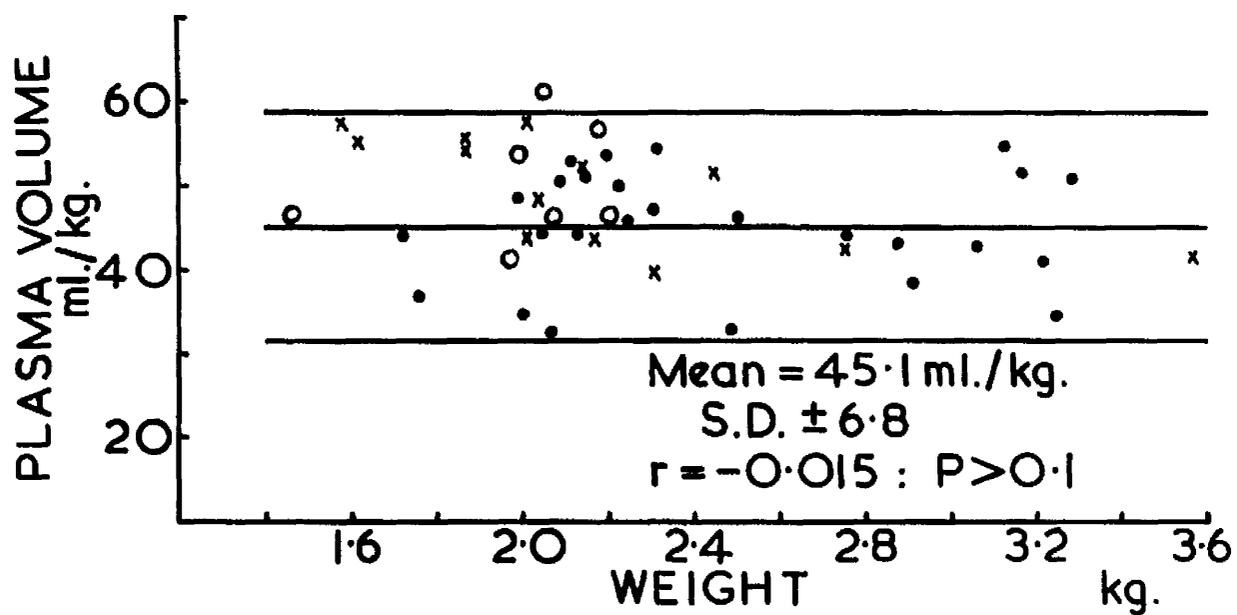
Subjects denoted as in Figure 4.5.

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 ($\pm$ 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

$$Y = 292.32 + 47.204 \; x - 6.359 \; x^2 :$$

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

FIGURE 4.8.
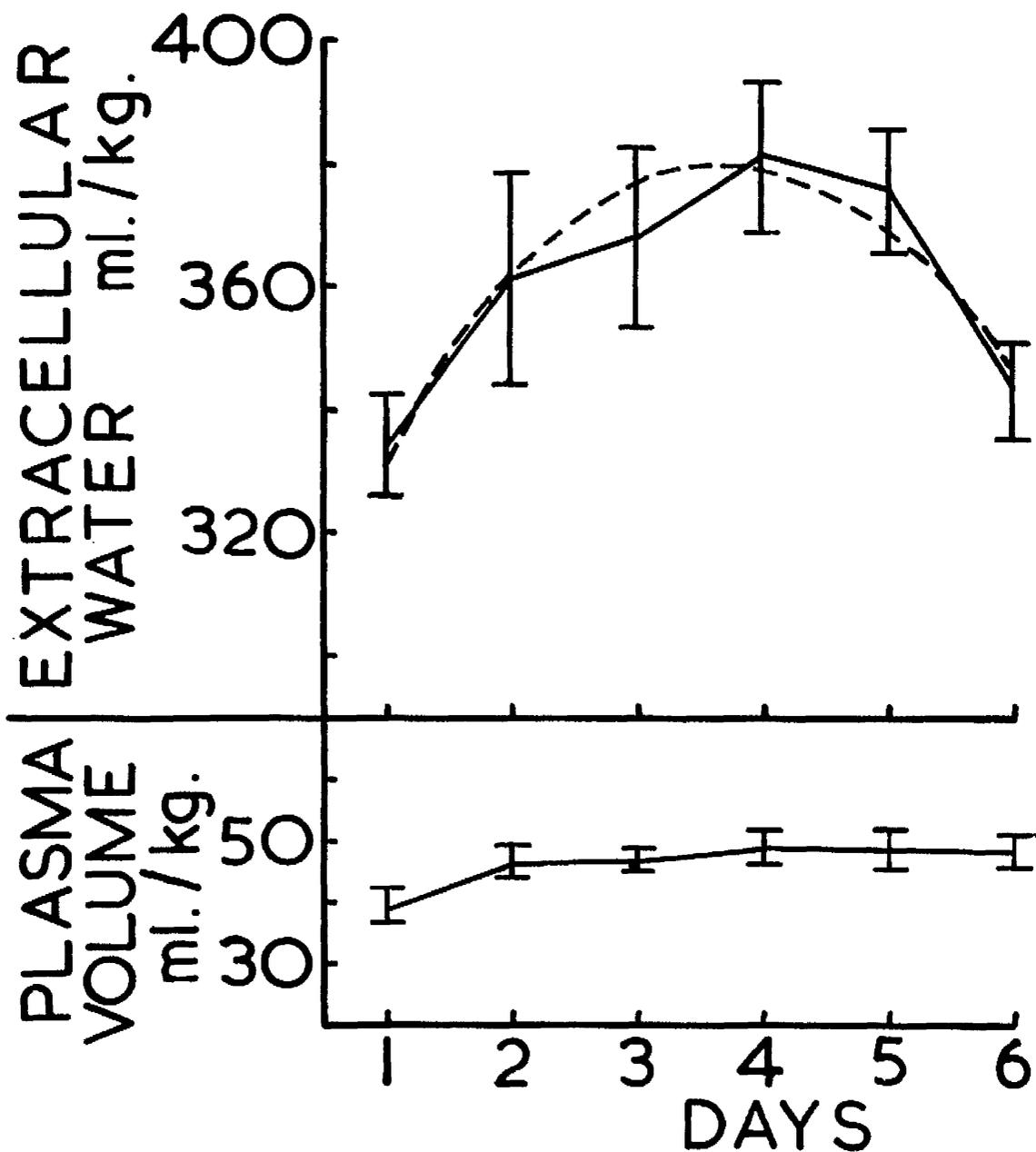
Results from 39 infants (mean
+ S.E.) plotted against time
The broken line represents the
calculated parabolic regression
curve which has a maximum at
3.7 days.

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

Day of Life	Number of Observations	Observed E.C.W. Mean ± S.E. ml./kg.	Calculated Mean E.C.W. from Regression Equn. ml./kg.	Body Wt. Mean ± S.E. Gm.	Calculated Mean Absolute E.C.W. ml.
1	8	334 ± 8	333	2400 ± 76	799
2	6	362 ± 17	361	2310 ± 73	834
3	5	368 ± 15	377	2250 ± 74	848
4	7	382 ± 12	379	2260 ± 76	857
5	6	376 ± 10	359	2290 ± 82	845
6	7	343 ± 8	347	2320 ± 78	805

FIG.4.9.

99

BODY WEIGHT
(Mean±S.E.)

I-C WATER
+'SOLIDS'

E-C WATER

DAYS
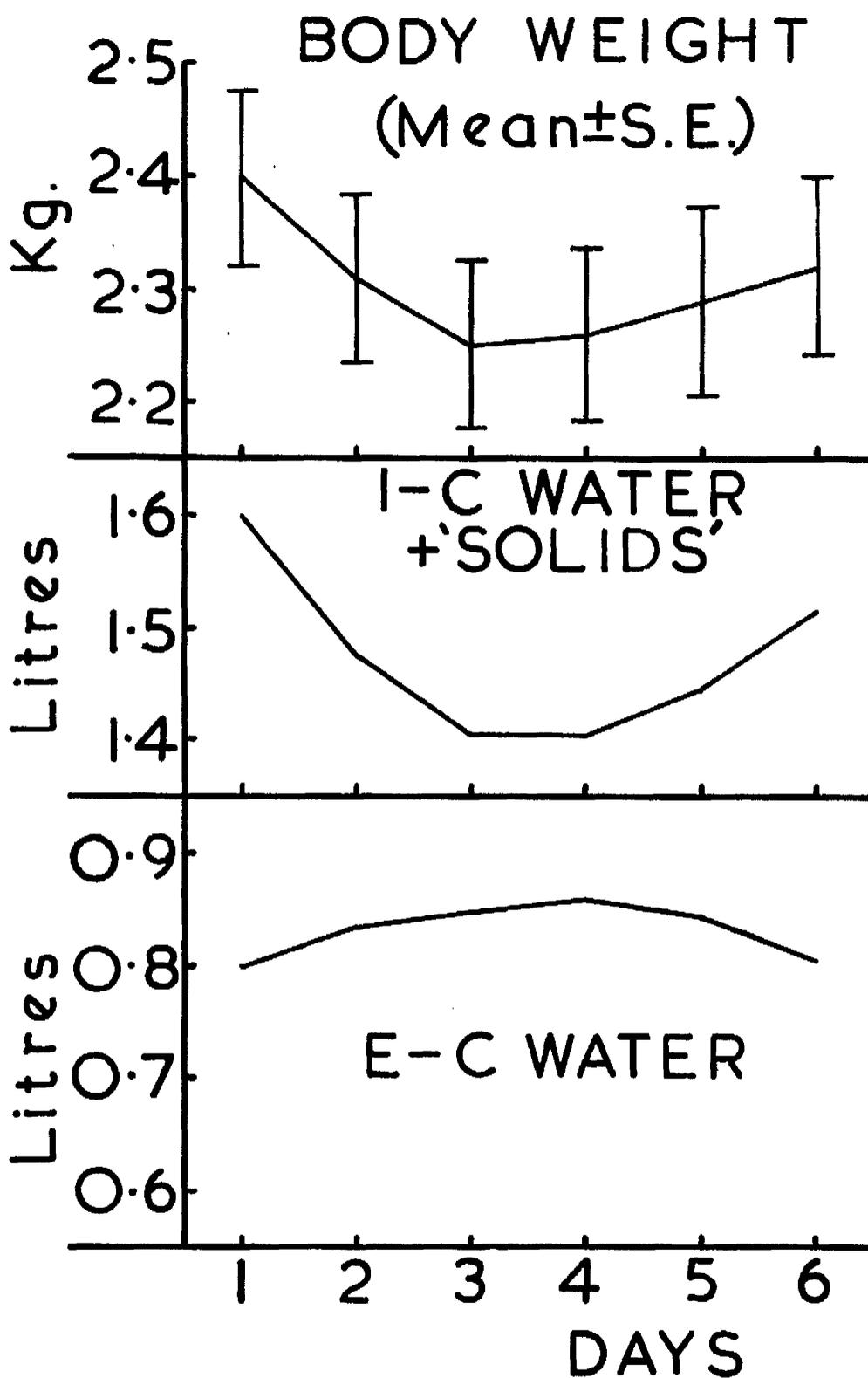
Upper curve represents daily
changes in observed body weights.
Lower curve derived as in Table
4.2.   Middle curve represents
body weight minus extracellular
water.

intracellular 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, $F = 2.93$, $P \doteqdot 0.10$).

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

FIG. 4.10
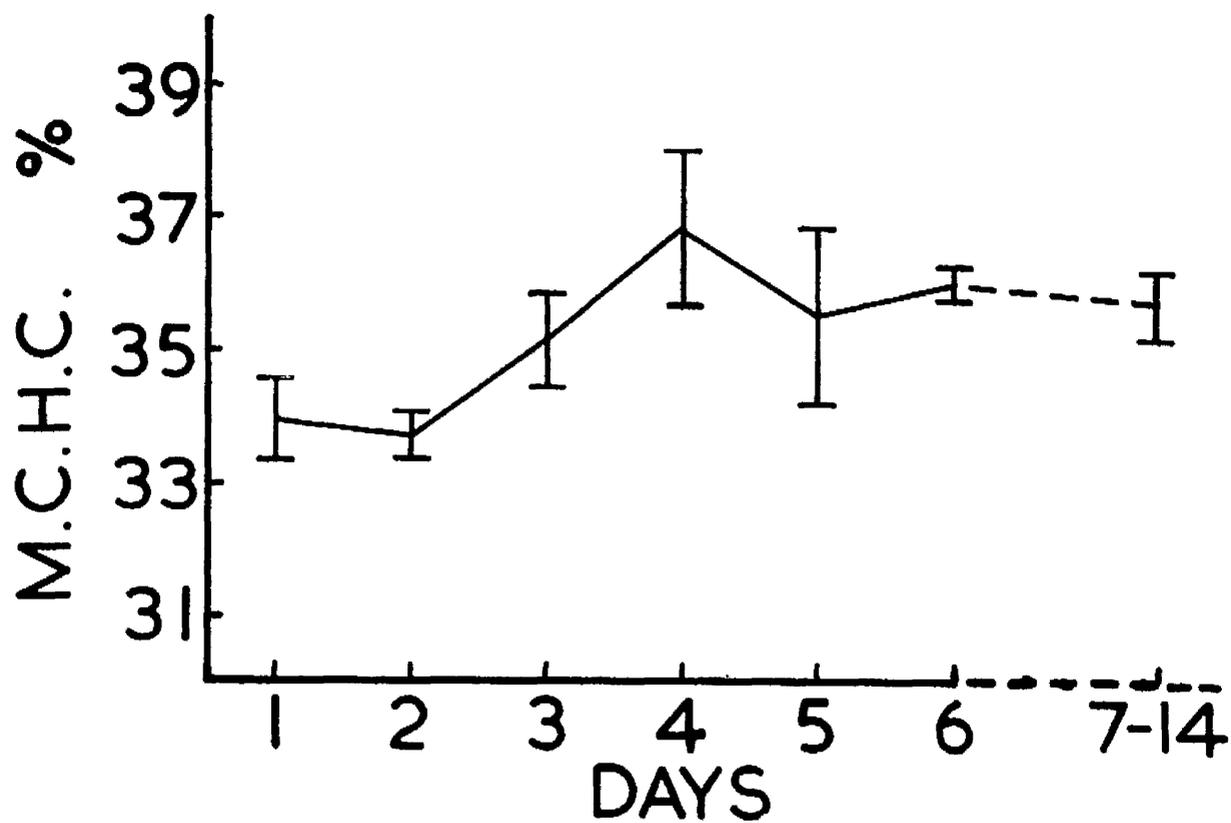
Results from 34 infants indicate a peak
on Day 4 corresponding with the maximum
for extracellular water (Figure 4.8.)

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.

FIG. 4.11
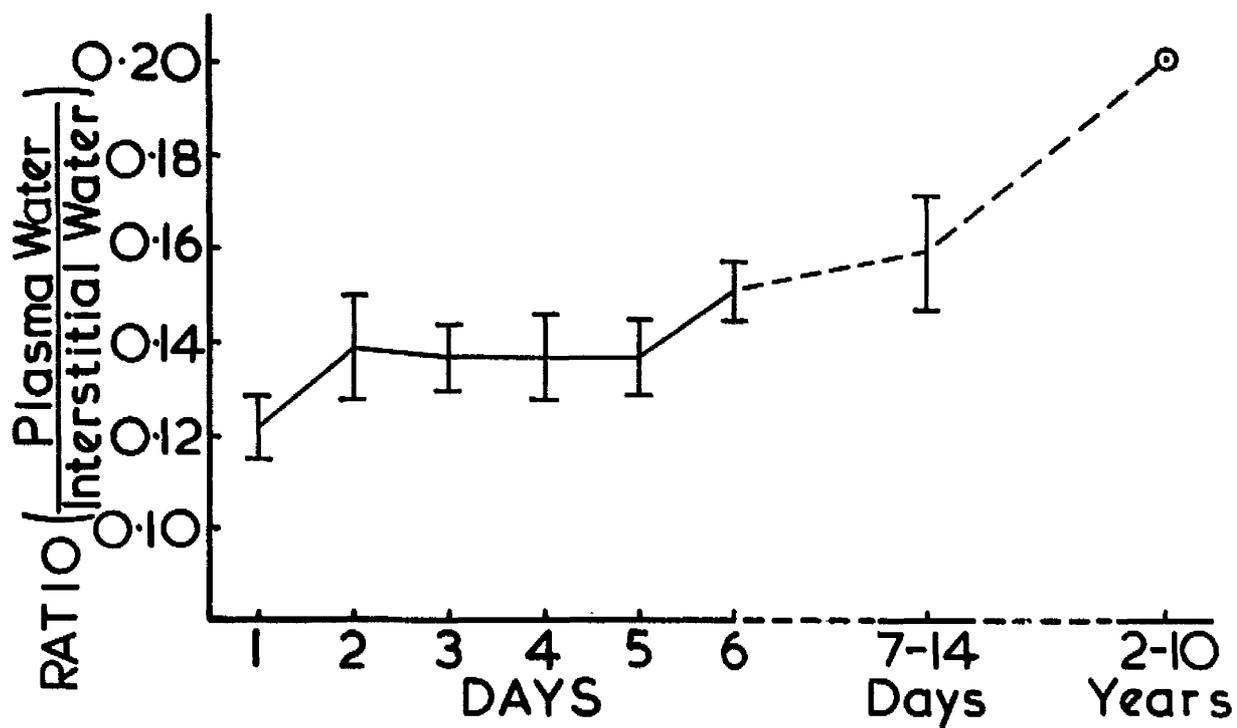
The increase in the ratio shown here is
due to decrease in relative size of the
interstitial compartment.   Value for
older children derived from results given
in Chapter 3.

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 anti-diuretic 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 intra- 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 osmolarity 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 osmolarity 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 proportionatoly 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.(196,87,50,165). 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 brest-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-1824 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 same, 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 <u>urea</u> was estimated by the micro-Kjeldahl method, with Nesslerization.   The usual modification of this method (217) was used to estimate serum <u>total proteins</u>.

Serum <u>electrophoresis</u> 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 <u>total osmolarity</u> 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.

Estimation	Mean (S.D.)
Sodium	138.7 (4.17) meq./l
Potassium	4.57 (0.535)meq./l.
Chloride	103.7 (2.80) meq./l.
Urea	32.9 (9.05) mg./100 ml.
Total protein	6.82 (0.50) Gm./100 ml.
Total osmolarity	284.5 (4.61) mOsm./kg.water

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 6 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 differences 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

Subject	Sodium meq./l.	Potassium meq./l.	Chloride meq./l.	Urea mg./100ml.	Protein Gm./100ml.	Osmolarity mOsm./kg.water
A.B.	138.8 (1.39)	5.32 (0.30)	103.5 (1.51)	32.6 (4.05)	7.50 (0.306)	294.0 (4.96)
B.B.	141.1 (1.56)	4.91 (0.53)	104.9 (1.65)	27.9 (3.41)	7.66 (0.158)	290.1 (5.20)
J.M.	141.1 (2.28)	4.84 (0.22)	105.4 (1.07)	30.5 (2.91)	7.44 (0.089)	291.1 (.305)
W.D.	142.1 (1.46)	4.46 (0.29)	105.1 (0.84)	30.8 (3.14)	7.28 (0.310)	292.9 (4.16)
Total	140.8 (2.07)	4.88 (0.46)	104.7 (1.41)	30.4 (3.65)	7.47 (0.264)	292.0 (4.47)

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 5.3.

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

	Sodium		Potassium		Chloride		Urea		Protein		Osmolarity	
	s^2	P	s^2	P	s^2	P	s^2	P	s^2	P	s^2	P
Between Subjects	14.6		0.99		5.33		30.0		0.20		24.0	
	5.50 <0.01		7.35 <0.01		3.11 <0.05		2.59 N.S.		3.62 <0.05		1.23 N.S.	
Within Subjects	2.99		0.13		1.71		11.6		0.056		10.5	

N.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 unstabling 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 Luetscher
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:

$$T.P.O.(mOsm./l.) = 2(Na + K) + Urea - 8$$

Sodium and potassium are expressed in meq./l. and
urea in m.Mols./l i.e., $\frac{mg./100ml.}{6}$

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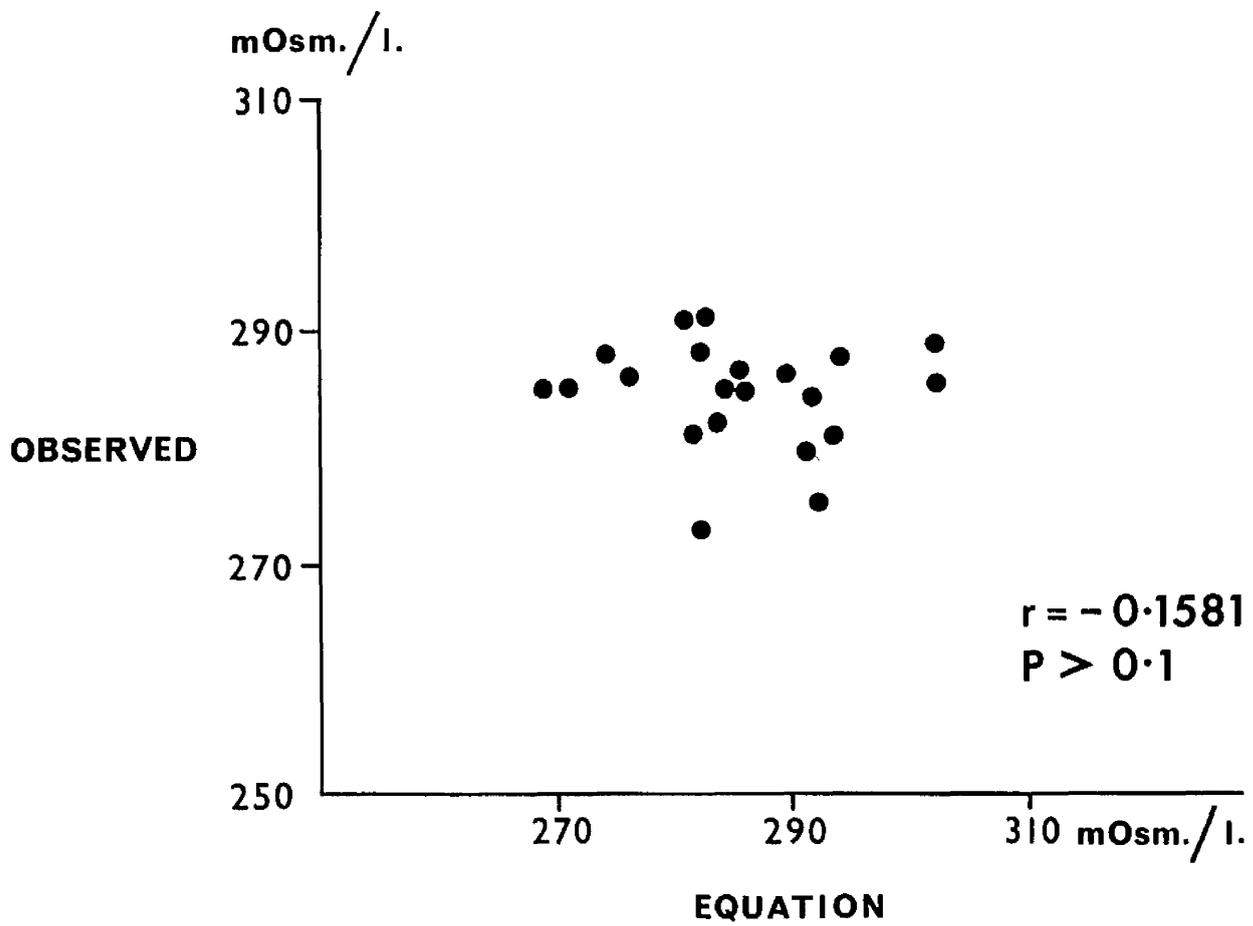
sufficiently different to suggest significantly differing parent populations. Hence it is not surprising that there is no correlation between the results ($r = -0.1581$, $P > 0.1$.)

