CIBA FOUNDATION
COLLOQUIA ON AGEING

**Vol. 4.   Water and Electrolyte Metabolism in Relation
to Age and Sex**

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VOLUME 4

Water and Electrolyte Metabolism in Relation to Age and Sex

Editors for the Ciba Foundation

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With 85 Illustrations

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PREFACE

THIS volume represents the fourth colloquium in the Ciba Foundation's programme for the encouragement of basic research relevant to processes of ageing which was initiated by the Trustees early in 1954. In line with the series of conferences begun earlier on Endocrinology, these meetings are arbitrarily described as Colloquia to distinguish them from the single conferences on isolated subjects which are known as Symposia.

This colloquium on Water and Electrolyte Metabolism in Relation to Age and Sex brought together a number of people working on these problems from very different angles, with what success the reader may judge for himself. Membership had to be limited to a small group, as usual, but it is hoped that the published proceedings will have a world-wide readership, and will prove to be of value to those workers in this field who could not be asked to participate on this occasion, as well as to others not so closely associated with such research.

Professor McCance, who directed the meeting with firm but friendly skill and split-second time-keeping, also gave much valuable help to the Deputy Director in its organization and planning. He and Dr. Widdowson have continued their assistance with some much appreciated advice on editorial matters.

To those to whom this book serves as an introduction to the activities of the Ciba Foundation it should be explained that it is an international centre which owes its inception and support to CIBA Ltd. of Switzerland. Under the laws of England it is established as an educational and scientific charity and is administered independently and exclusively by its eminent British Trustees.

The aim of the Foundation is to improve co-operation in medical and chemical research between workers in different countries and different disciplines. At its 200-year-old house in the medical centre of London the Foundation provides accommodation for scientists of all nationalities, organizes conferences, conducts a medical postgraduate exchange scheme between Great Britain and France, arranges a variety of informal discussions, awards two annual lectureships, and is building up a library service in special fields. In general, the Foundation assists international congresses, scientific institutions and individual research workers as much as lies within its power.

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28th–30th January, 1958

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CHAIRMAN'S OPENING REMARKS

R. A. McCance

When I first became interested in electrolytes some 25 or 30 years ago, there were not many other people interested in the subject. Indeed, if they had been collected together in this room for a symposium, they would have rattled about like peas in a pod. But we did not meet. The world was no larger then but there were no fairy godmothers like the Ciba Foundation to transport us from distant parts of the world to London in machines flying at hundreds of miles an hour in order that we might see each other. Now there are so many people interested in electrolytes that if all of them were to come to a meeting, we should have to hold it in Trafalgar Square, or if it were wet, in the Festival Hall.

We owe our fairy godmother a lot of thanks.

The subject of electrolyte metabolism has developed enormously. We realize now that electrolytes enter into practically every reaction that takes place in the body, but we still know very little about a great many of them. The functions of magnesium, for example, are still very much of a mystery, and if anybody here can throw any light on this element it would be very stimulating. We still know extremely little about how and why the total amounts of the various electrolytes in the body are maintained; why and how their relationships change with age; what part each individual cell is playing and what effect a change in the rest of the body may have on an individual cell. That brings me to the object of this colloquium. If you look at your programme you see that we have been asked to try to put together our knowledge and information about water and electrolyte metabolism in relation to age and sex. You will see how the days have been divided up. The first day will be devoted to "General principles". Then we

have "The developing organism", and lastly "Senescence and disease". I recognize the problems that arise when a collection of "experts" get together: some people who are going to speak today may not have any experience at all of the newborn baby or of the effect of age on electrolyte metabolism—except perhaps on their own, and I hope they have not had too much of that! Prof. Wallace can hardly be expected to be very interested in old age; he would prefer, I dare say, to listen to a paper about congenital heart failure rather than the one about congestive heart failure which Dr. Fejfar is going to give. One of the objects of the symposium, however, is that he shall do it. People speaking on Thursday, moreover, may not have thought about a newborn baby's renal function since they were one themselves! At the same time it is very useful to have a collection of experts brought together like this, if they—so to speak—play to the title. We must always try to keep before us the object for which we have been brought together, that is to say to pool our knowledge so far as possible about the metabolism of electrolytes in relation to age and sex.