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 osmolarity 290) had means of 307.0 (observed) and 297.2 (calculated). These means are significantly different ($t = 2.898$, $P < 0.01$), 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
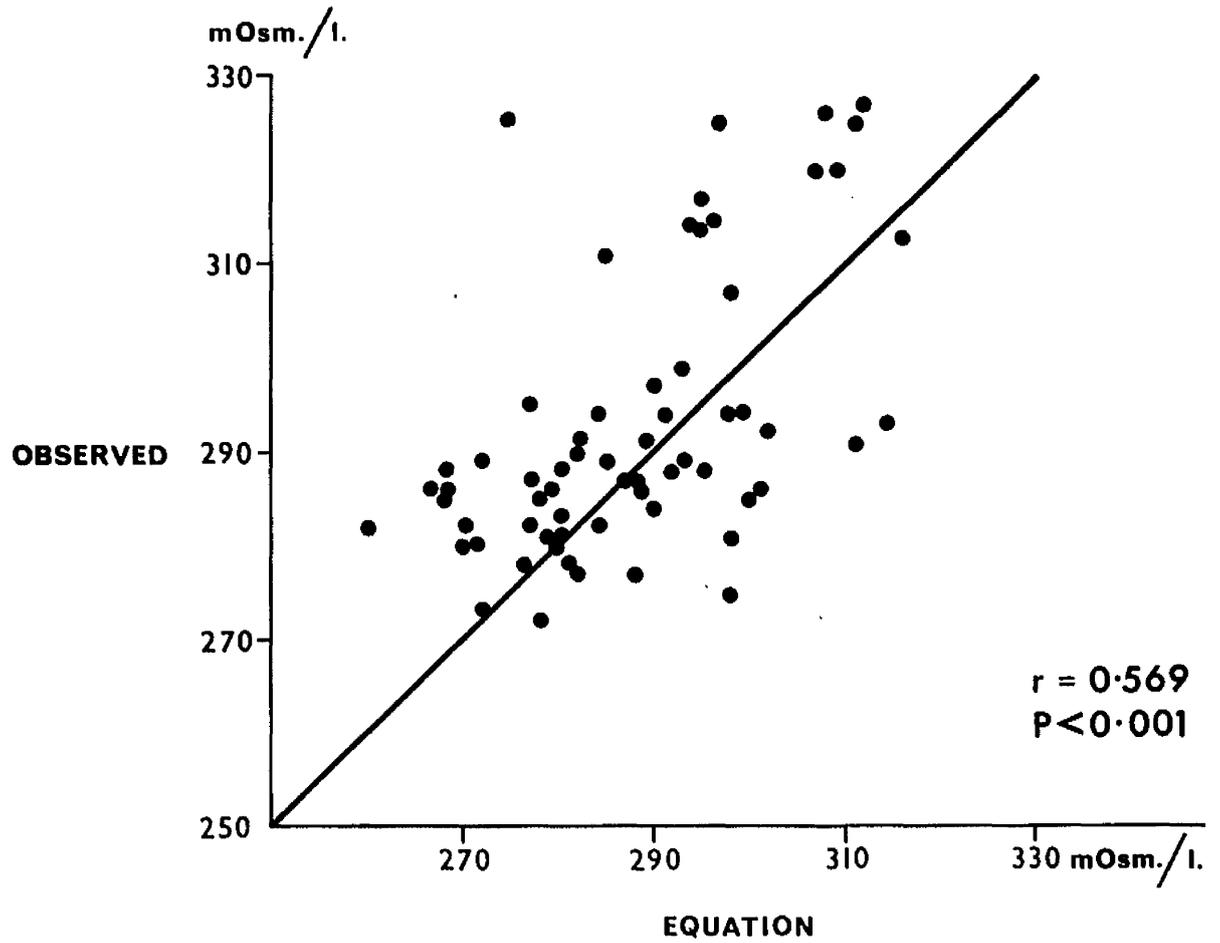
However, although the correlation is now highly significant ($P<0.001$) it is in fact so poor ($r = 0.5690$) that it is difficult to regard this equation for calculating osmolarity as being of any value whatsoever.

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 Wells 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
urea, 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

140

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

to heart failure.

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 haematocrit 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 Merrill 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 intramascular 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

149

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 those 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 AGN 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.

151

ACUTE NEPHRITIS

Changes in Body Weight . 168 Patients
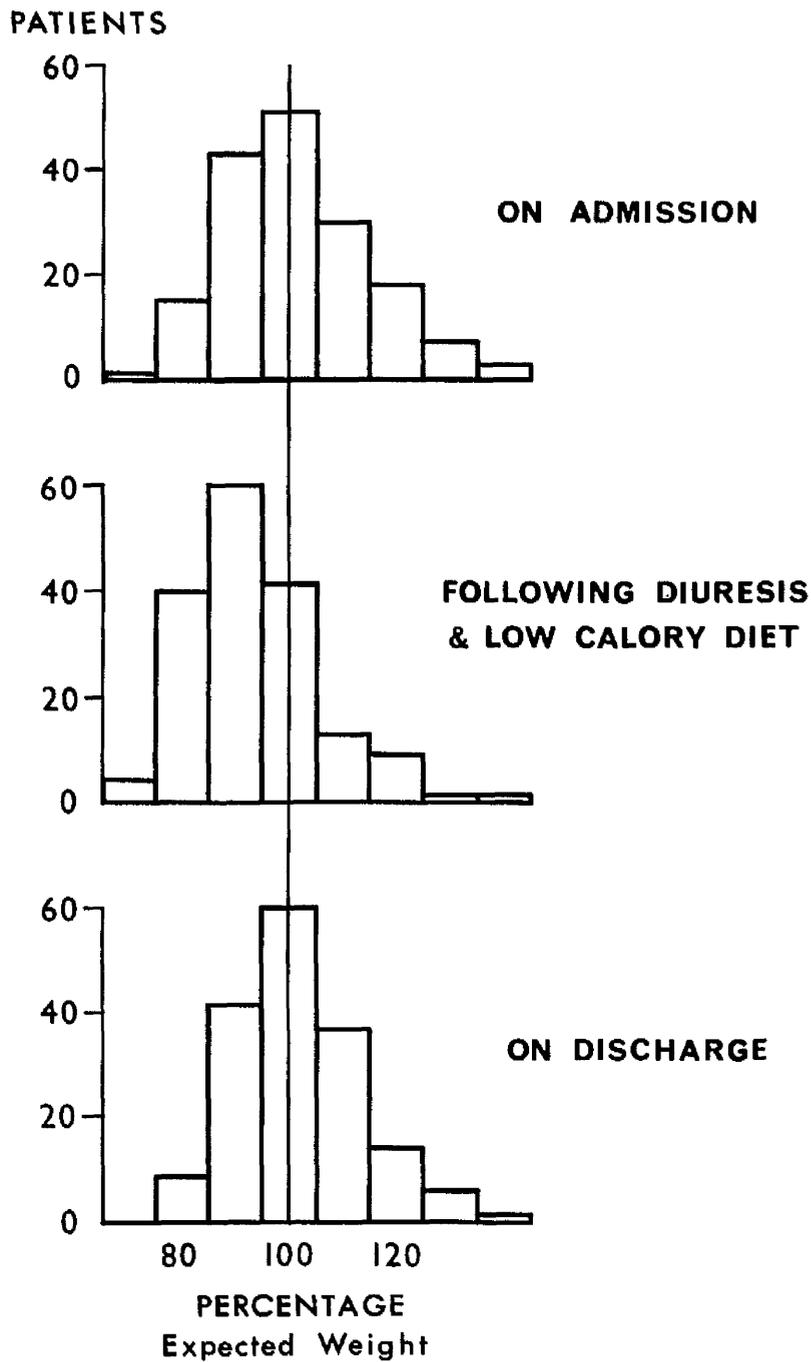
1. On admission.   2. Lowest weight.   3. On dismissal

    100.92%   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 $\pm$ S.E.):

TABLE   7.1.

	Weight 1	Weight 2	Weight 3
Oedema Present	104.0(1.63)	93.2(1.06)	101.2(1.27)
Oedema absent	96.2(1.62)	92.0(1.53)	100.0(1.42)

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 104 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.E.) of the percentage weights
for this group is as follows:

Weight 1	Weight 2	Weight 3
98.1 (2.31)	91.5 (1.96)	102.6 (2.19)

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):

FIG. 7.2.

155

(a) AGE

(b) HOSPITAL DAYS

(c) DAY OF ADMISSION

(d) BLOOD UREA

BLOOD PRESSURE

(e) Systolic

(f) Diastolic
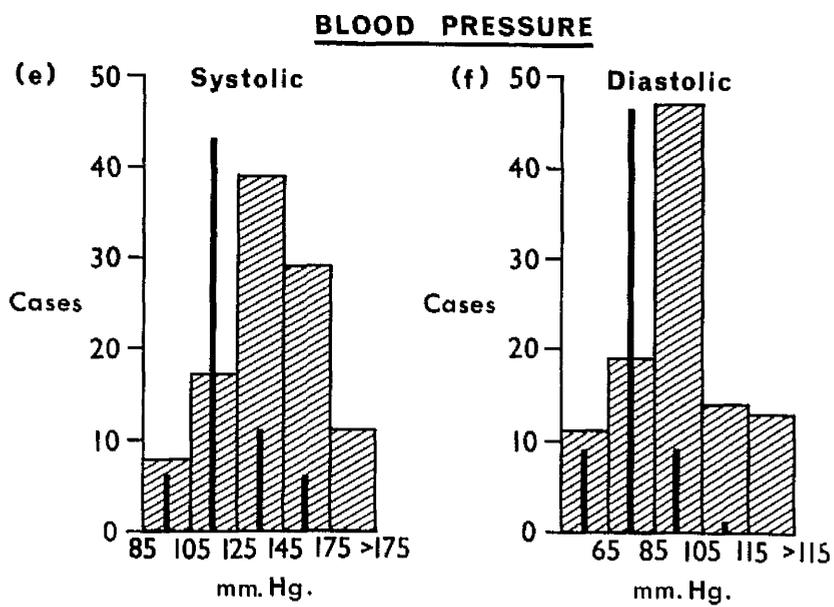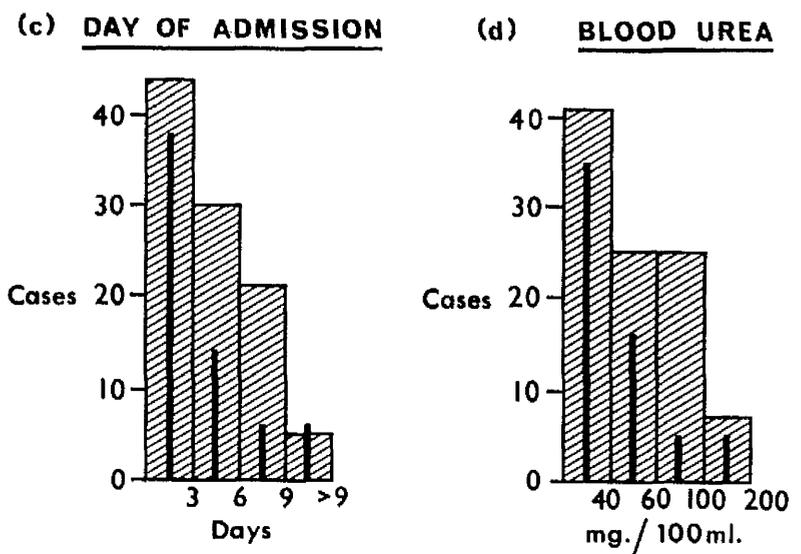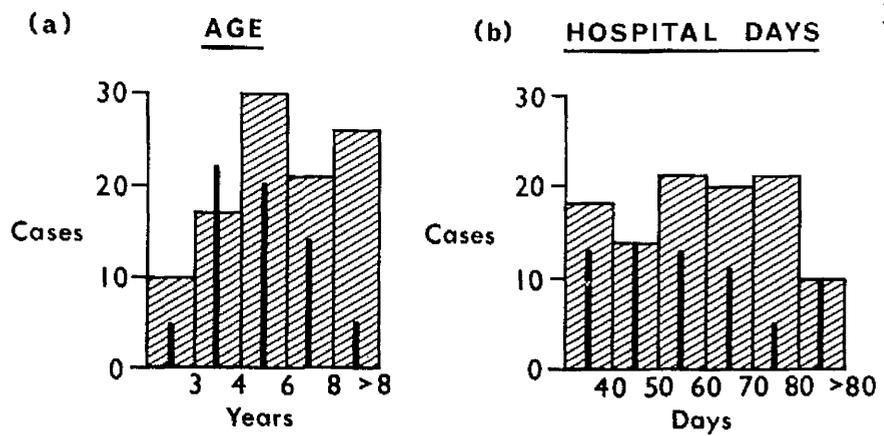
Oedematous cases (hatched blocks) compared with non-oedematous (solid lines).

TABLE 7.2.

	Age (years)				
	3	3-4	5-6	7-8	8
Oedema present	10	17	30	21	26
Oedema absent	5	22	20	14	5

These distributions are significantly different
($\chi^2 = 12.024$, $0.025 > P > 0.01$). 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.

	Throat	U.R.I.	Ear	Nil
Oedema present	54	10	9	27
Oedema absent	29	8	7	19

These distributions are almost identical
($\chi^2 = 1.098$, $0.8 > P > 0.7$.)

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 7.4.

	Days after onset of symptoms			
	1-3	4-6	7-9	9
Oedema present	44	30	21	5
Oedema absent	38	14	6	6

There is a trend in these results which fails to reach significant level ($x^2 = 7.09$, $0.1 > P > 0.05$). 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 χ^2 analysis is more informative:

TABLE 7.5.

	Blood urea (mg./100ml.)			
	40 and less	41-60	61-100	101-200
Oedema present	41	25	25	7
Oedema absent	35	16	5	5

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 ($x^2 = 7.88$, 0.05 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-oedematous group were normal (40 mg./100ml.), whereas of the 36 children originally oedematous, 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 - 30; 3 - 30.

Analysis of the results shows no significant difference for either measurement.   In the case of the red cells, the distributions between the oedomatous and non-oedomatous 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 f. and are as follows:

TABLE 7.6

	Systolic B.P. (mm.Hg)				
	85-104	105-124	125-144	145-174	174
Oedema present	8	17	39	29	11
Oedema absent	6	43	11	6	0

$$\chi^2 = 47.28, \quad P < 0.0005$$

TABLE 7.7.

	Diastolic B.P. (mm.Hg)				
	65	65-84	85-104	105-114	114
Oedema present	11	19	47	14	13
Oedema absent	9	47	9	1	0

$$\chi^2 = 56.5, \quad P < 0.0005$$

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 (x^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 (x^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 (x^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.

	Categories		
	1	2	3
Oedema present	78	12	5
Oedema absent	49	7	4

$$\chi^2 = 0.94, \quad 0.7 > P > 0.6$$

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 (78%) 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

Series Case No.	1	2	3	4	5	6	7	8	9	10
	K.E.	B.B.	W.D.	G.R.	W.W.	J.S.	K.L.	A.O'N.	A.M.	J.B.
Sex	F	F	M	M	M	M	F	F	M	M
Age (Yrs,Mths)	6.4	3.10	9.7	6.2	4.10	6.1	7.10	6.0	3.8	9.1
Prev. infection	Throat	Throat	Throat	Throat	Throat	Throat	Both ears	Throat	L.ear	?
% Exp. Wt. 1.	96	101	110	128	104	110	104	93	99	171
% Exp. Wt. 2.	92	91	97	105	101	99	92	82	85	155
% Exp. Wt. 3.	99	100	104	113	106	109	102	90	92	166
Days after onset	1	?	6	3	2	6	?	1	4	2
Oedema	0	2	2	2	0	1	2	2	2	1
Highest B.P.	110/65	100/70	170/110	190/120	120/80	130/100	170/120	170/120	135/100	150/110
Urine albumin	2	2	1	2	2	2	2	2	1	1
Urine-R.B.C.	3	3	1	3	3	3	3	1	1	1
Urine-Casts.	1	2	1	3	2	1	1	1	1	1
Blood urea (mg.%)	33	27	40	73	40	53	33	67	40	37
A.S.O. (u/ml)	2500	2500	2500	333	833	500	2500	125	333	200

169

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-1824 and of thiocyanate

FIG. 8.1.

171

ACUTE NEPHRITIS
Body Water Distribution

Kg/Litres.
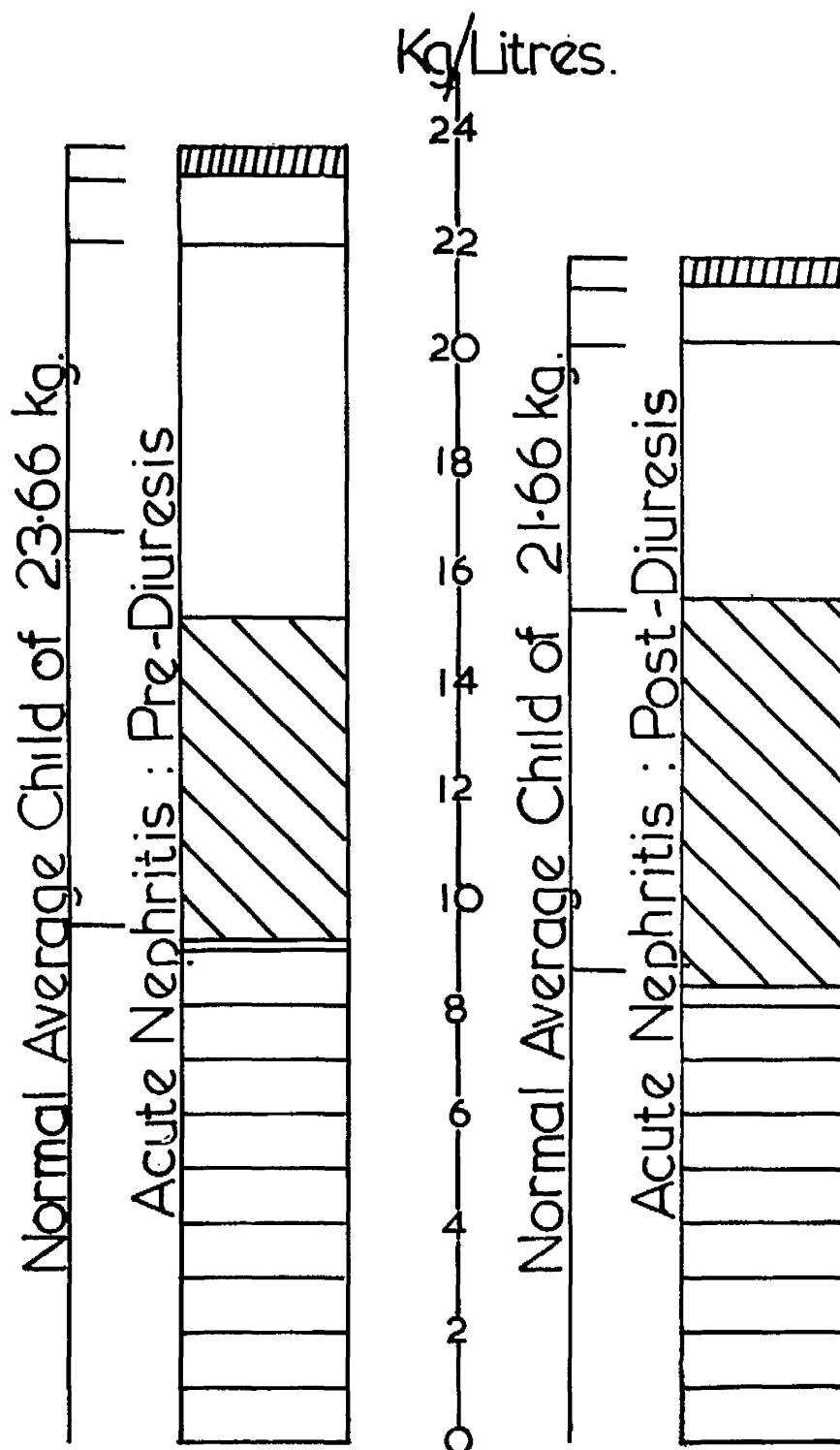
Block diagrams representing mean body water distribution of ten children with AGN before and after diuresis. Comparison is made in each case with water distribution in an average normal child of appropriate size. The compartments, in order downwards are: red cell mass, plasma volume, interstitial water, 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 µc. of $_{24}$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 8.2

BODY WATER DISTRIBUTION IN AGN

		Body Weight kg.	T.B.W. Litres	E.C.W. Litres	I.C.W. Litres	P.V. ml.	C.V. ml.	B.V. ml.	Plasma Proteins Gm.%
1. Y.B.	1.	19.24	10.90	6.14	4.76	982	426	1408	5.85
	2.	19.12	10.40	5.16	5.24	940	426	1366	7.02
2. B.B.	1.	16.00	10.47	6.70	3.77	1027	423	1450	7.08
	2.	14.40	9.54	4.02	5.52	761	258	1019	7.75
3. W.D.	1.	31.65	22.25	10.54	11.71	1913	698	2611	6.10
	2.	28.00	18.37	7.29	11.08	1360	691	2051	7.71
4. G.R.	1.	26.00	14.10	9.18	4.92	1335	580	1915	5.65
	2.	22.30	12.93	5.52	7.41	1247	565	1812	6.58
5. W.W.	1.	18.40	11.02	5.91	5.11	793	369	1162	6.53
	2.	18.50	10.70	5.47	5.23	780	364	1144	6.45
6. J.S.	1.	21.44	13.80	7.25	6.55	1157	507	1664	6.79
	2.	20.24	13.23	5.08	8.15	1159	455	1614	6.82
7. E.L.	1.	24.70	13.94	9.00	4.94	1330	455	1785	7.10
	2.	21.95	13.00	6.32	6.68	1200	430	1630	6.81
8. A.O'N	1.	18.50	12.68	6.29	6.39	942	439	1381	6.76
	2.	16.55	12.91	4.43	8.48	859	427	1286	5.42
9. A.M.	1.	15.80	8.91	5.89	3.02	677	263	940	5.49
	2.	13.70	8.50	4.28	4.22	615	260	875	5.68
10. J.B.	1.	44.90	25.00	11.80	13.20	2240	1044	3284	6.72
	2.	41.85	23.30	9.96	13.34	1560	878	2438	7.42
Means	1.	23.66	14.31	7.87	6.44	1240	520	1760	6.41
	2.	21.66	13.29	5.75	7.54	1048	475	1523	6.77

renders the use of such a proportional expression
meaningless.

It is clear that, whereas the post-diuresis
results are substantially normal, the initial or
prediuretic 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, 1kg.
of which is water. The loss of 1 kg. solid weight
following a week's semi-starvation seems not unreasonable.

FIG. 8.2.

175

ACUTE NEPHRITIS
Changes in Body Weight &
Water Distribution
10 Cases
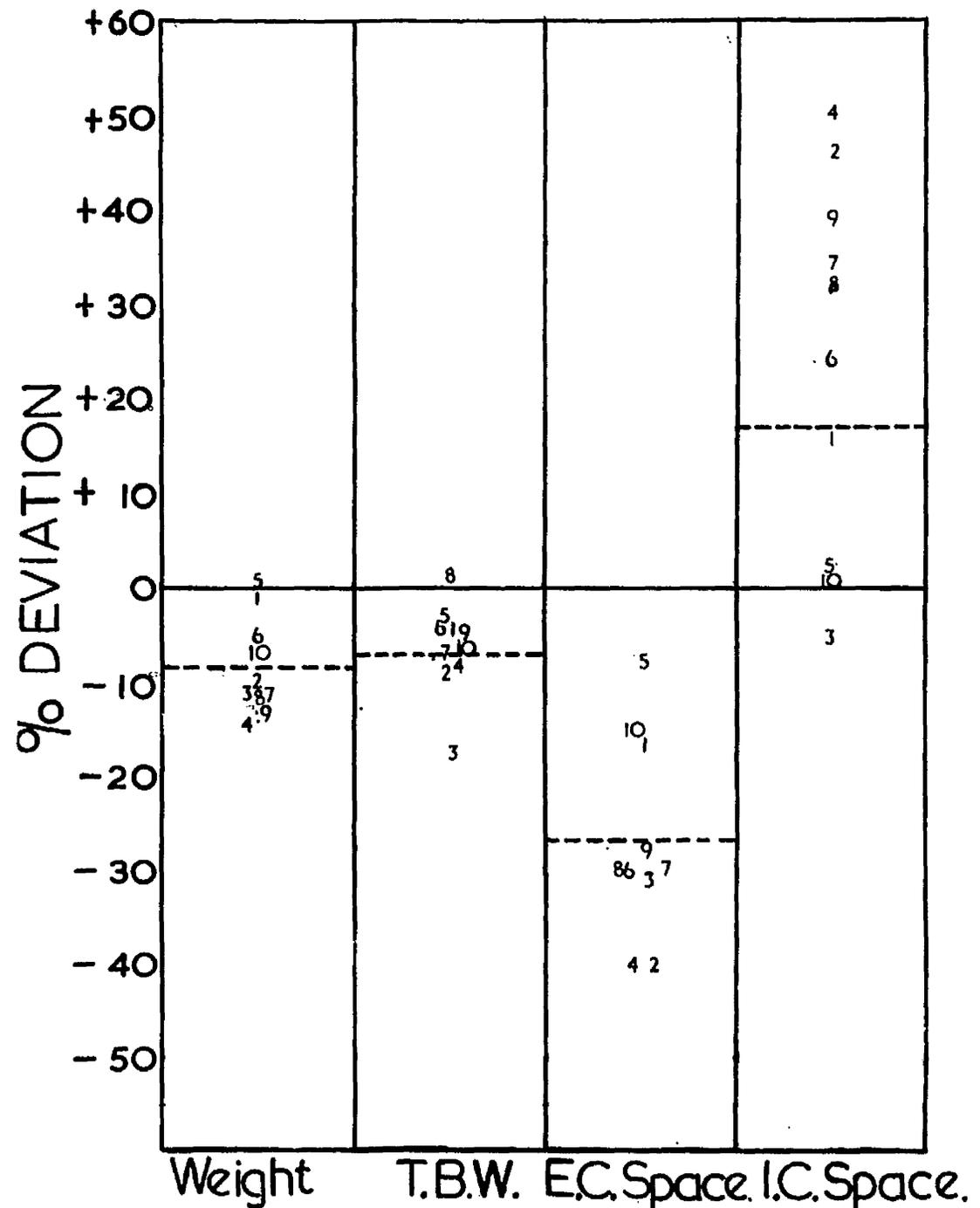
Individual changes in body water
distribution occurring with diuresis.
Small figures refer to case-numbers.

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.C.W. 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.W.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

		T.B.W.	E.C.W.	I.C.W.	P.V.	C.V.	B.V.
Nephritics 1.		607.6	342.6	265.0	52.1	21.9	74.0
Nephritics 2.		622.2	271.4	350.8	49.5	21.8	71.3
Normal		598.4	269.8	328.9	47.4	25.0	72.4
1.	F.	0.237	41.675	4.461	3.108	5.475	0.053
	P.	N.S.	<0.01	<0.05	N.S.	<0.05	N.S.
2	F.	0.223	0.049	0.370	0.022	4.876	0.017
	P.	N.S.	N.S.	N.S.	N.S.	<0.05	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:

    "Adjusted mean" variance
    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
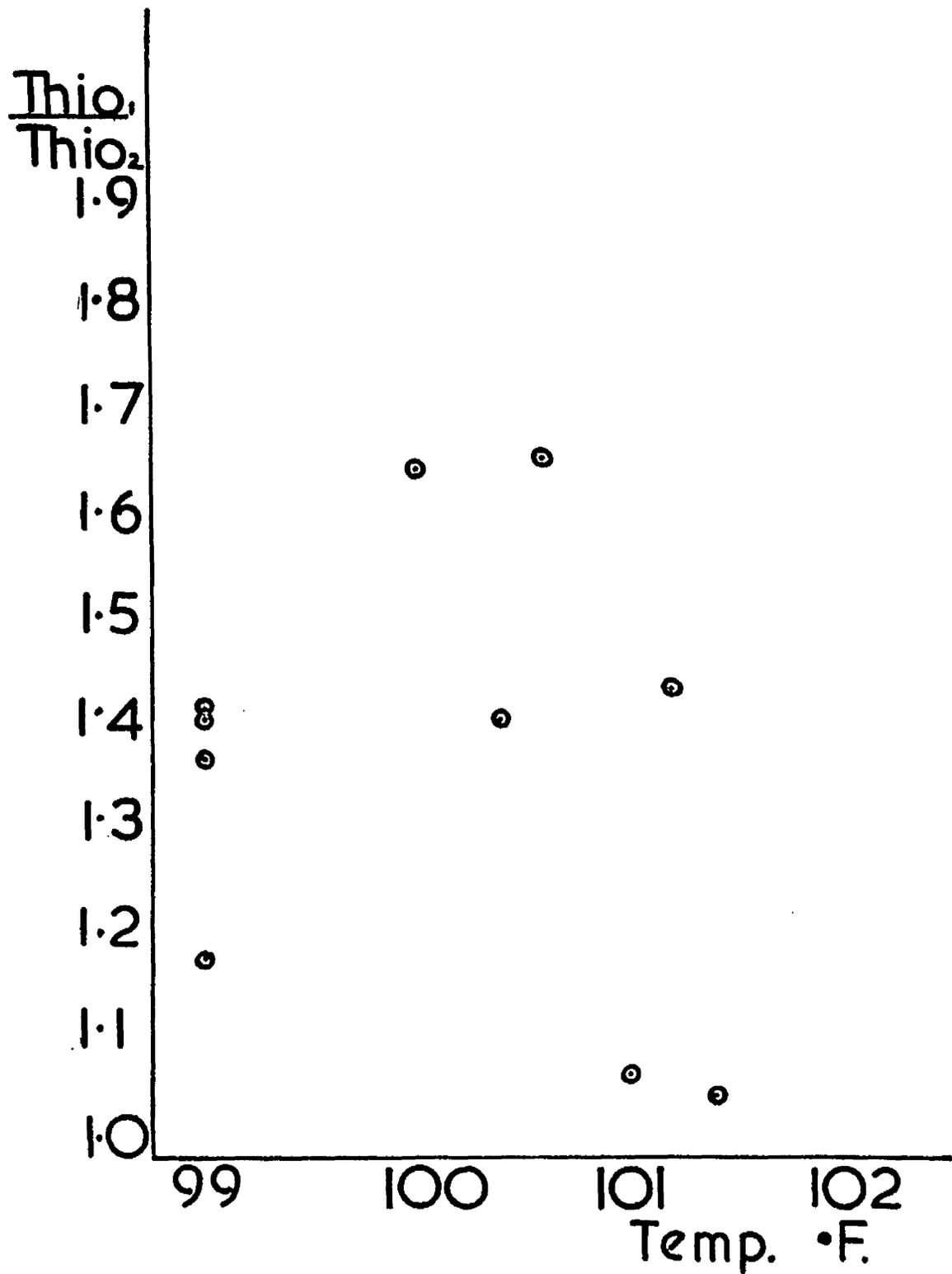
ACUTE NEPHRITIS

Changes in thiocyanate volume of dilution
showing no correlation with body temperature.

ACUTE NEPHRITIS

O   no oedema
1   oedema of face only
2   mod. gen. oedema
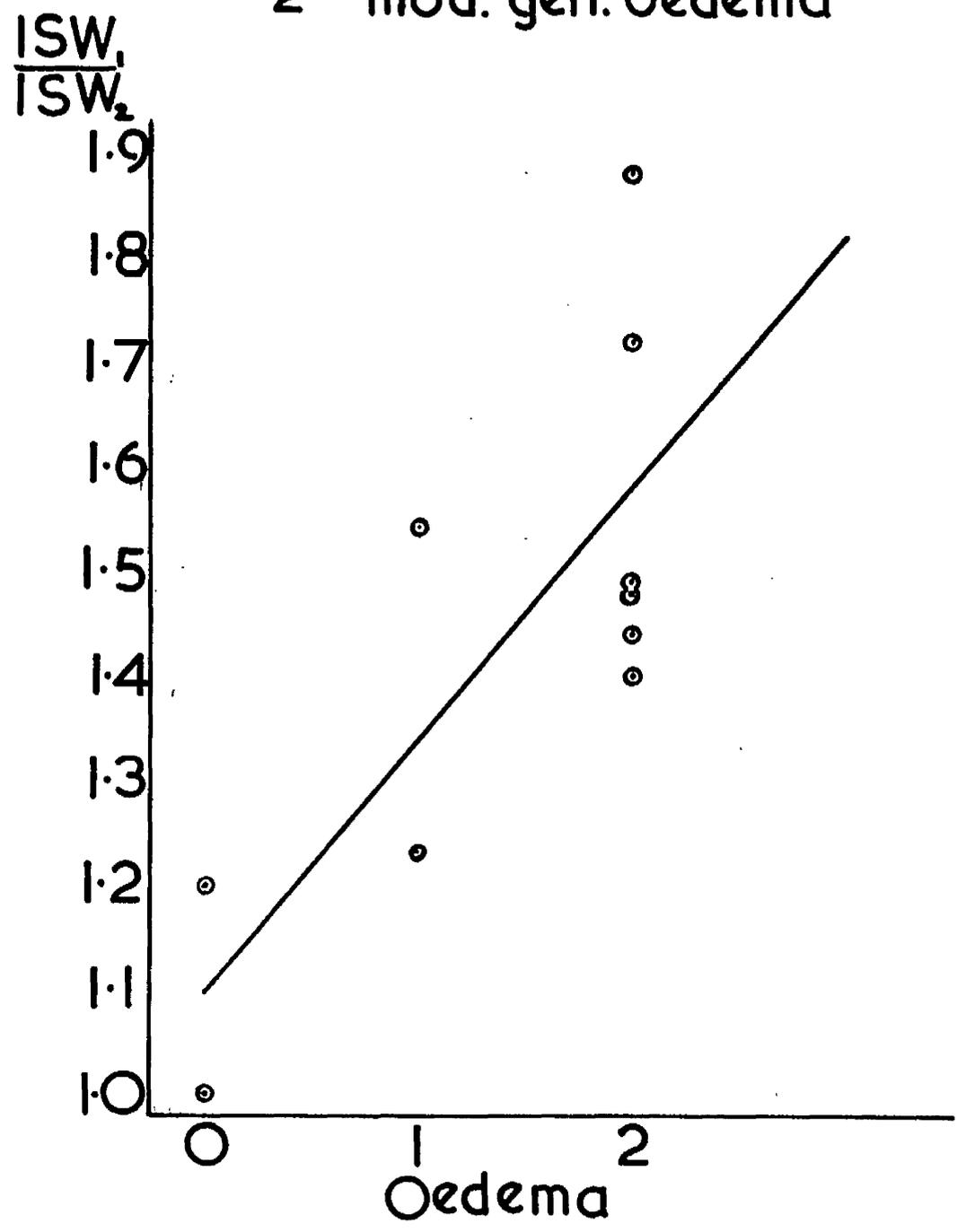
Rough correlation demonstrated between changes in observed interstitial space and degree of clinical oedema.

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 λ,
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:

FIG. 8.5.

189

ACUTE NEPHRITIS
EVAN'S BLUE ELIMINATION
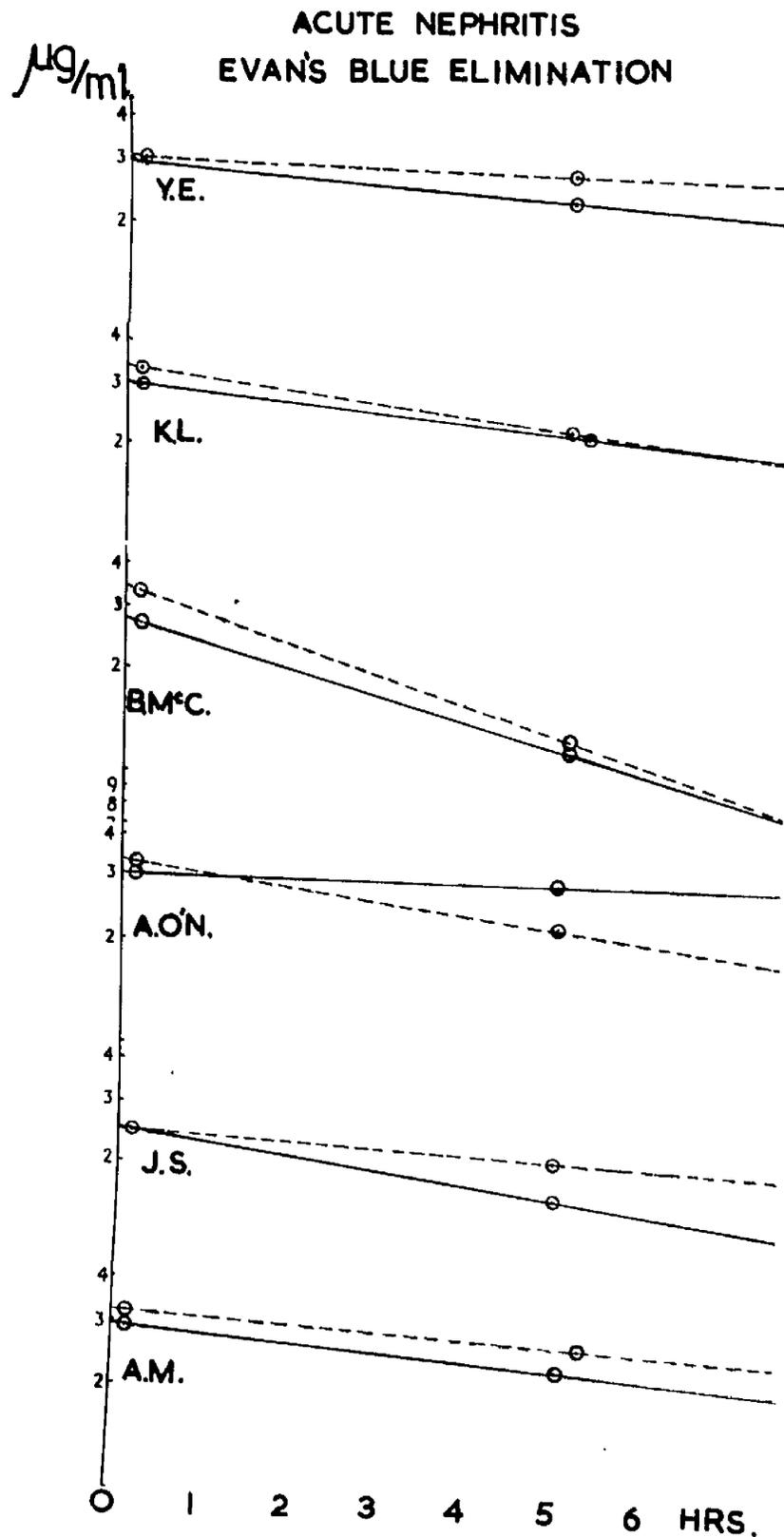
Individual T-1824 elimination
curves before diuresis (solid
lines) and after (broken lines)

FIG. 8.6.

190

ACUTE NEPHRITIS
THIOCYANATE ELIMINATION
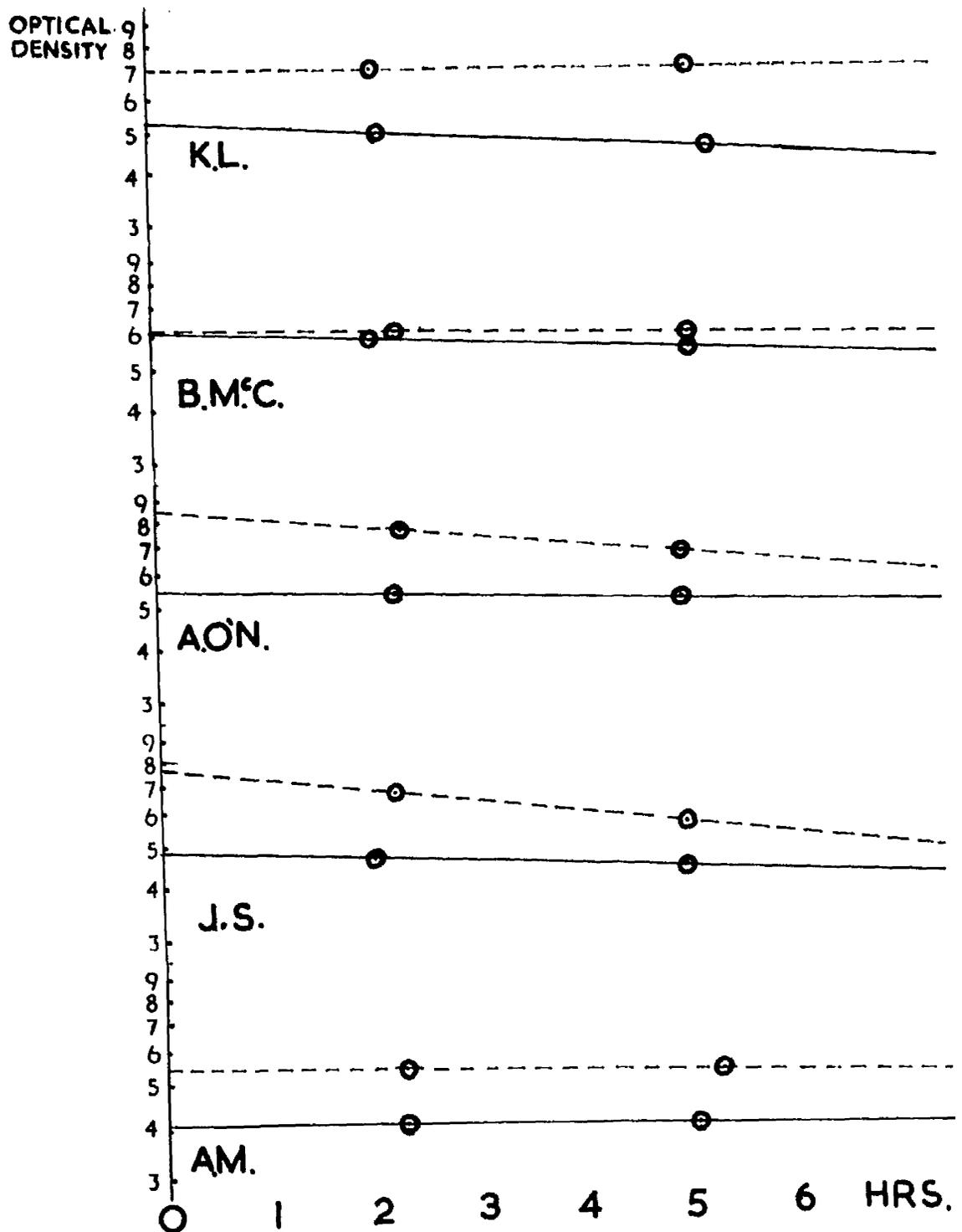
Individual thiocyanate elimination curves before diuresis (solid lines) and after (broken lines)

T - 1824: $t = 0.9898,$ $P > 0.3$

Thiocyanate: $t = 1.053,$ $P > 0.3$

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. $Na_e = 2015$ meq. (44.7 meq./kg.)

2. $Na_e = 1550$ 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 $Na_s = 146 \times 2.24$ (P.V. in litres)

$= 327.0$ meq.

The concentration of sodium in interstitial

fluid is given by:

$$\left(Na_{is}\right) = Na_s) \ x \ \frac{95}{93.28}$$

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):

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

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

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

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

and $x = (Na_s)$

By substituting in equation (1) above,

the values for K_e 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

Series Case No.	11	12	13	14	15	16	17	18	19	20	21
	J.McD.	W.C.	H.C.	G.B.	J.L.	D.G.	P.McC	D.W.	P.M.	L.B.	N.C.
Sex	M	M	F	F	M	M	M	M	M	F	F
Age(Yrs.,Mths)	9.4	9.2	9.6	7.2	8.8	8.7	2.4	12.4	6.5	7.1	8.4
Prev.infection	Throat	Throat	Throat	Throat	Throat	-	-	-	Throat	Throat	Throat
% Exp. Wt.1.	113	107	110	113	112	966	not weighed	102	119	114	112
% Exp. Wt.2.	101	85	95	100	102	87	93	93	101	105	94
% Exp. Wt.3.	106	92	98	106	104	102	102	98	113	106	100
Days after onset	2	3	2	2	1	1	3	6	7	4	7
Oedema	1	3	2	1	1	0	2	2	1	2	3
Highest B.P.	160/110	175/115	140/90	140/95	160/85	115/70	105/40	160/112	145/100	140/100	150/100
Urine-albumin	3	3	3	2	3	3	2	3	2	1	3
Urine-R.B.C.	2	2	3	2	3	1	1	2	3	2	3
Urine-Casts	1	2	1	1	1	1	1	2	3	2	3
Blood urea (mg.%)	107	180	79	70	77	202	96	83	43	50	74
A.S.O.(u./ml.)	1250	200	1250	500	500	500	500	50	2500	500	50

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.

200

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 $4^{o}C$. 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:

Fluid   1 litre (Half-strength milk).

Calories   600

Sodium   14 meq.

Potassium   4 meq.

Chloride   20 meq.

(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 $36^{o}C$ $(68^{o}F)$ 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 $\pm$ 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 severla 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

205

ACUTE NEPHRITIS

EARLY DIURETIC PHASE

Urine : 8 Cases
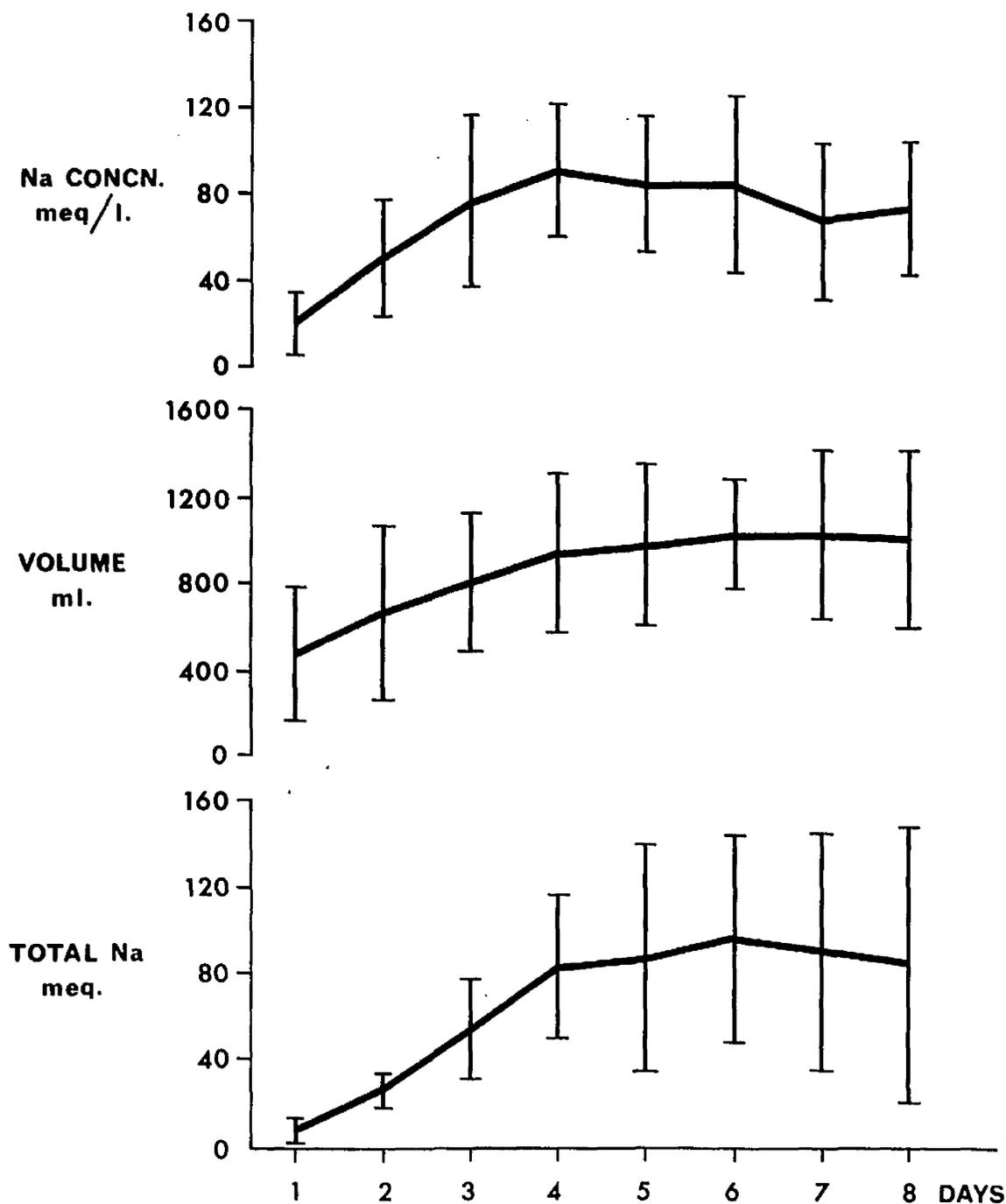
N.B.   The graphs throughout this
       chapter represent daily
       mean ± S.D.

during the first two days of the study there is a
profound degree of sodium retention, and that the
sodium duiresis 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
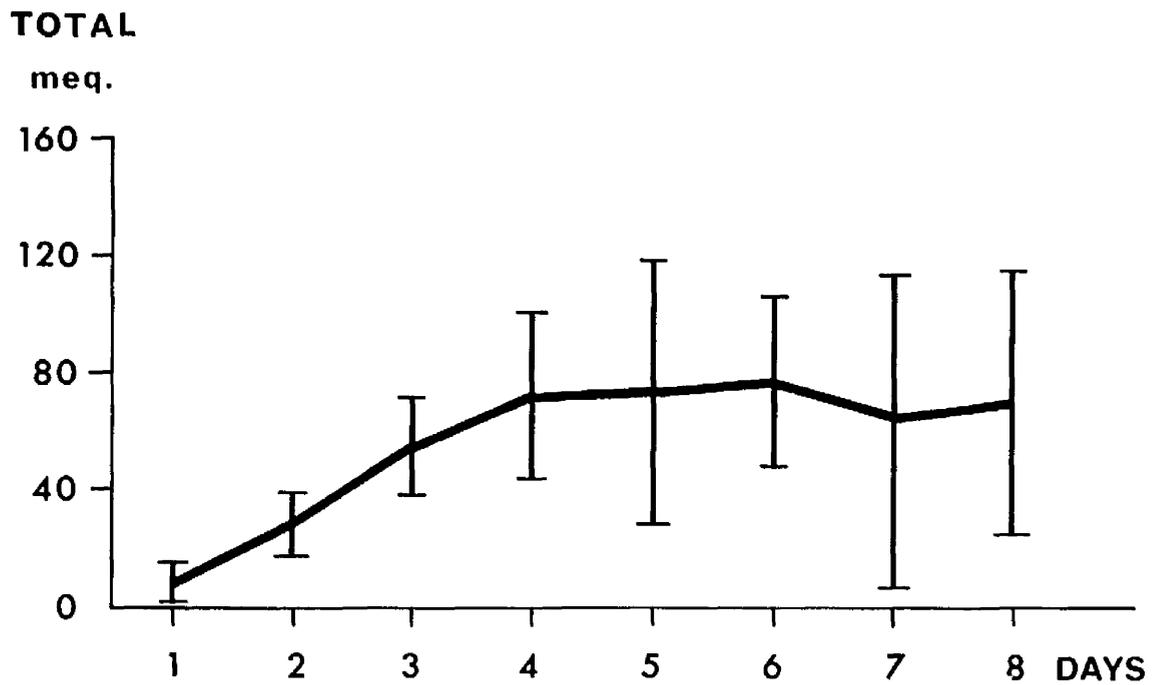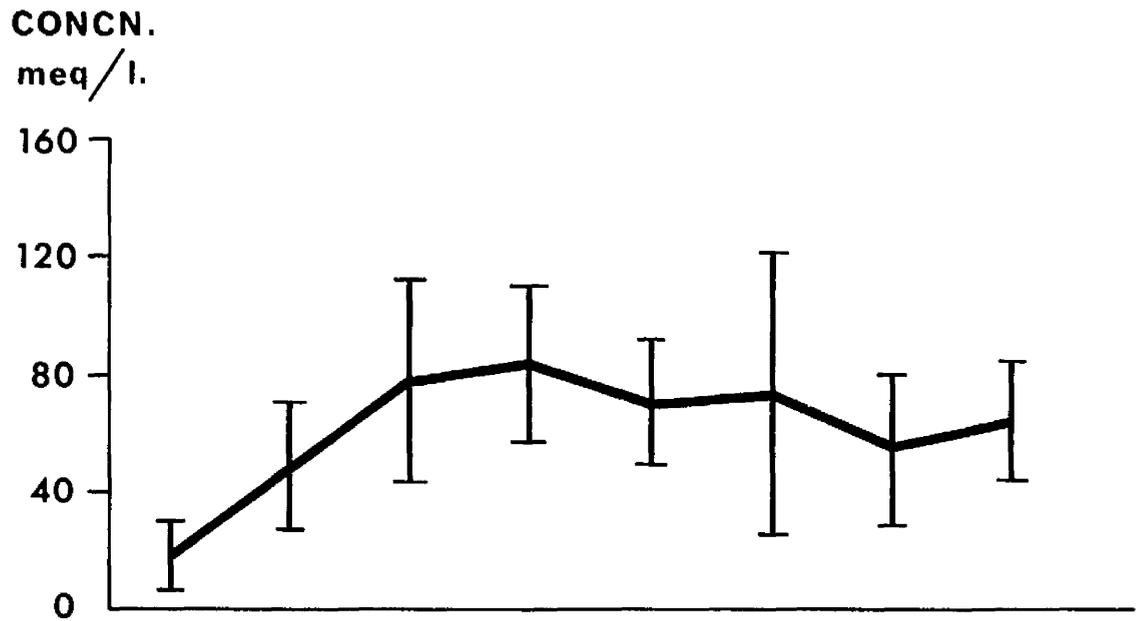
the onset of diuresis.   It would further appear clear
that diuresis was not complete at the end of the study
period in this group.

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

FIG. 9.5.

ACUTE NEPHRITIS
EARLY DIURETIC PHASE

Urinary Potassium : 8 Cases
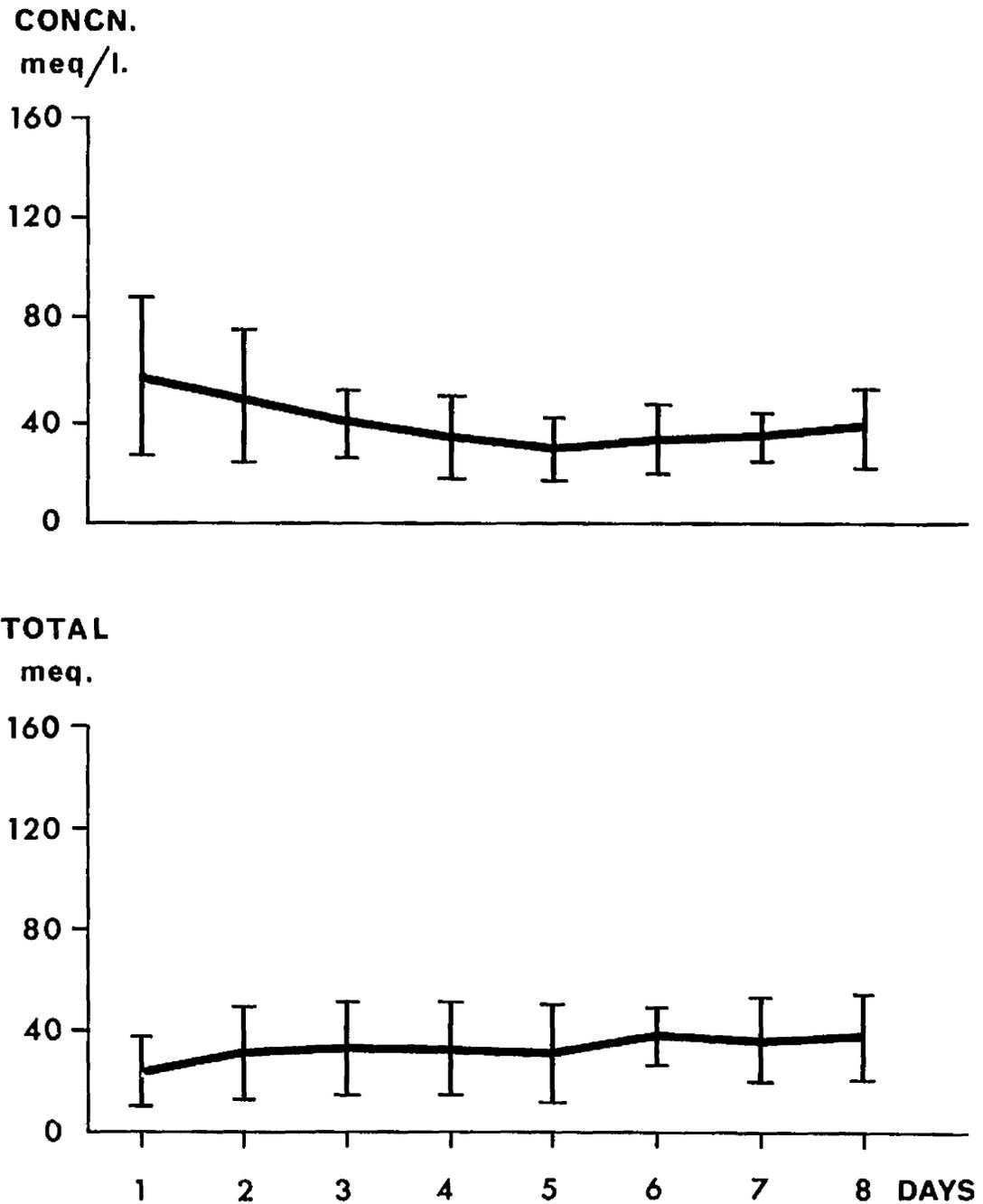
Note relative constancy of potassium excretion as compared with sodium and chloride

EARLY DIURETIC PHASE

Sodium Concn. : 5 Cases

SERUM
meq/l.

URINE
meq/l.
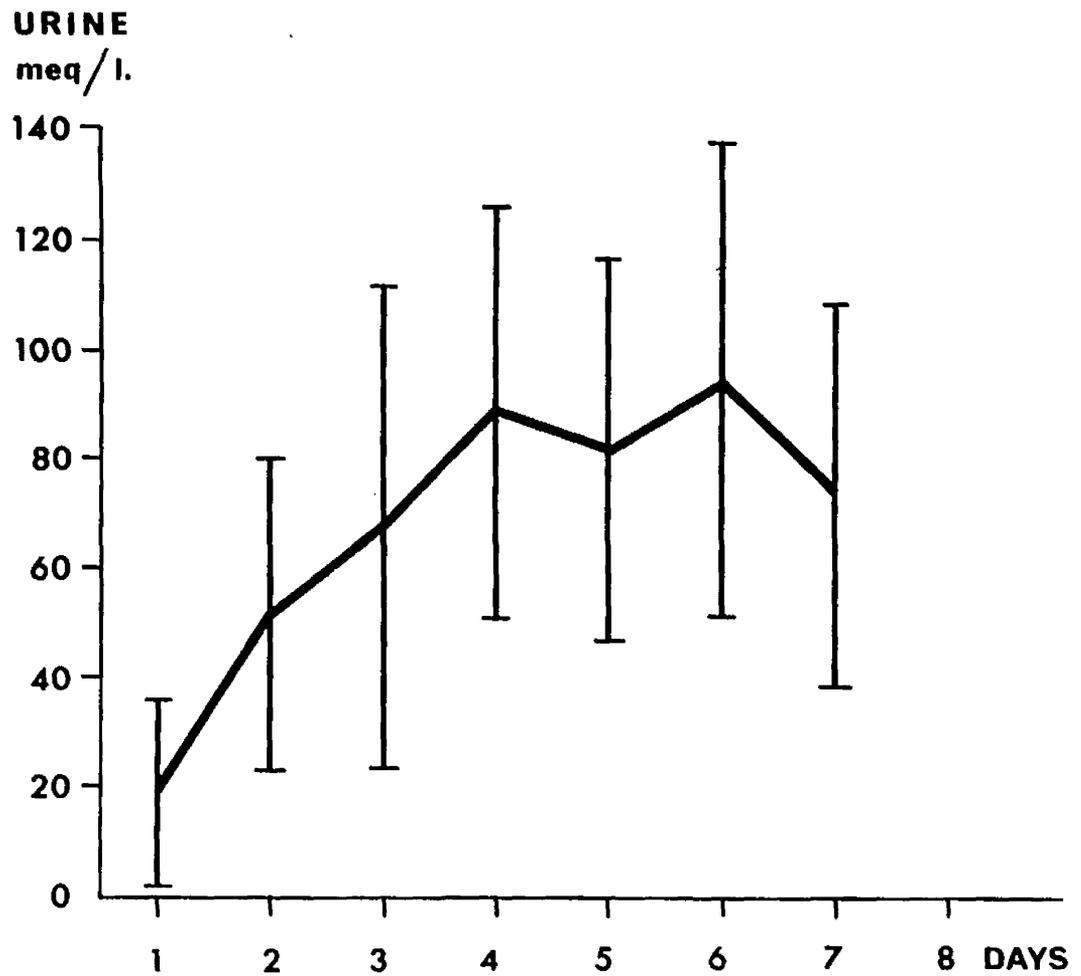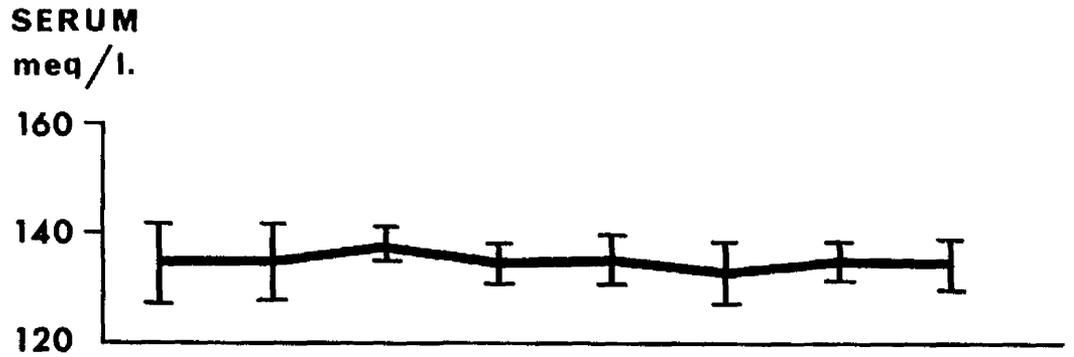
Marked increase in urinary sodium
concentration while the plasma sodium
remains constant

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

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 285 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 Concn. : 5 Cases

FIG. 9.7.

215

ACUTE NEPHRITIS
EARLY DIURETIC PHASE

meq./l. **Serum Calcium : 5 Cases**

meq./l. **Serum Magnesium**
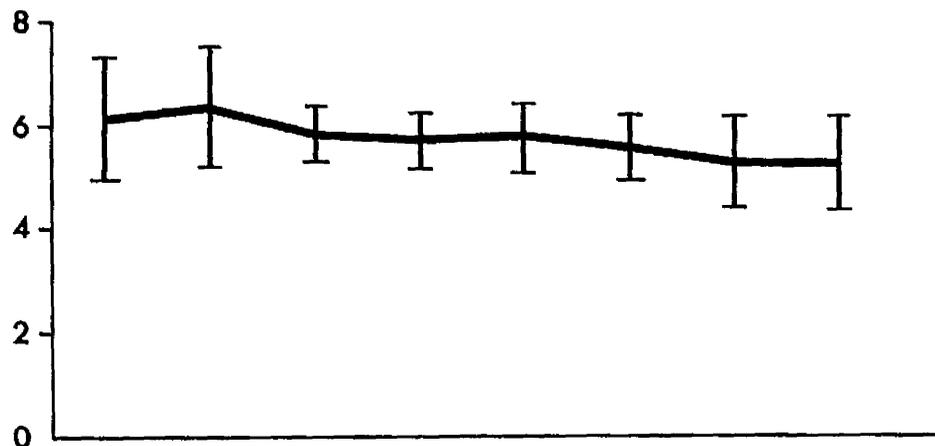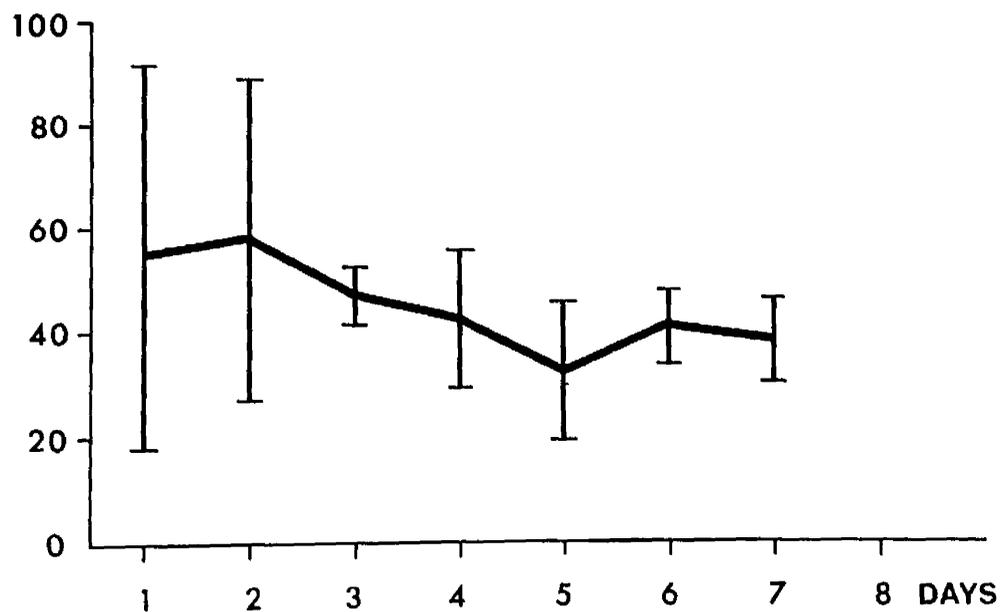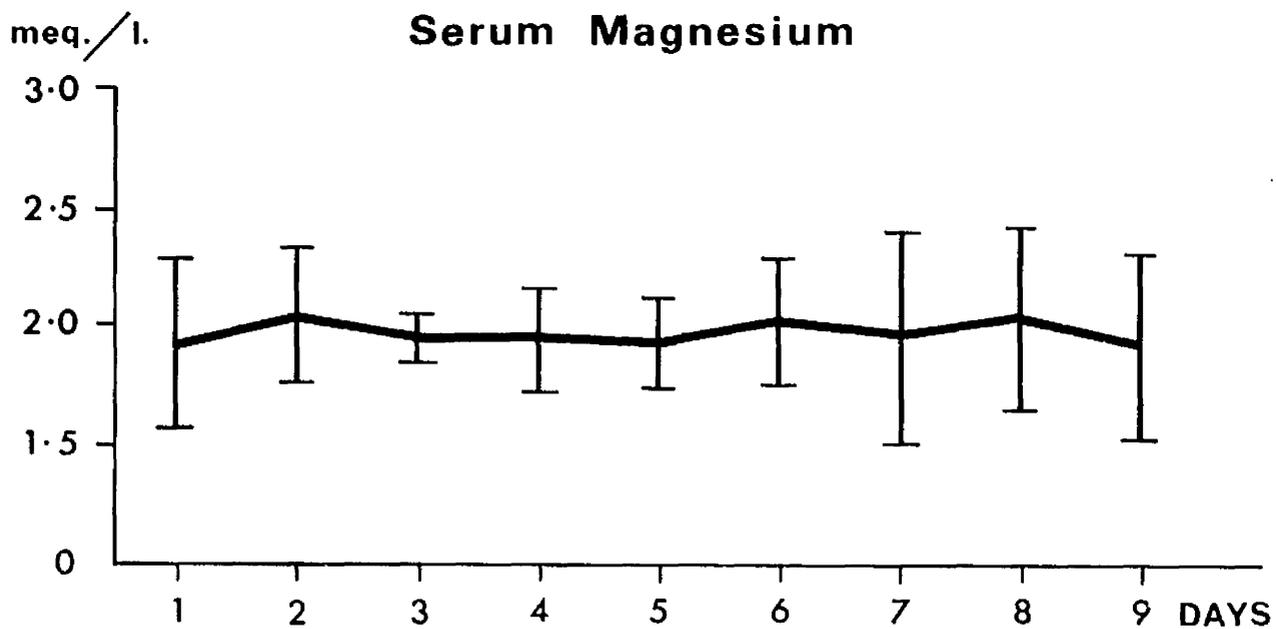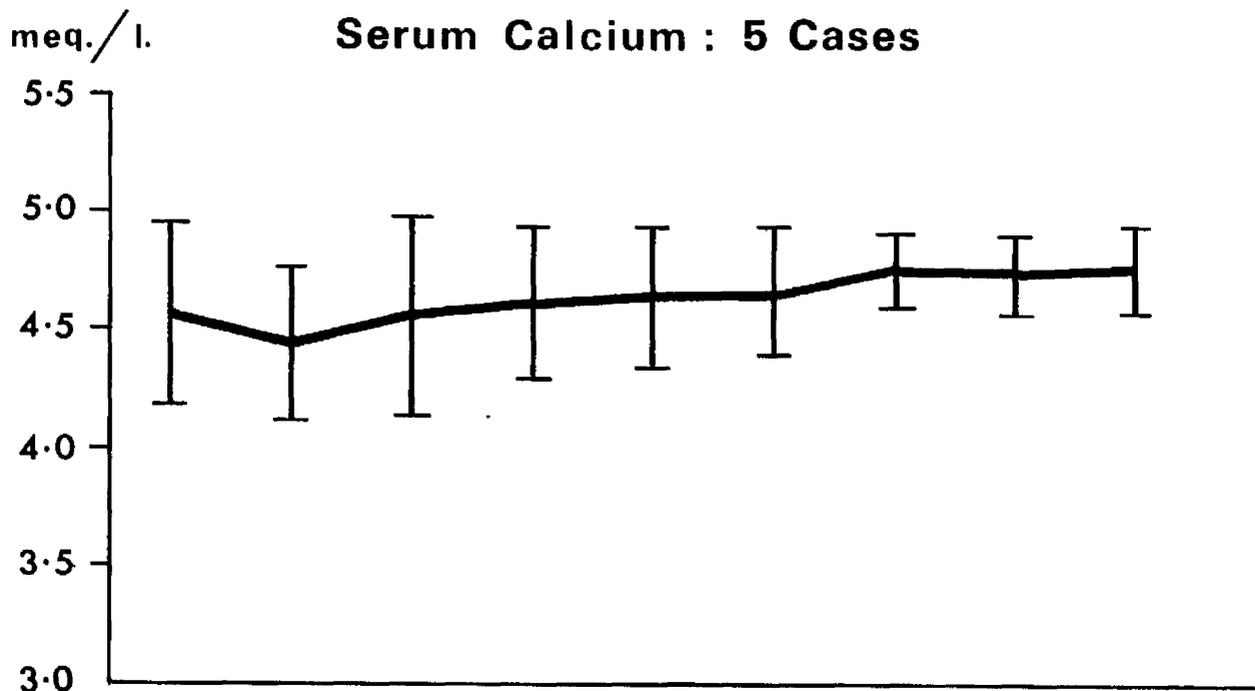
FIG. 9.8.

ACUTE NEPHRITIS
EARLY DIURETIC PHASE

PLASMA UREA
mg./ 100 ml.

5 Cases

FIG. 9.9   217
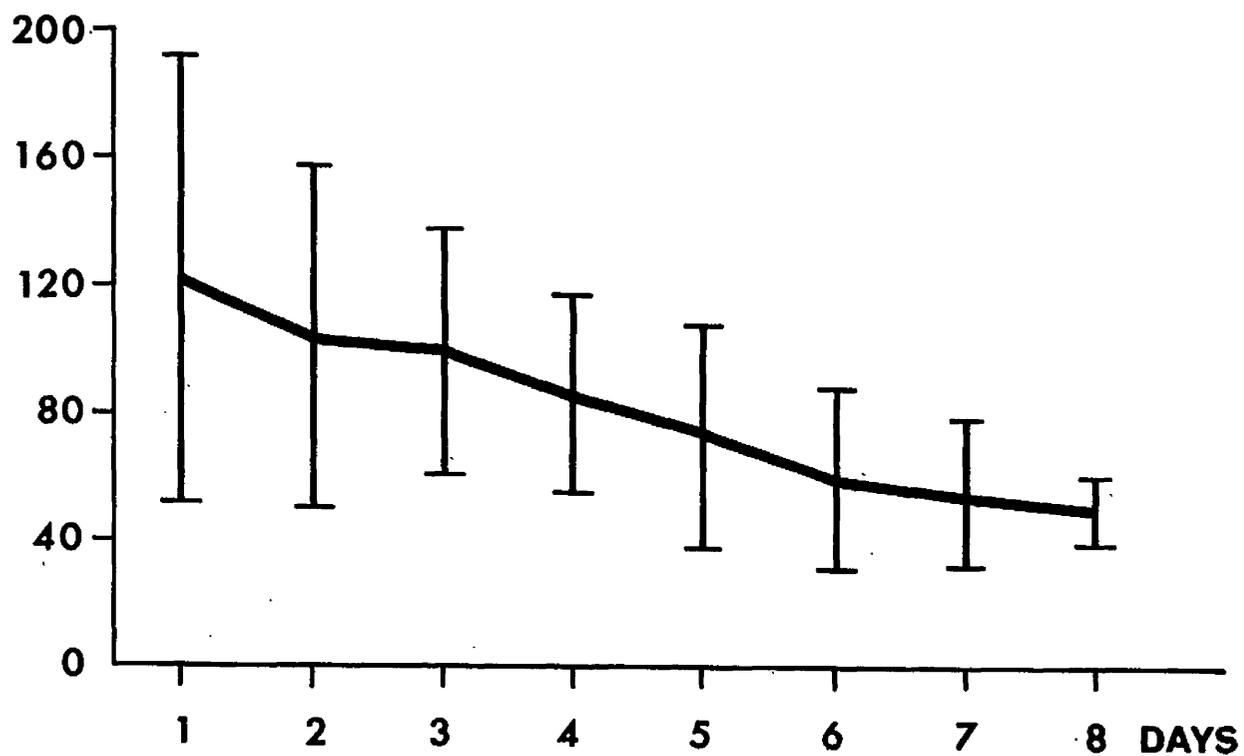
# ACUTE NEPHRITIS

EARLY DIURETIC PHASE

Total Plasma Osmolarity

5 Cases
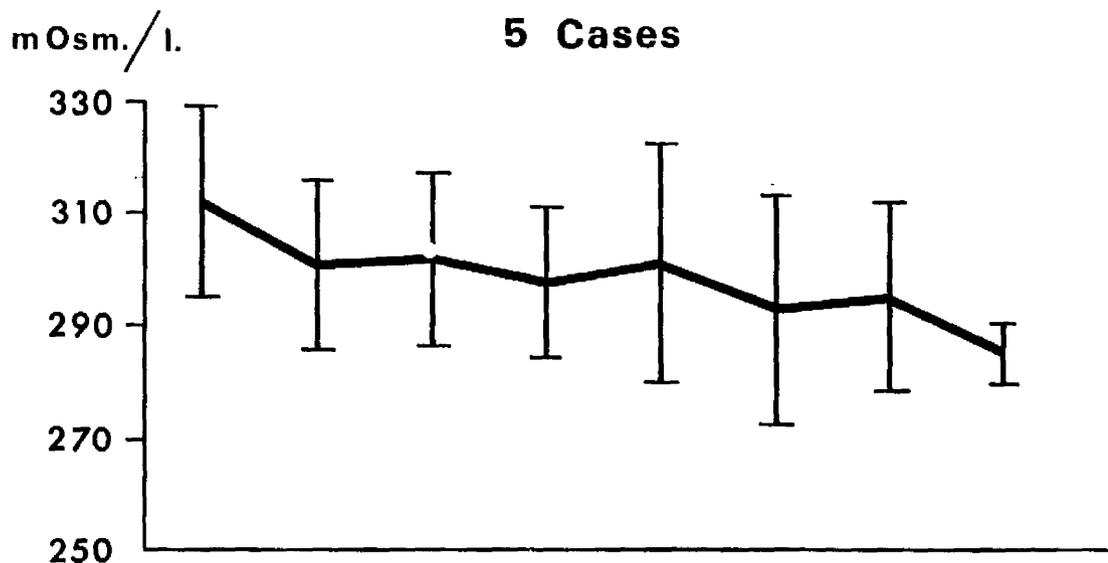
### Osmolarity minus (Urea + Proteins)
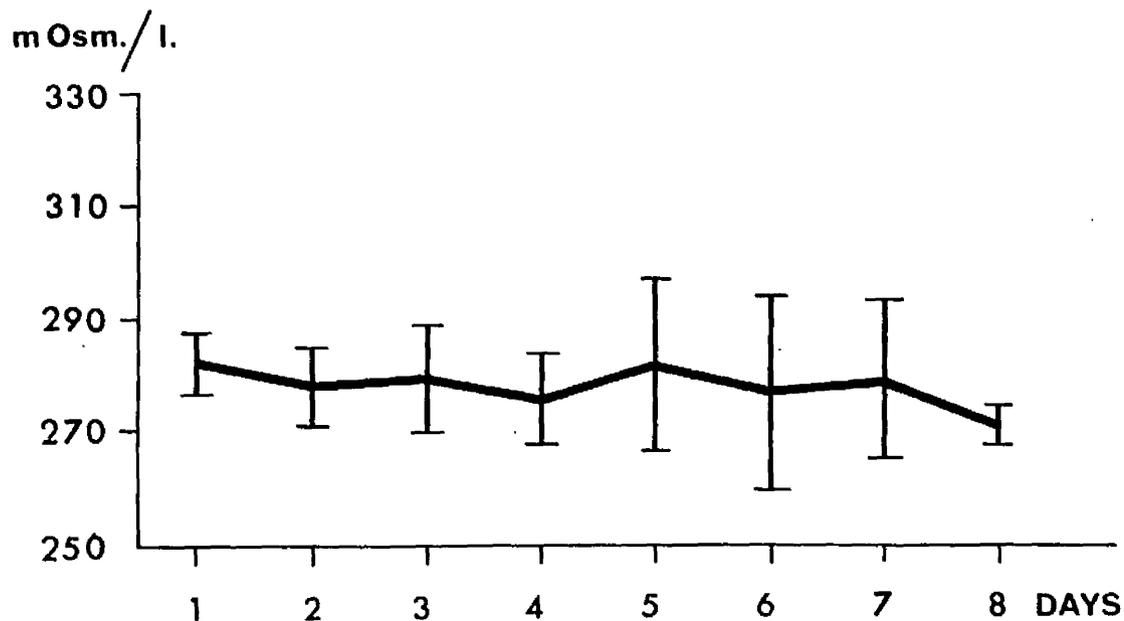
For explanation see text

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
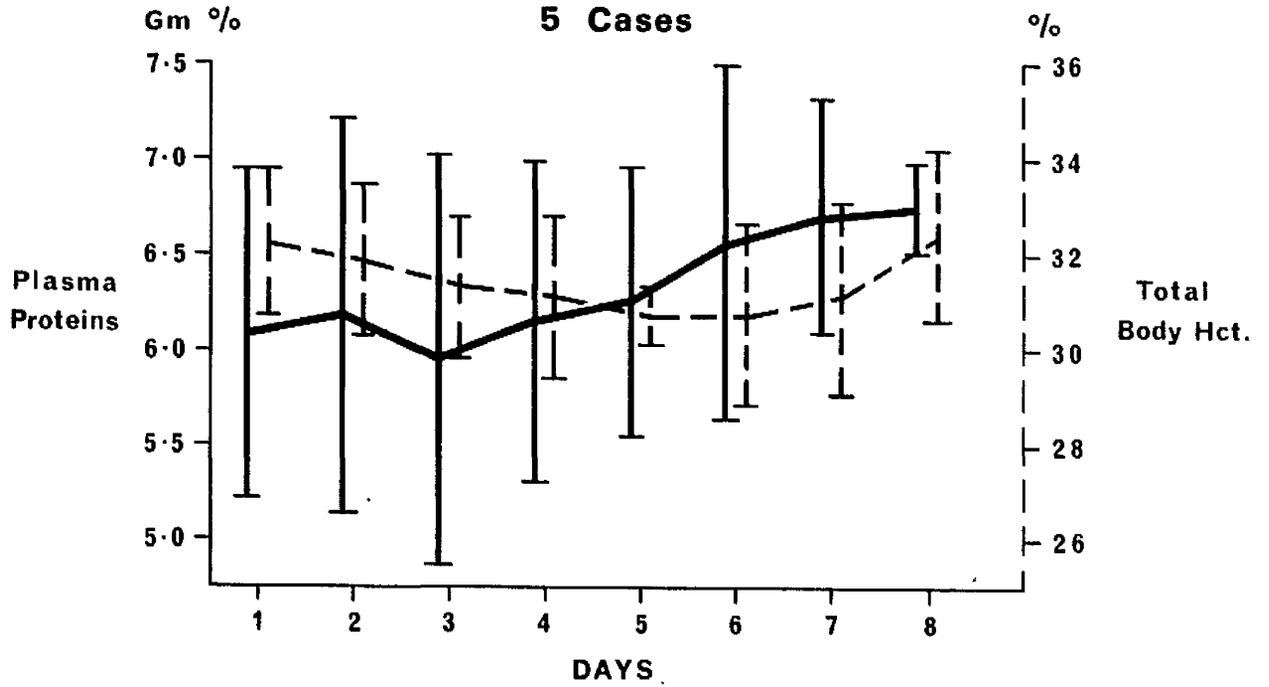
FIG. 9.10   219

ACUTE NEPHRITIS
EARLY DIURETIC PHASE

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

FIG. 9.11

221

ACUTE NEPHRITIS
LATE DIURETIC PHASE

Urine Volume : 5 Cases
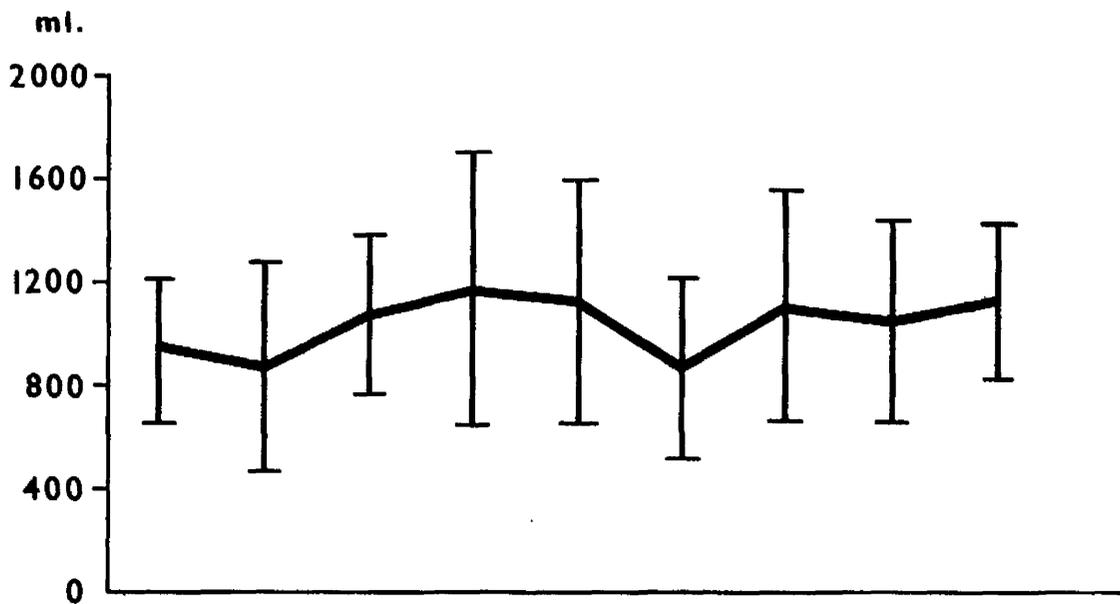
Urine Total Sodium
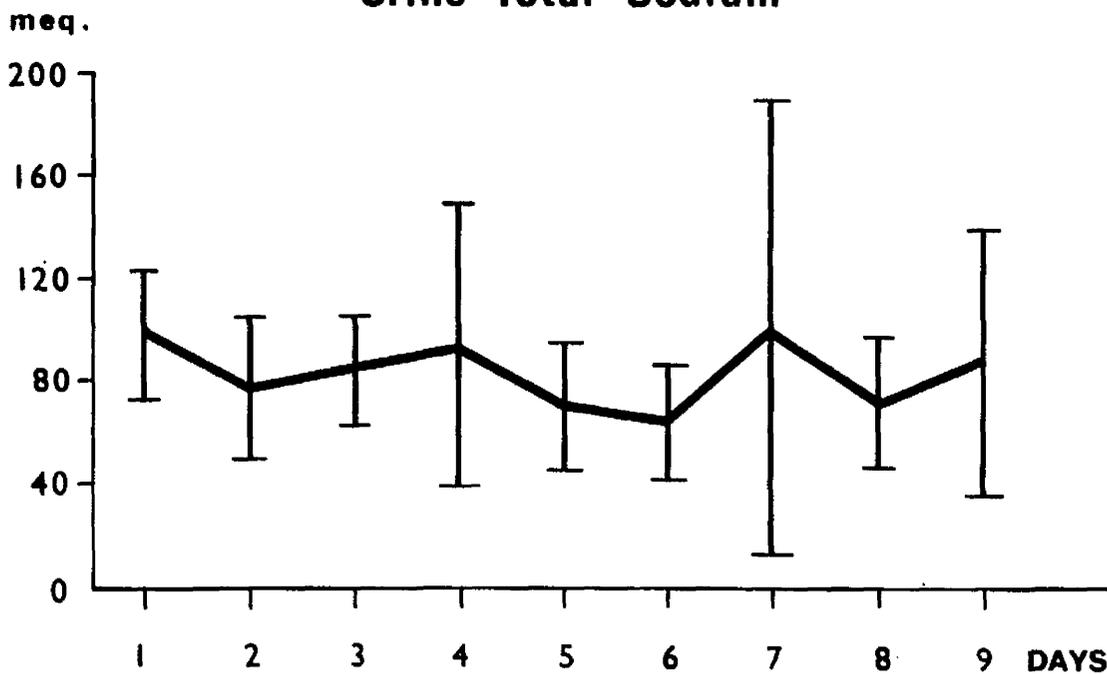
As compared with the early diuretic phase,
no overall pattern is here evident.

FIG. 9.12

ACUTE NEPHRITIS

222

LATE DIURETIC PHASE

Serum Chloride Concn. : 5 Cases
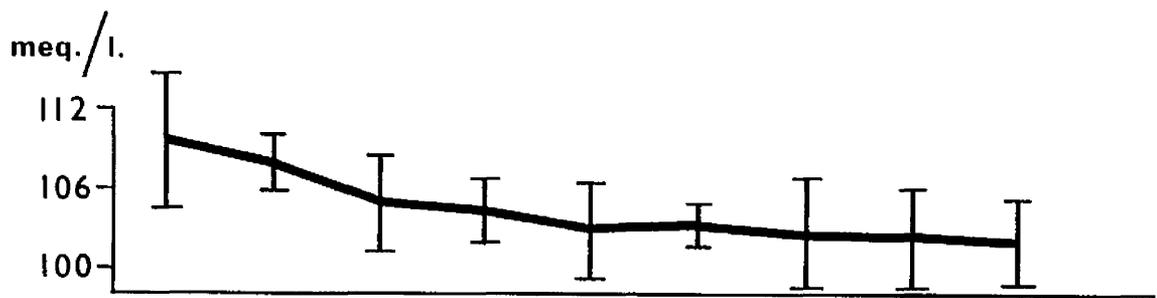
Urine Chloride Concn.
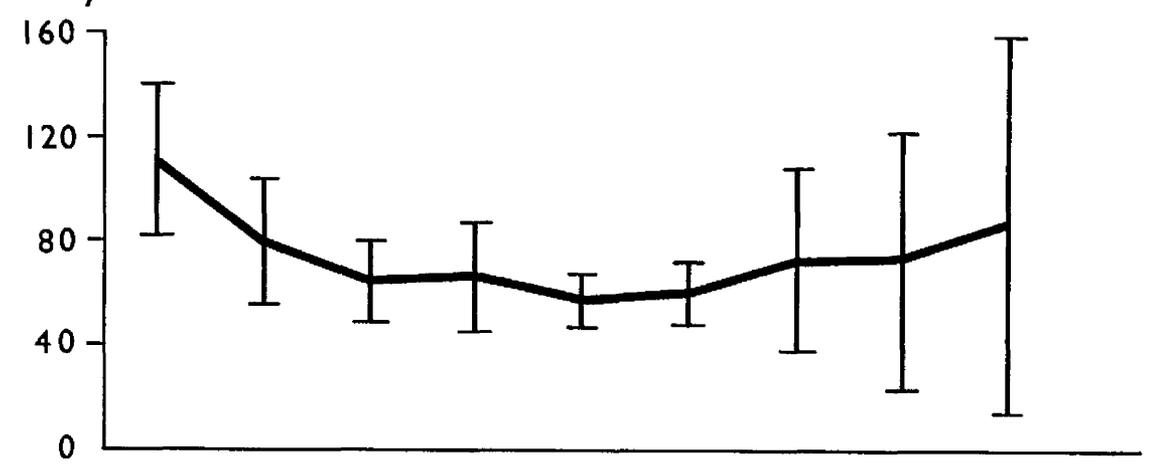
Urine Total Chloride
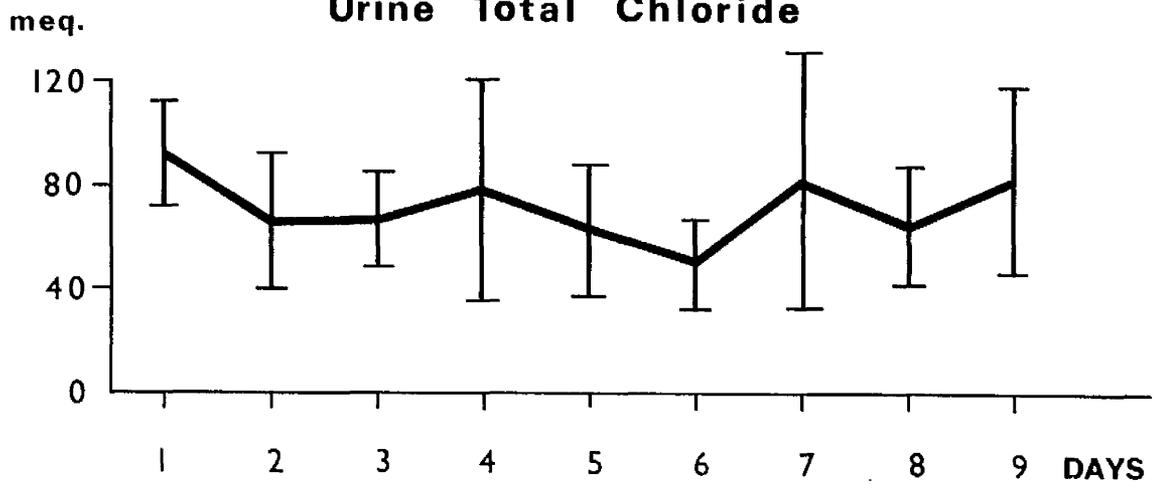
Note progressive fall in serum chloride
level

FIG. 9.13

ACUTE NEPHRITIS
LATE DIURETIC PHASE

Serum Potassium Concn. : 5 Cases

Urine Potassium Concn.

Urine Total Potassium

FIG. 9.14

224
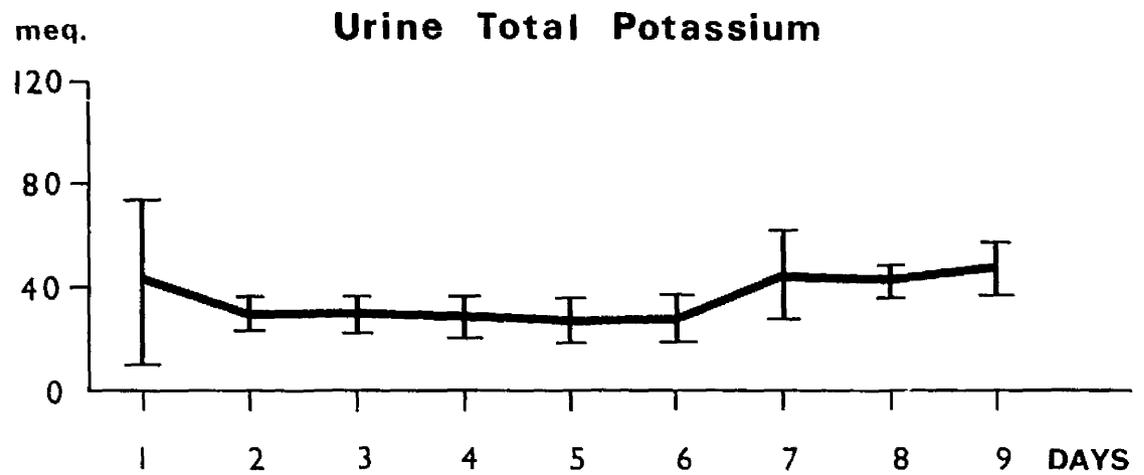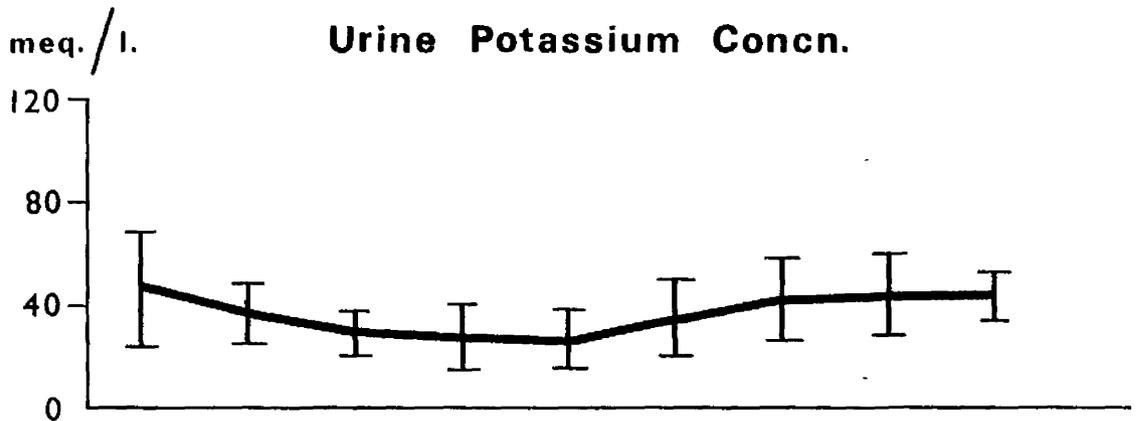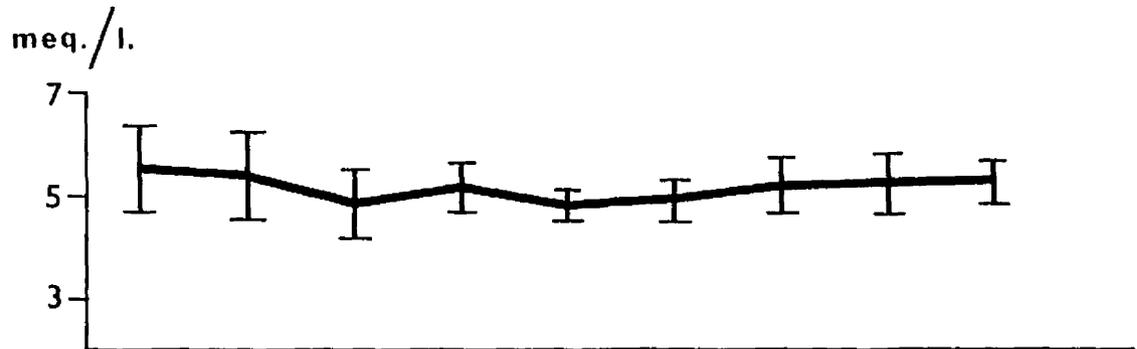
ACUTE NEPHRITIS
LATE DIURETIC PHASE

Serum Sodium Concn. : 5 Cases
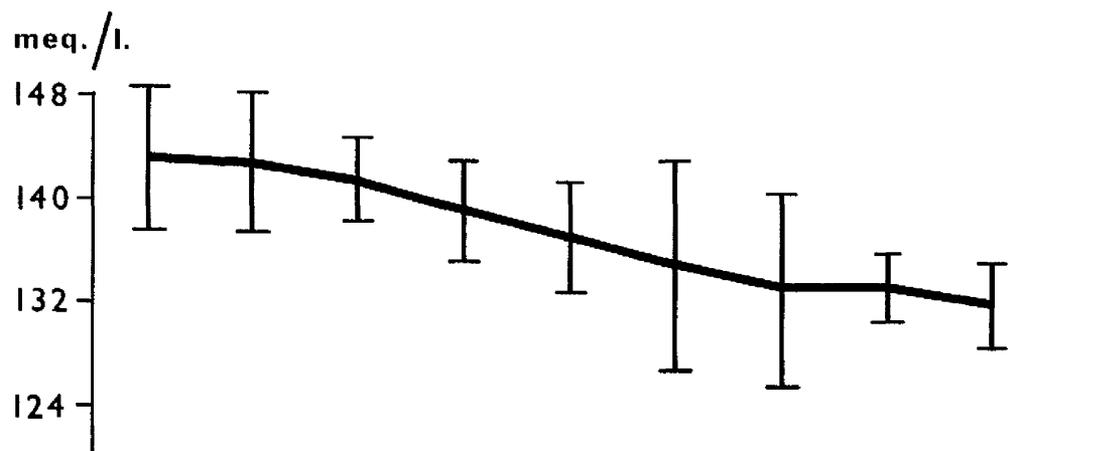
Urine Sodium Concn.
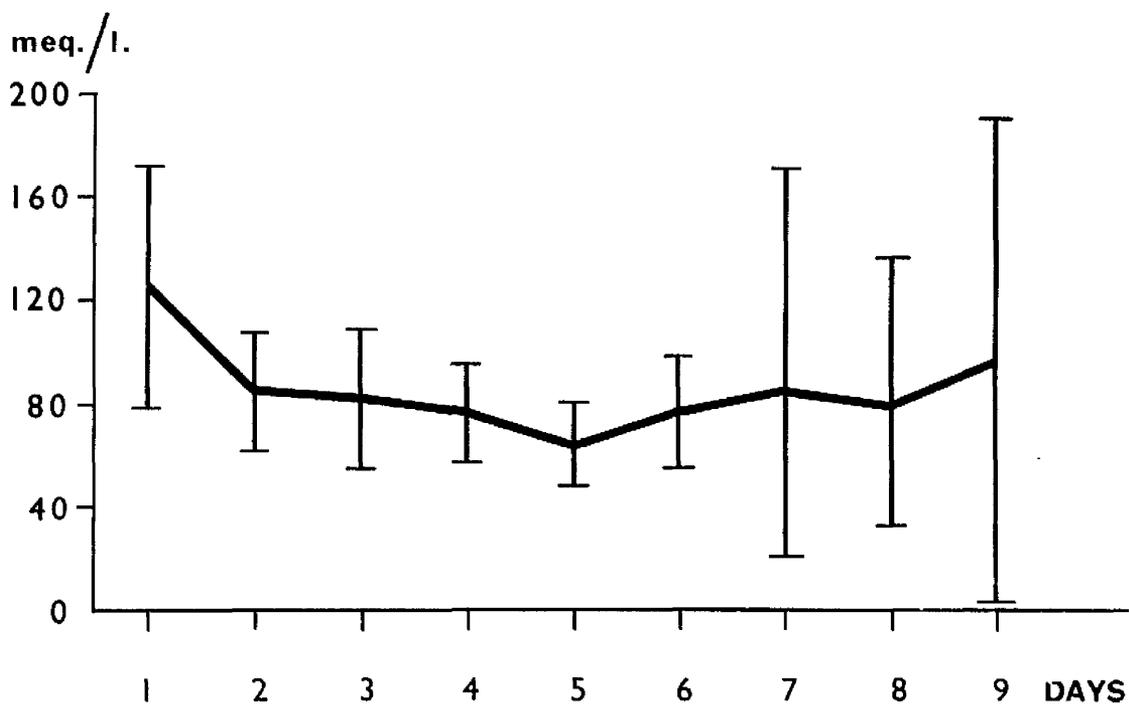
The progressive fall in serum sodium concentration shown here is highly significant.

($r = -0.5973$, $P < 0.001$). 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
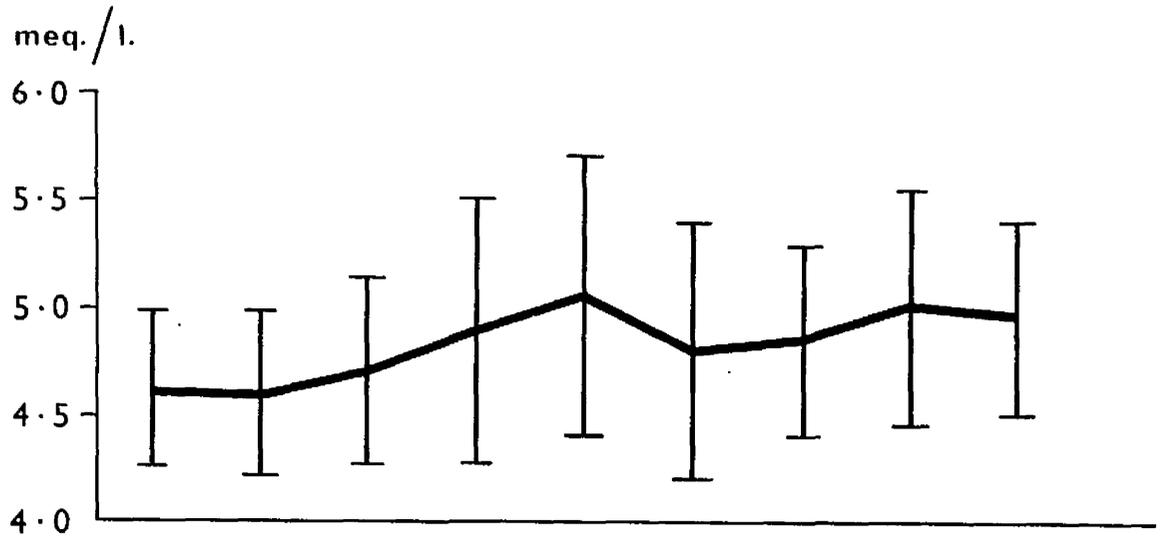
Serum Magnesium
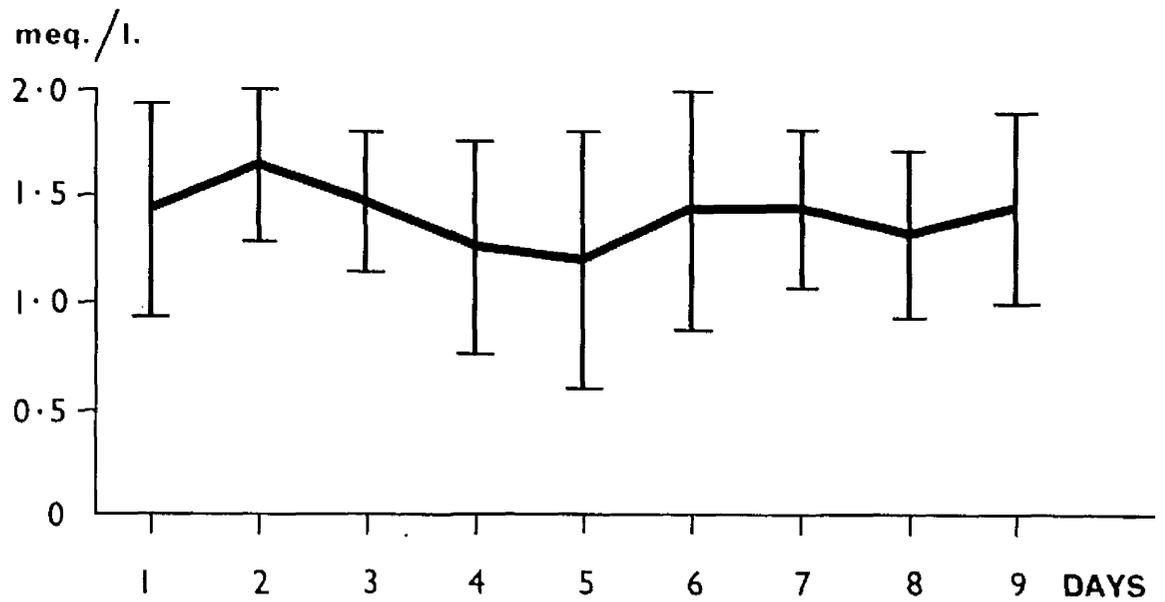
FIG. 9.16

ACUTE NEPHRITIS
LATE DIURETIC PHASE

Plasma Urea : 4 Cases
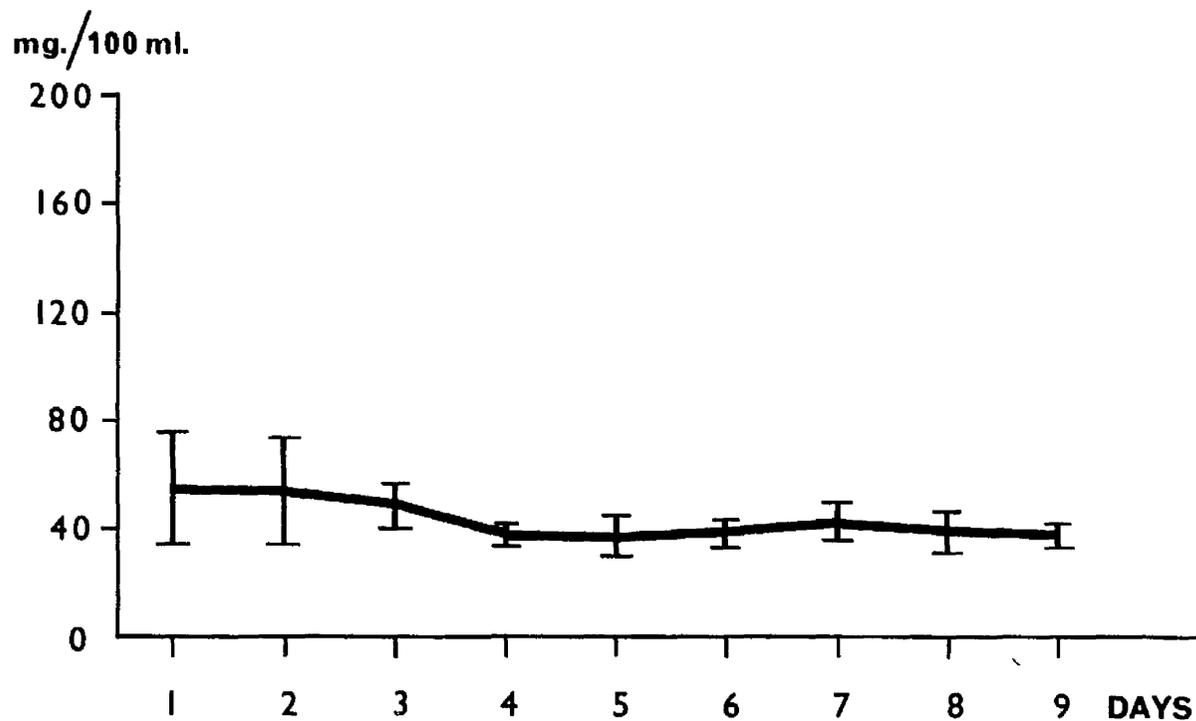
The much lower values shown here should
be compared with those in Fig. 9.8

FIG. 9.17

228

ACUTE NEPHRITIS
LATE DIURETIC PHASE

Total Plasma Osmolarity : 4 Cases
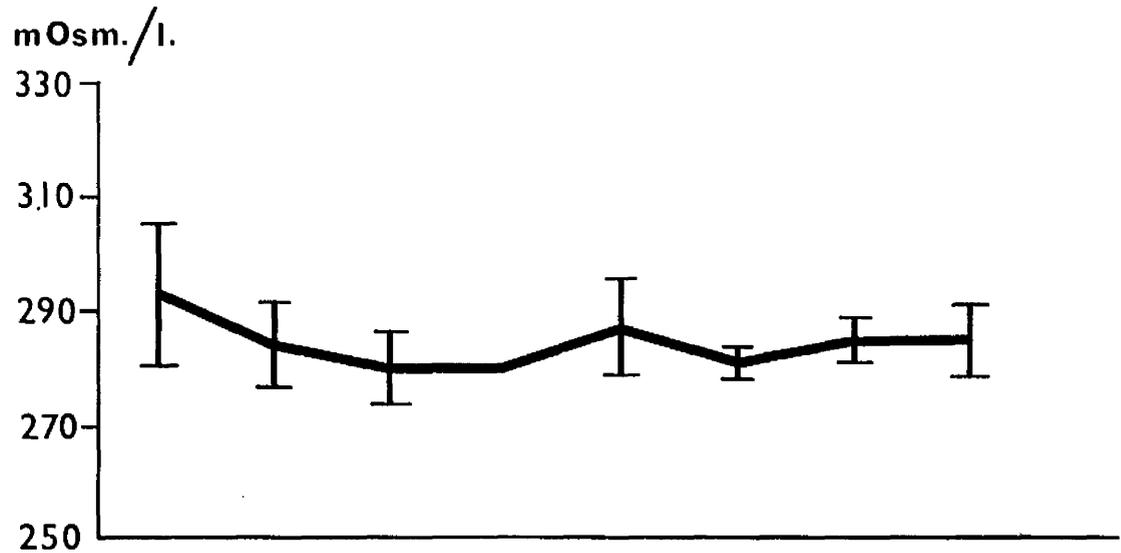
Osmolarity minus (Urea + Proteins)
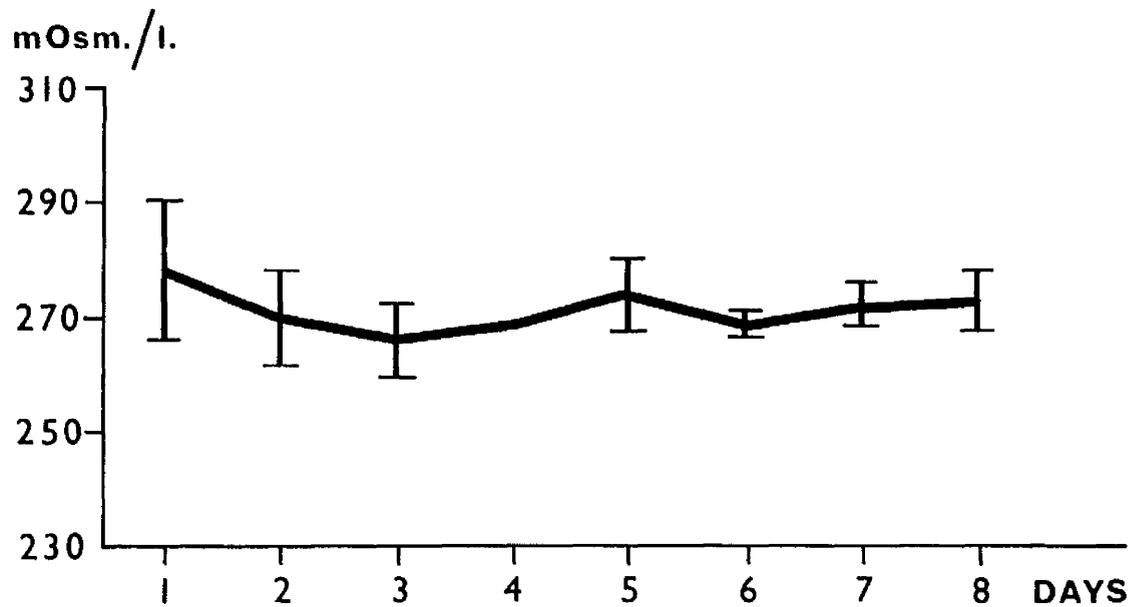
Insufficient observations were obtained for day 4 to render meaningful the use of S.D.

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.

FIG. 9.18

ACUTE NEPHRITIS
LATE DIURETIC PHASE
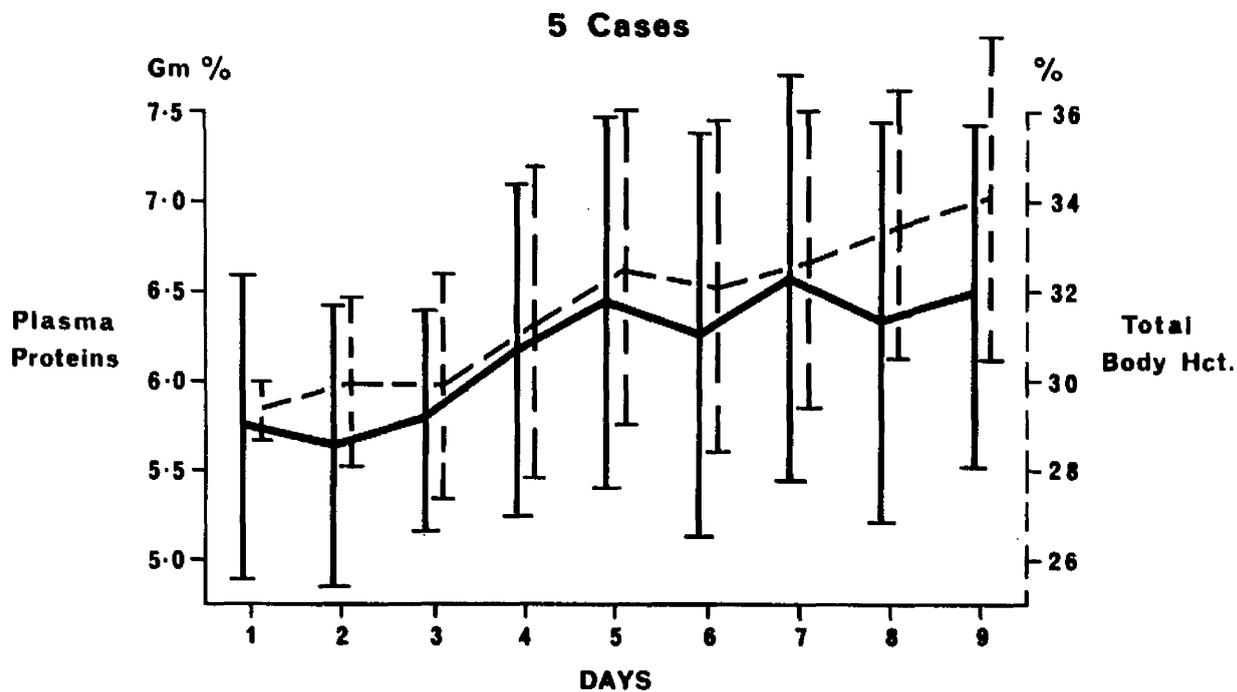
5 Cases

Plasma protein concentrations (solid)
compared with haematocrit determinations
(broken)
The correlation is highly significant
(r = 0.752)
Cf. Figure 9.10

FIG. 9.19

231

ACUTE NEPHRITIS

MEAN % WEIGHT CHANGES
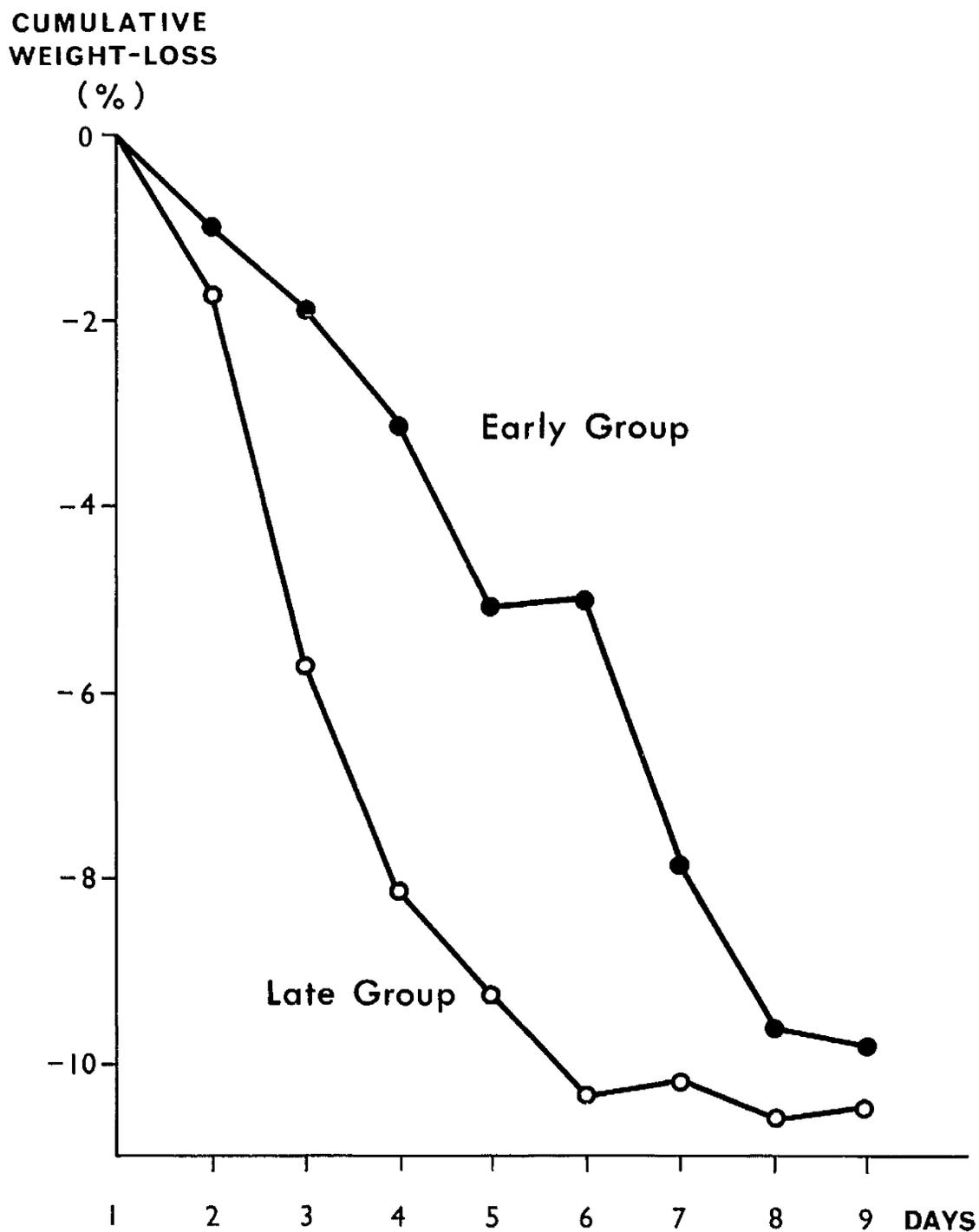
While the ultimate total weight loss of the early and late diuretic groups is similar, the timing of the loss is seen here to be markedly different.

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.

<center>CHAPTER   10</center>

<center>OTHER ASPECTS OF ACUTE GLOMERULONEPHRITIS</center>

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. Chiosura (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 $\pm$ one S.D. are indicated for each fraction and

FIG. 10.1   236

ELECTROPHORETIC PATTERN
2O Normal Children
T. P. = 6·82 ±O·5O
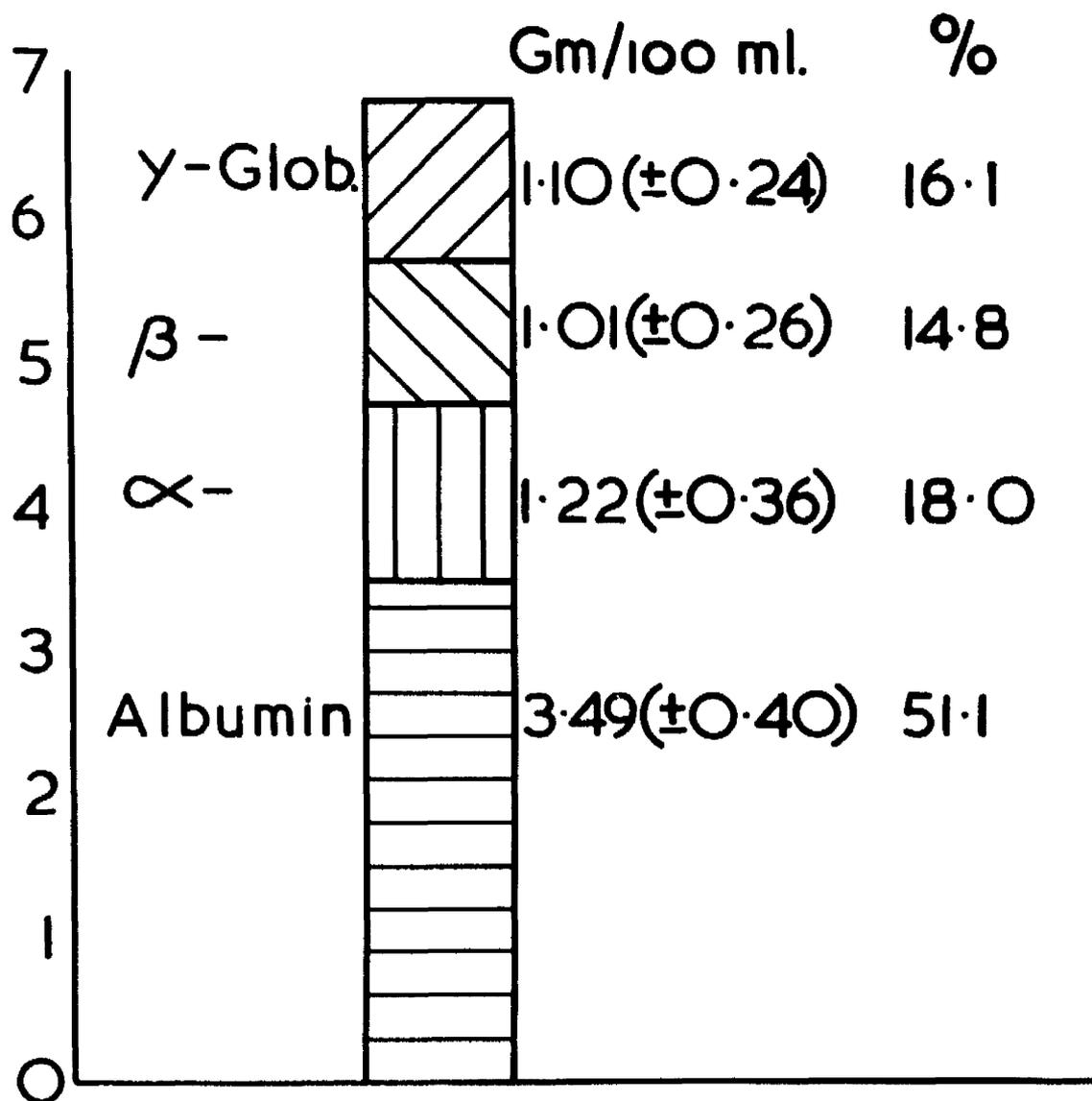
Block diagram representing mean normal electrophoretogram.

compare well with the results of published series.

Where the degree of separation was sufficient to allow separate scanning, the mean α_1-globulin was found to be 0.23 Gm./100 ml. and the mean α_2 0.99 Gm./100 ml., giving a mean α_2/α_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 α-globulin, the β-globulin is normal and the γ-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

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

α-globulin: No significant difference

$(t = 0.086, P > 0.90)$.

FIG. 10.2   238

ACUTE NEPHRITIS

39 Children
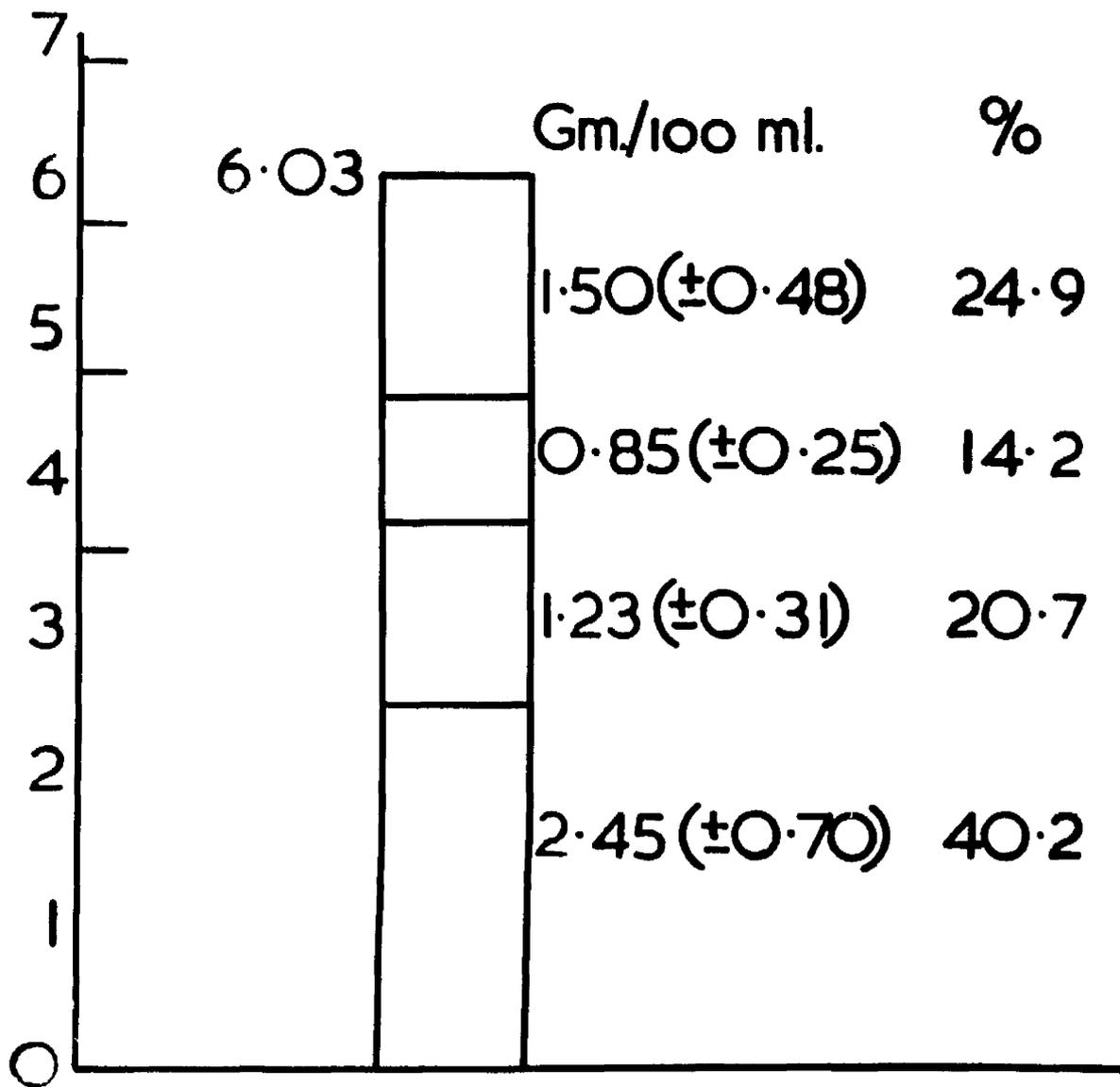
Mean electrophoretogram for 39 children at various stages of AGN. Fractions depicted in same order as in Figure 10.1 Normal pattern is indicated on left.

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

FIG. 10.3

ACUTE NEPHRITIS

A.S.O. & Y-Globulin

r = 0·602
P < 0·001

FIG. 10.4   241
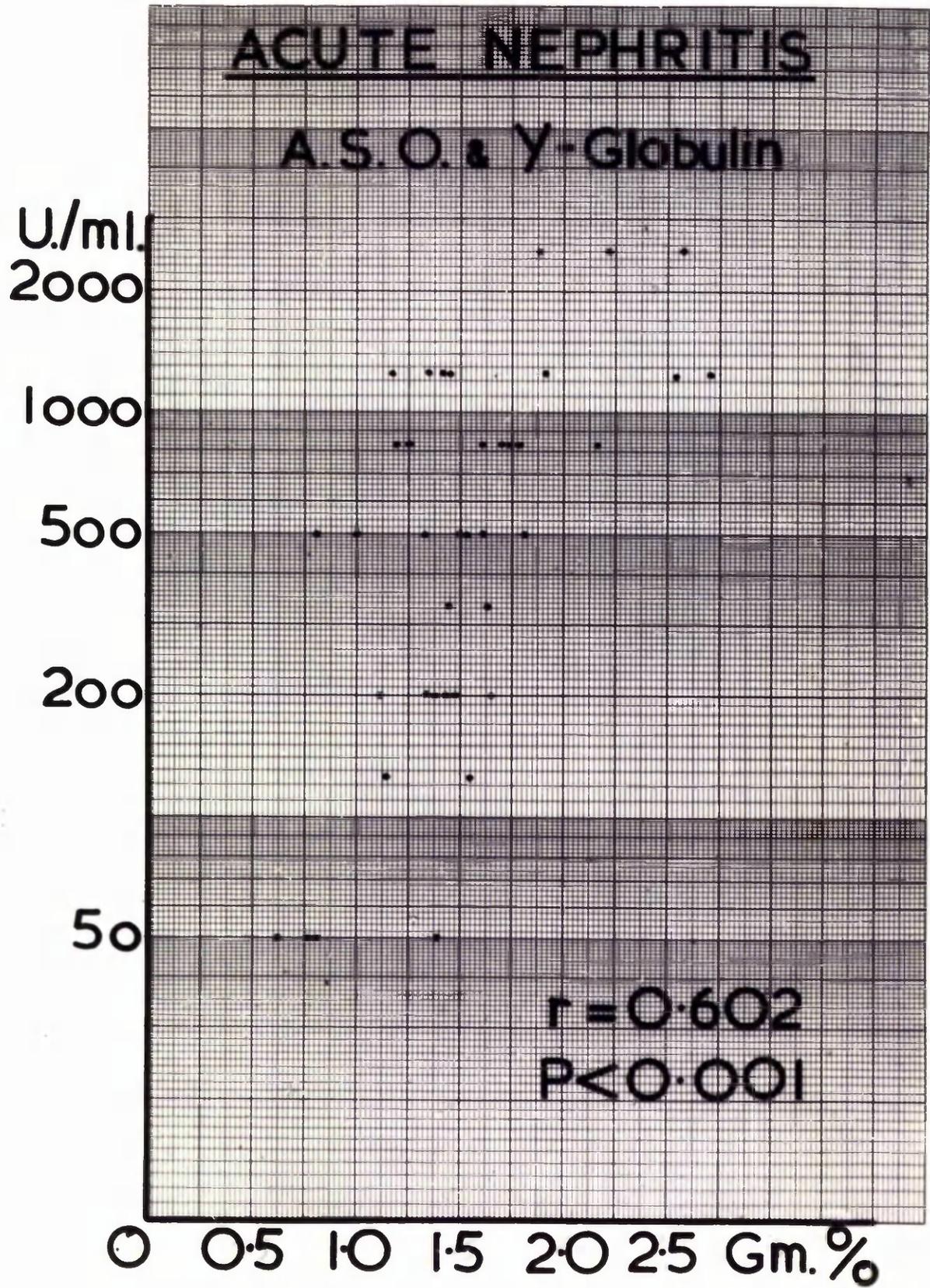
ACUTE NEPHRITIS
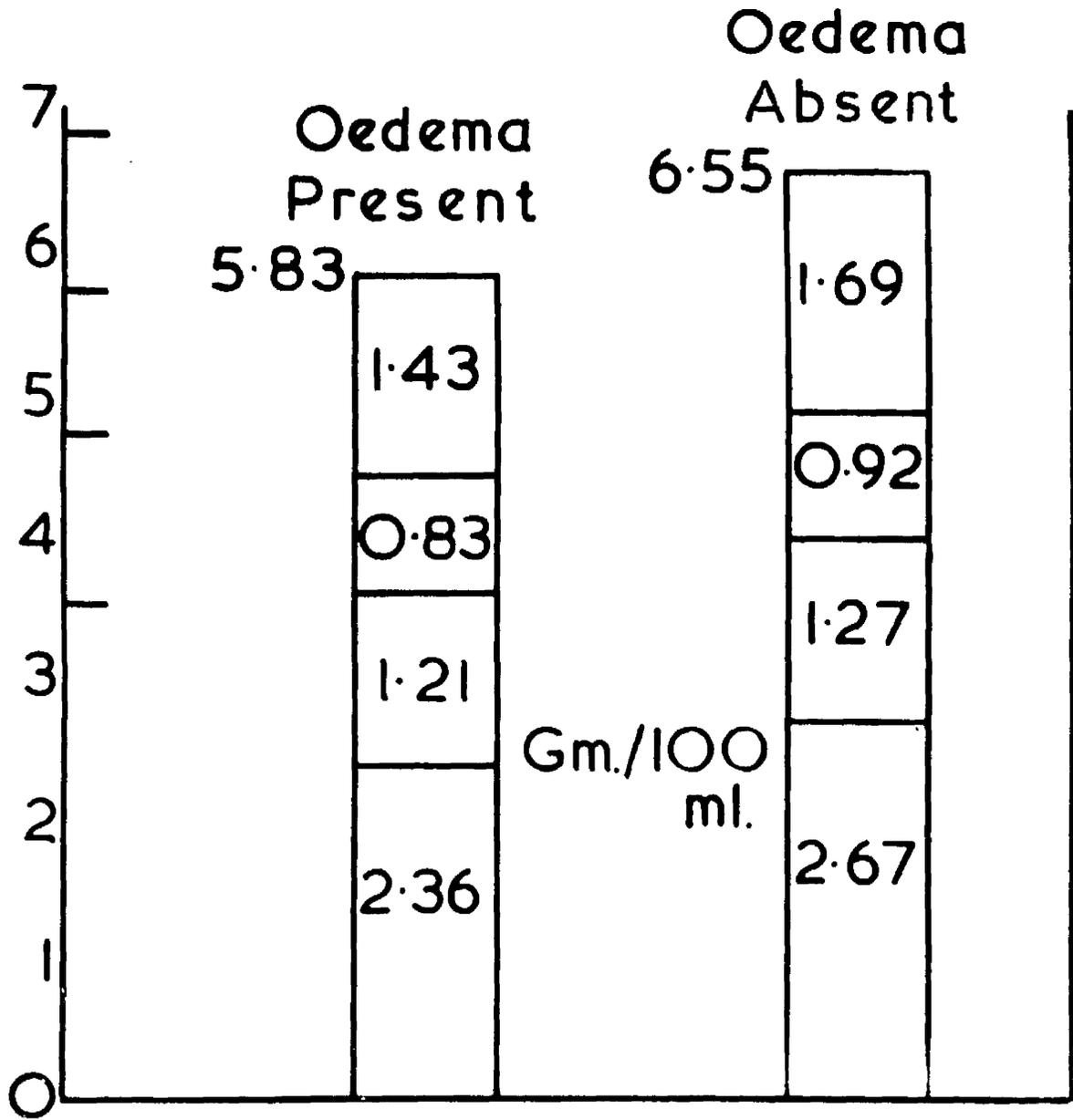
Association with oedema.   Normal
pattern is shown on left-hand scale.

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)

	Oedema present	Oedema absent
Total protein	H.S. Low(< 0.001)	N.S.(> 0.2)
Albumin	H.S. Low(< 0.001)	H.S.Low(< 0.001)
α-globulin	N.S. (> 0.7)	N.S.(> 0.6)
β-globulin	S.Low(> 0.02)	N.S.(> 0.4)
γ-globulin	H.S.High(< 0.005)	H.S.High(< 0.001)

In thirteen of the total of 39 estimations adequate
separation of the α-globulin into α_1 and α_2 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 α_1 fraction with
reduction of the α_2. 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

CHANGES IN α_2/α_1 RATIO

	Mean ($\pm$ S.E.)
AGN (13 cases)	2.6 ($\pm$ 0.34)
Normals (10 cases)	4.3 ($\pm$ 0.47)
AGN - Oedema present (8 cases)	2.4 ($\pm$ 0.35)
AGN - Oedema absent (5 cases)	2.9 ($\pm$ 0.74)

P < 0.01
P < 0.005
N.S.

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, β- and γ-globulins all
increase, while the α-globulin decreases slightly.
The percentage increases of the β- and γ-globulins
are very similar being 7% and 4% respectively.   The
increase of albumin is somewhat greater - 27%.

FIG. 10.5.   244

ACUTE NEPHRITIS

Changes with Diuresis
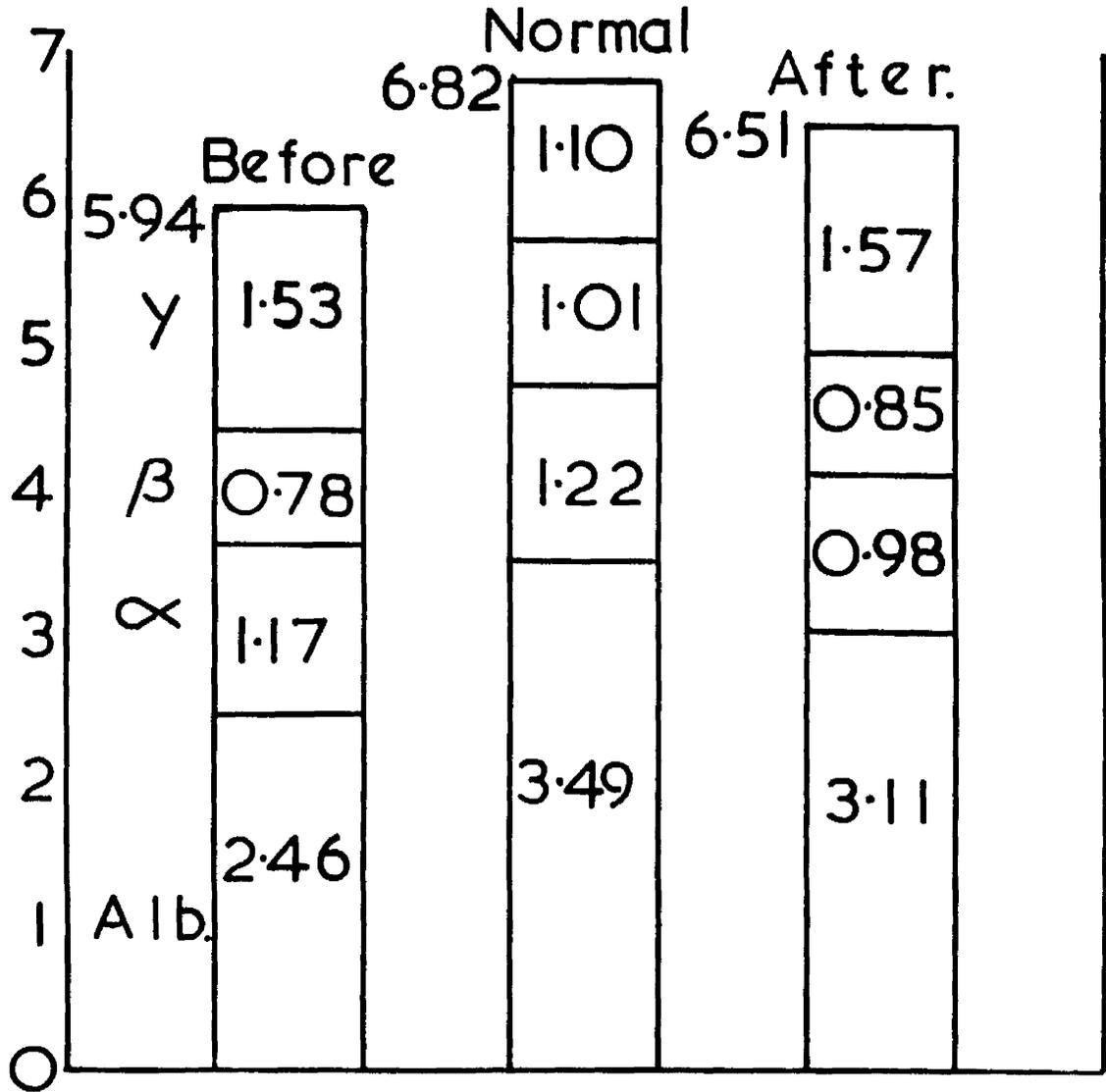
It may be presumed that a number of factors
are likely to be concerned in these changes.   For
example the alteration of plasma volume taking place
with diuresis is certainly of importance.   A
reduction of the water dilution factor would be expected
to cause a uniform rise of all the protein fractions.
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 $\beta-$ and
$\gamma-$globulin fractions rise less than would be expected,
albumin rises rather more, but that the behaviour of
the $\alpha-$fraction is quite different in that it
decreases below the level of the pre-diuretic value.

FIG. 10.6.   246

ACUTE NEPHRITIS

Plasma Protein Changes
14 Children
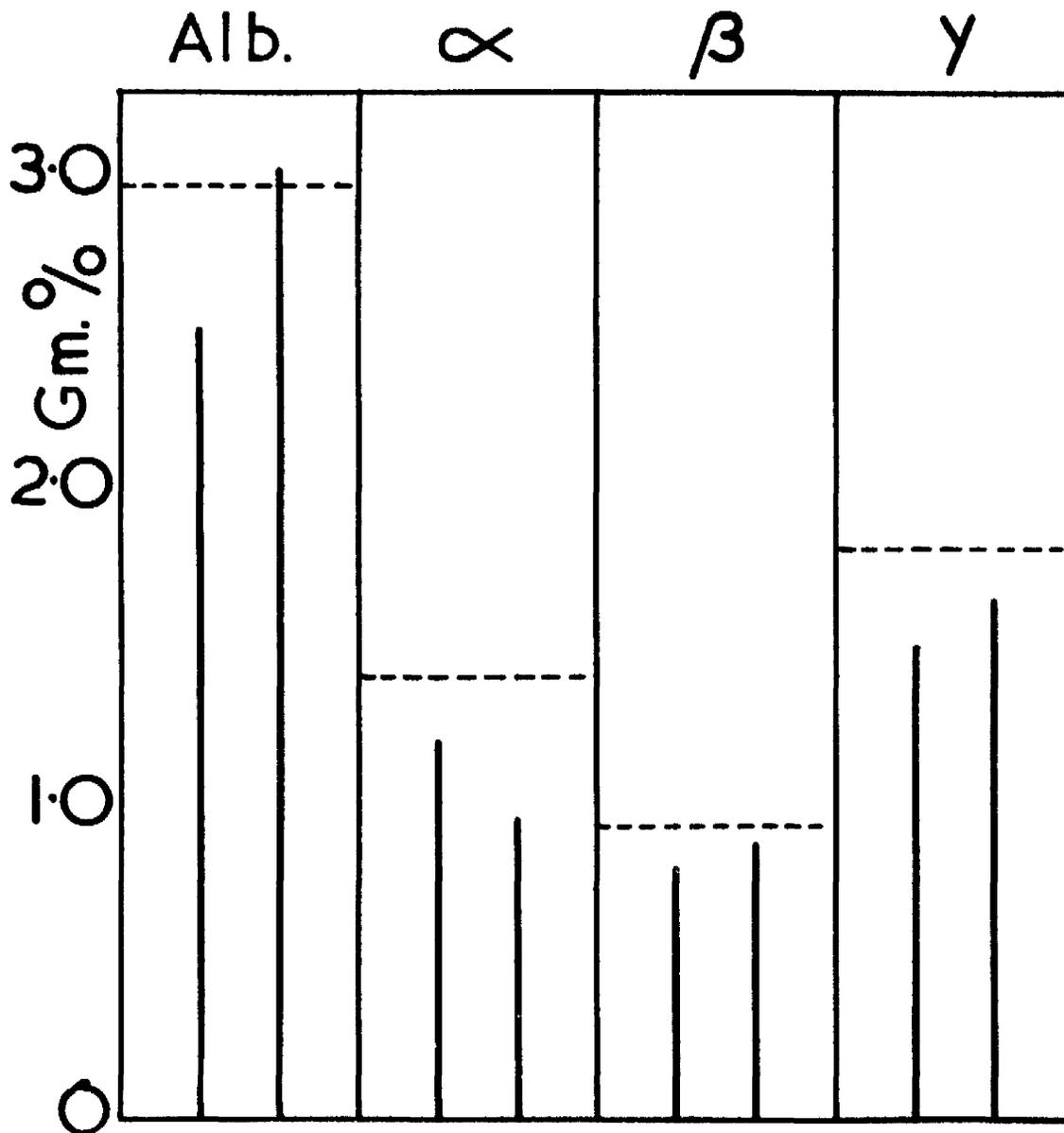
For each protein fraction, the left-hand
vertical line represents the mean pre-
diuretic level; the right-hand lines
represent the levels following diuresis.
The broken horizontal lines represent the
levels to be expected after diuresis
assuming an average plasma volume
decrease of 18%.

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
α-globulin together with a raised   γ-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
(1) 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 1, s and d are measured in cms.,

V (heart volume in ml.) $= 1 \times s \times d \times K$

K is a factor which includes the ellipsoid
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 $\pm$ 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

FIG. 11.1

(a)
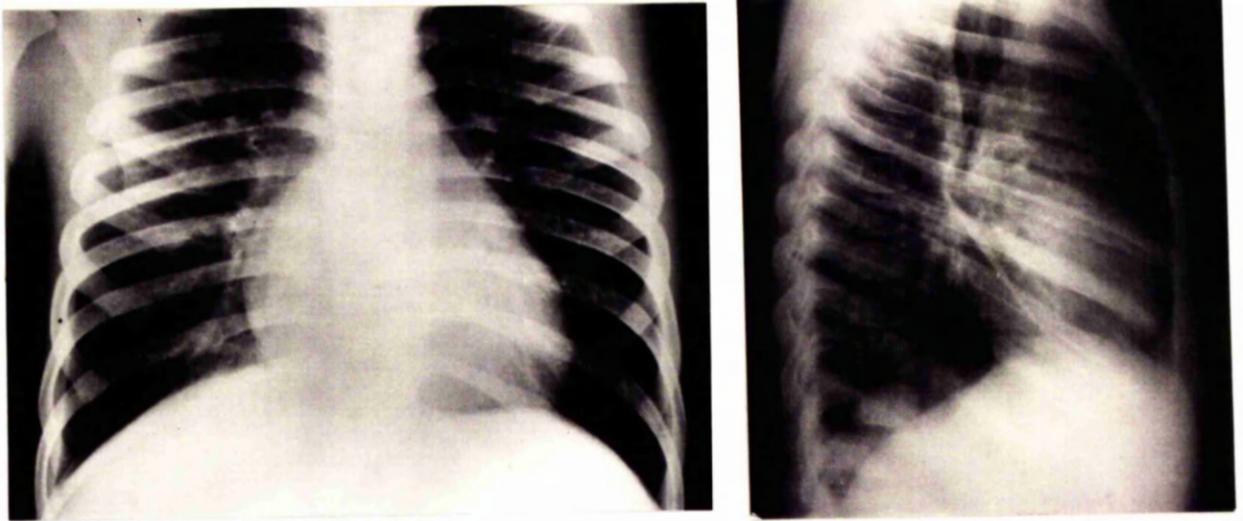
(b)
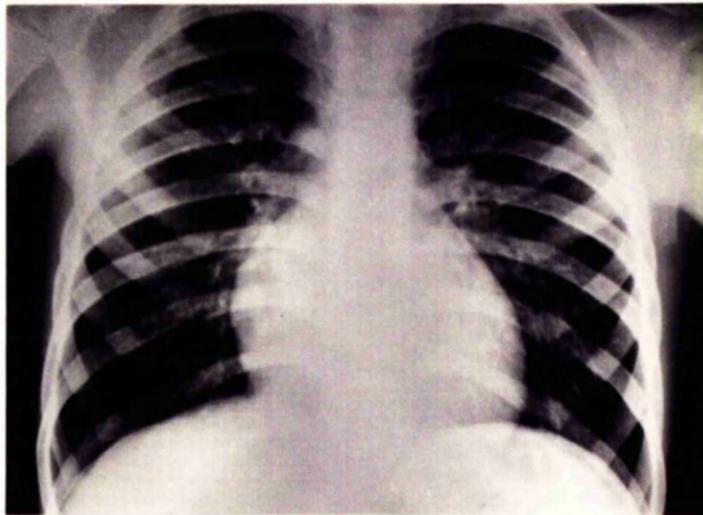
(a) Oedematous phase: cardiomegaly and
     pleural transudates are clearly
     visible.

(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.

Category	Numbers of Children	
	Oedema absent	Oedema present
Normal	25	13
Heart +. Lungs 0.	4	7
Heart 0; Lungs: V+	0	3
Heart 0; Lungs: PF+	0	2
Heart 0; V+, PF+	0	2
Heart +; Lungs: V+	2	7
Heart +; Lungs: PF+	1	8
Heart +; V+ PF+	0	15
Totals	32	57

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)

Heart Volume I (ml.)	Heart Volume II (ml.)
352 (209-552)	285 (192-452)
Mean difference =	67 ml. (19.1%)
$t = 6.921$	$P < 0.001$

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

FIG. 11.2. 257

ACUTE NEPHRITIS

Changes in Heart Volume
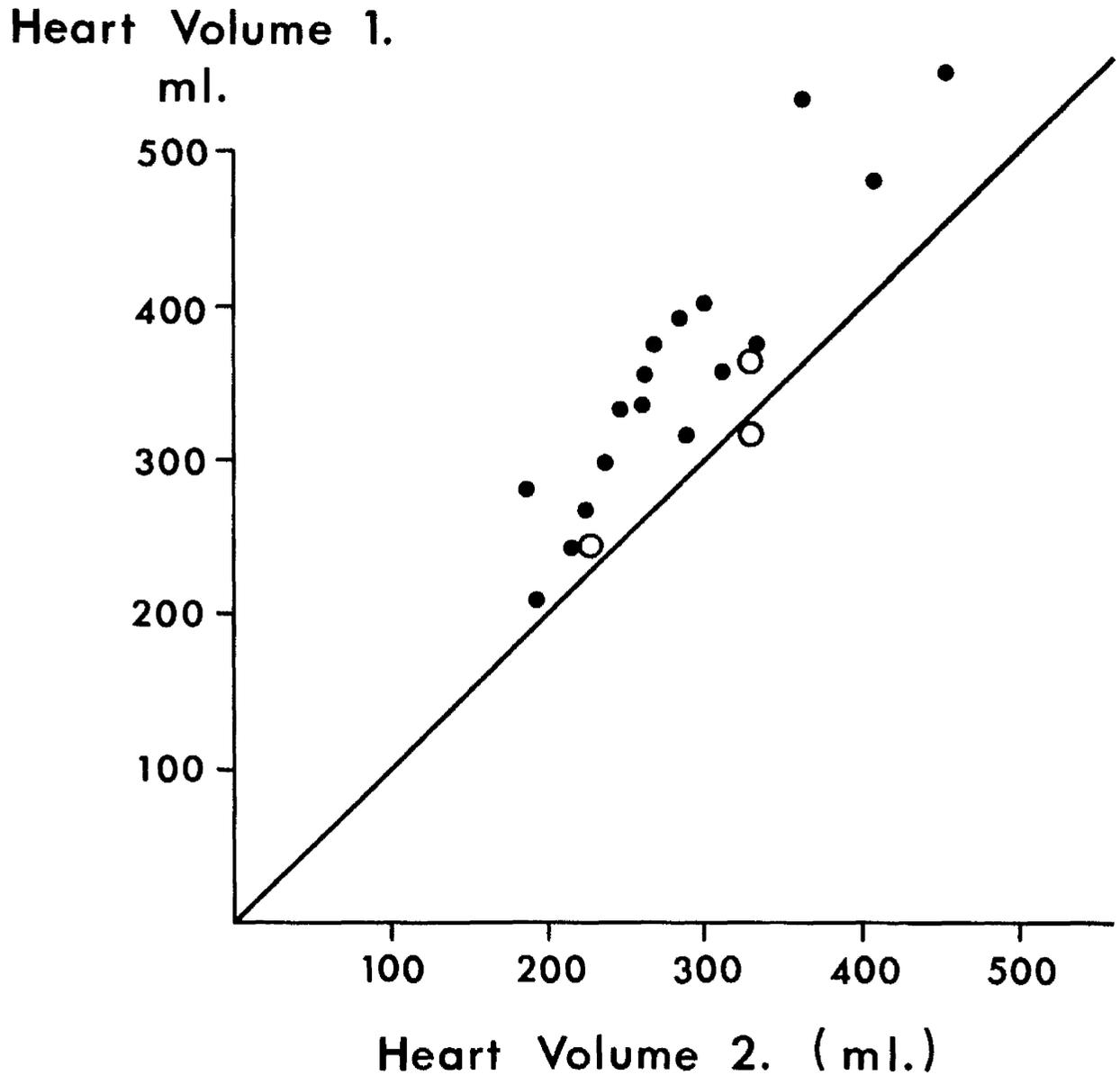
The open circles represent three
non-oedematous subjects.
The 45° line represents perfect
agreement between the two
determinations.

FIG. 11.3.

ACUTE NEPHRITIS
Changes in C-T Ratio
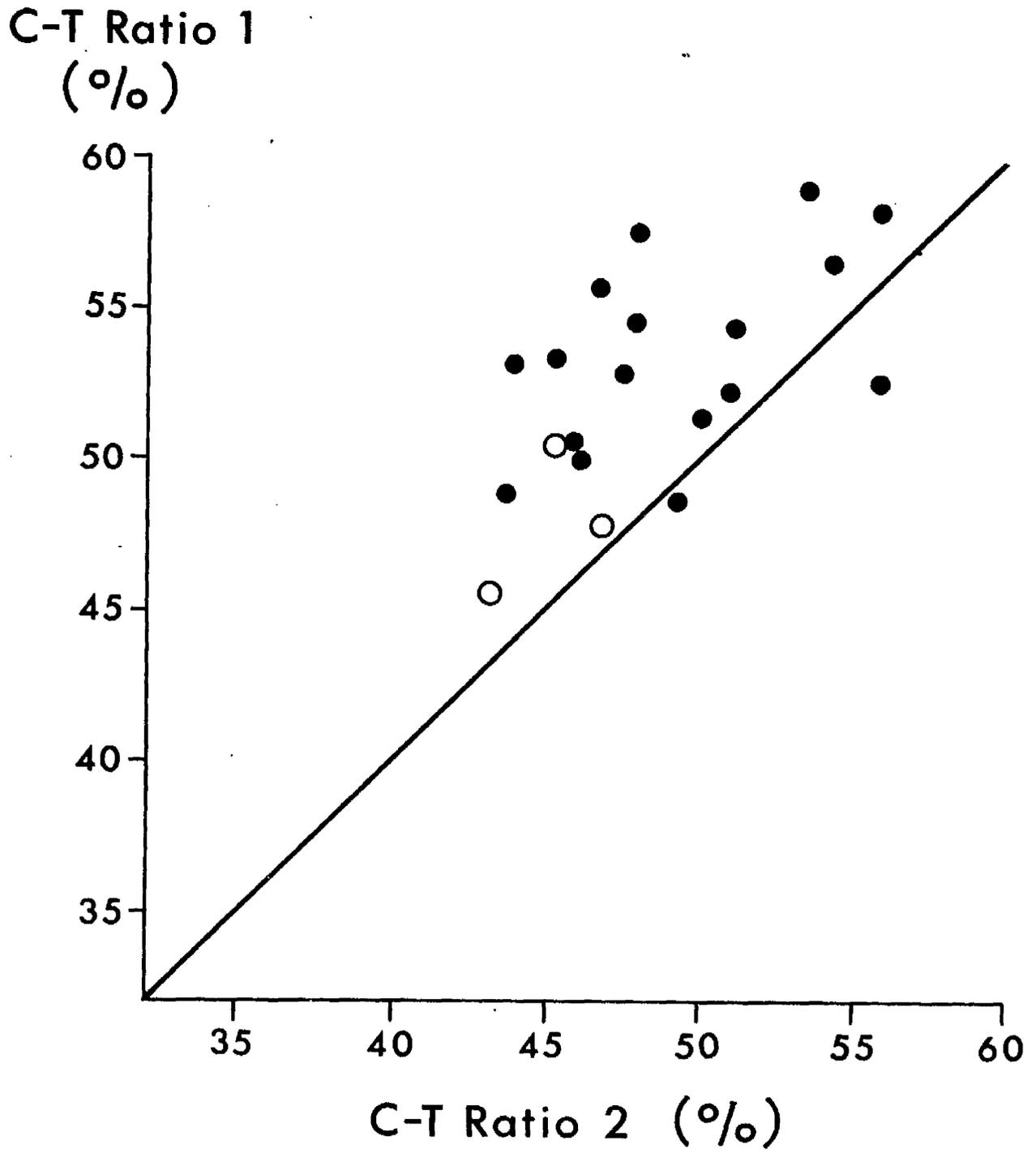
Details as in Figure 11.2

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

FIG. 11.4.

ACUTE NEPHRITIS
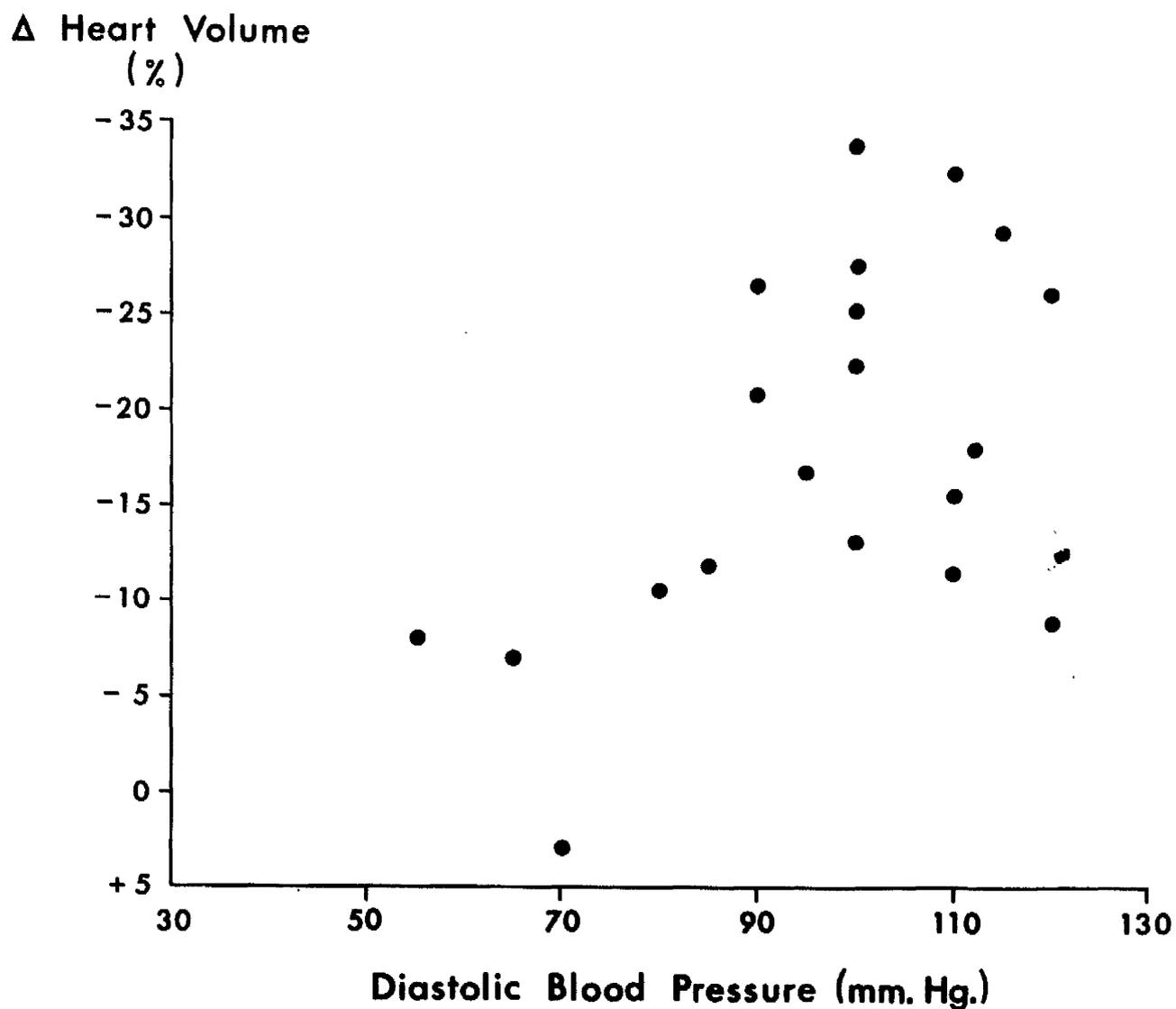
In general, larger changes in heart
volume occur in the hypertensive
patients. Nevertheless within this
group no correlation is seen.

FIG. 11.5.

ACUTE NEPHRITIS

Heart Volume
ml.

$r = 0.9136$
$P < 0.001$

MEAN Δ H.V. 69 ml. (19.9%)
MEAN Δ B.V. 346ml. (18.4%)

Blood Volume (litres)
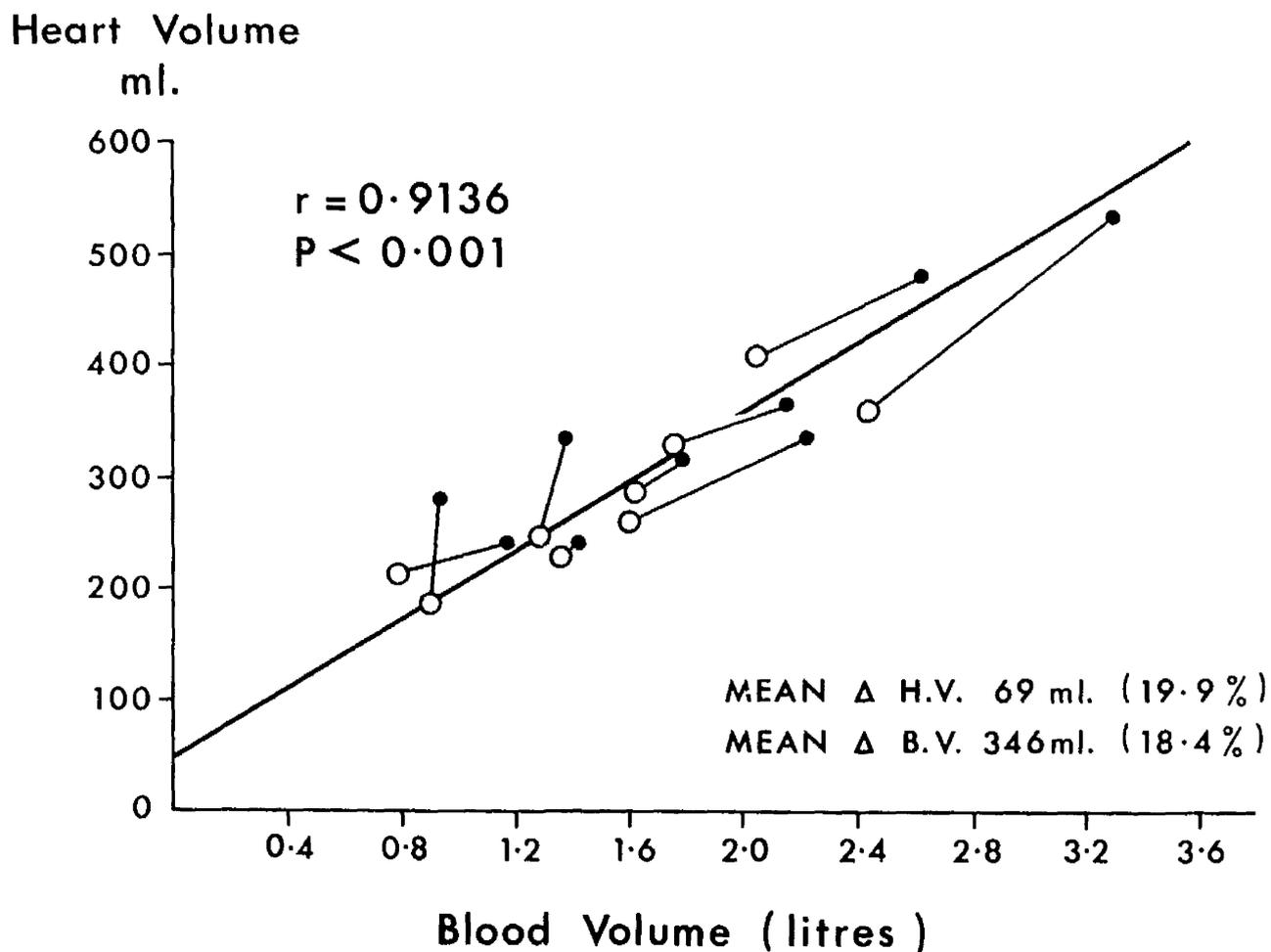
Determinations carried out in pre-
diuretic phase (closed circles)
linked with those done following
diuresis (open circles). The mean
changes are highly comparable.

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

FIG. 12.1.
265

ACUTE NEPHRITIS

Daily
Urine H₂O: 700 → 400 ml.
Na : 100 → 20 meq.

Plus
2 days

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-pyrine 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

FIG. 12.2

270

ACUTE NEPHRITIS   M.C. Female 8 yrs.
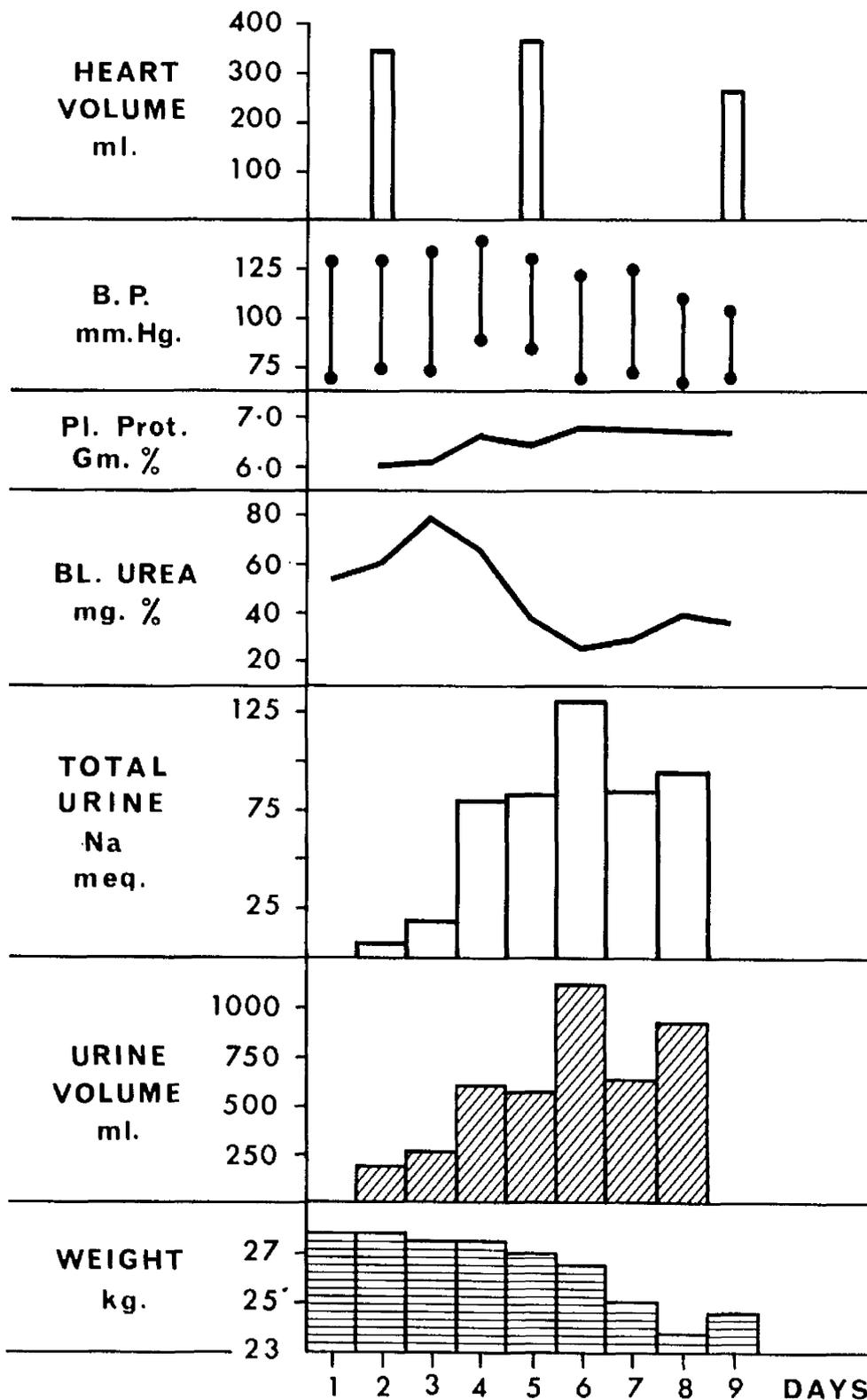
Summary of findings from child
in early diuretic group.

Fig. 12.3.

271

ACUTE NEPHRITIS   J.B. Male 9 yrs.
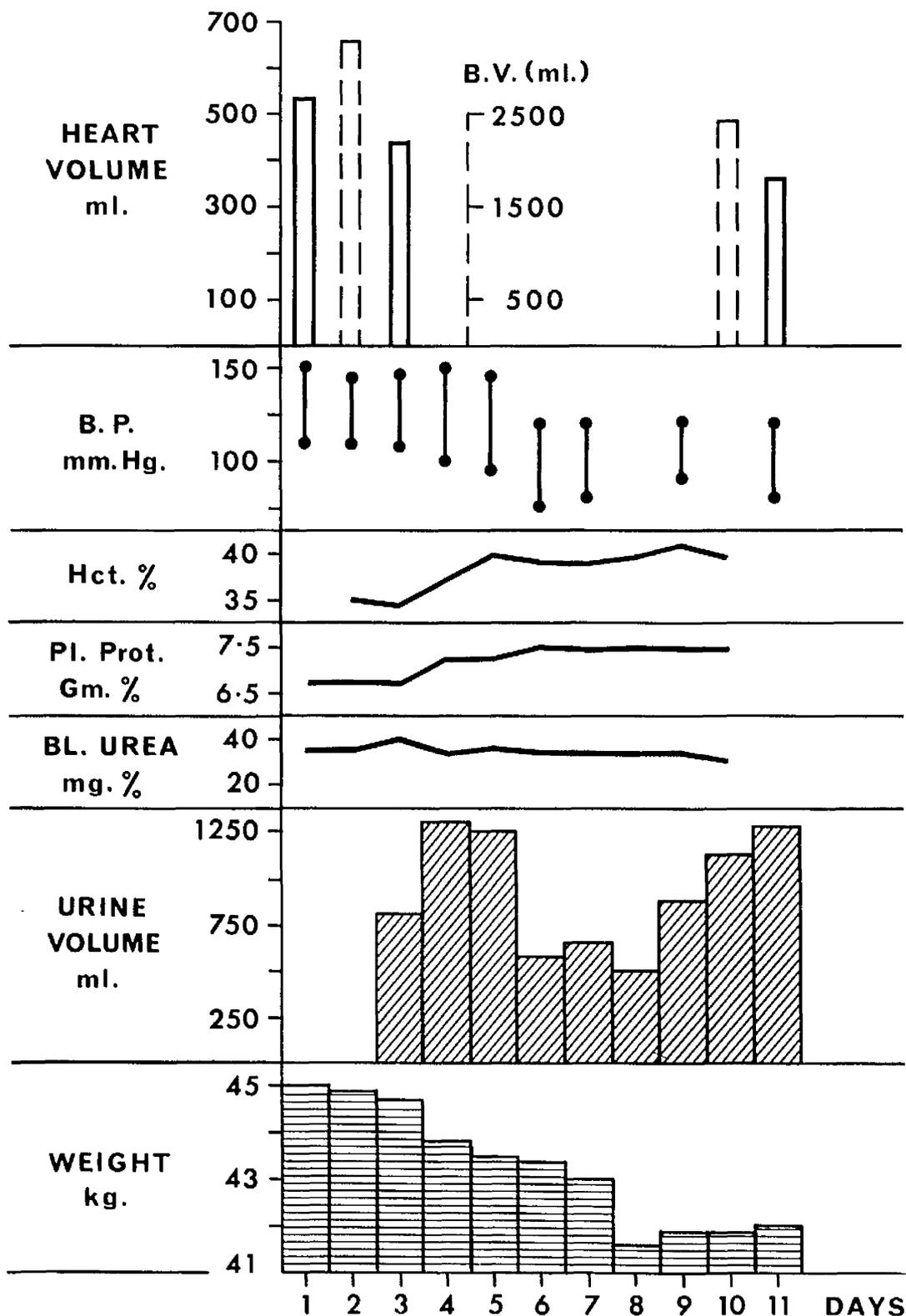
Summary of findings from
child in late diuretic group

(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 ⟵ HYPERTONIC SODIUM RETENTION ⟵
Hyponatriuria

E.C. Volume↑   E.C. Osmolarity↑

E.C.Space ⟵ I.C.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 luminar 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 those 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 concensus 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 at 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 considera

at present to be *sub indice*

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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