As a corpus for dealing with electrolytes we may be a little bit light on hormones. We could do with a few more specialists in this field—there may be some unknown ones here who will introduce themselves later—I hope there are! We shall require their assistance and I hope they will not be afraid of saying what they think, when they think it. They will have little chance of being contradicted!

It is a great pity that we shall have one absentee. I am very sorry that our colleague Kerpel-Fronius could not come. He is an old friend of mine and a very old friend of paediatrics and electrolytes. I saw him not so long ago and he was much looking forward to this international gathering. I personally think he would appreciate it very much indeed if we were to send him a letter as from the conference, saying how much we are missing him. With your permission I shall write a letter and send it off as from all of us.

THE DEVELOPMENT OF PHYSIOLOGICAL
REGULATION OF WATER CONTENT

E. F. Adolph

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University of Rochester, New York

The plan of this study is to single out one way of measuring
the physiological regulation of body water content. This way
will concern water exchanges, that is, water intakes and
outputs. By use of it, the ontogeny of regulatory responses to
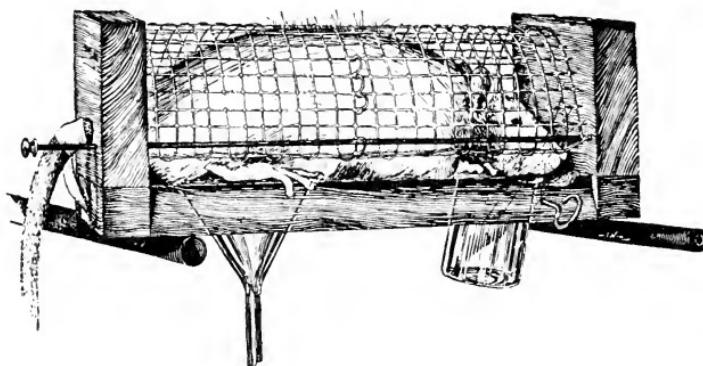
Fig. 1. Rat in restraint frame. Drinking water is available in
removable beaker; urine is shed into funnel. From Adolph,
Barker and Hoy (1954).

excesses and to deficits of water will be traced. We and others
found that at birth the responses whereby constancy of body
water is maintained are small compared to those of older
animals. The several relations involved in this regulation will
be described largely by means of data on laboratory rats.

Water exchanges vary chiefly in the excretion through the
urinary tract and in the drinking into the alimentary tract.
They are measured upon a rat confined to a frame (Fig. 1).
The urinary bladder is reflexly emptied when the rat and
frame are raised and lowered, whereupon the urine enters the

funnel and a tube held beneath it. Drink is taken from the
beaker, which can be freed from the frame and weighed at
intervals. The weight of the body, ascertained while the rat
is in the frame, measures any net change of body water
content, including evaporative losses.

When an adult rat has been forcibly given an excess of body
water, it promptly excretes water more rapidly than usual.
The urine flow varies linearly with the water excess present
in the body, as is shown when one plots the first hour's output
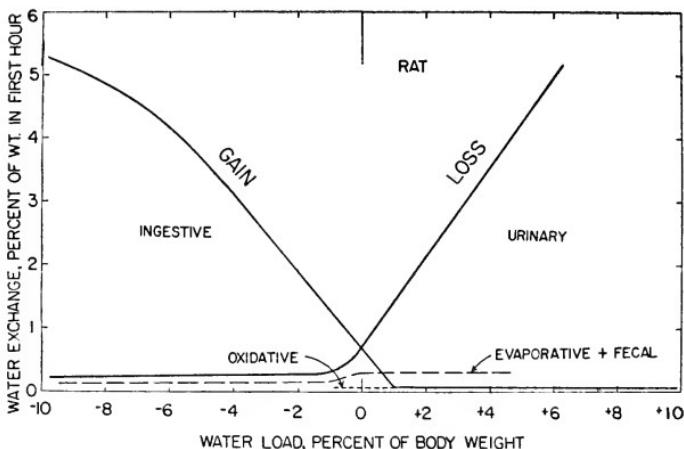
Fig. 2. Equilibration diagram for water exchanges of adult
rat. Constructed from data of Adolph (1956) and Adolph,
Barker and Hoy (1954).

of urine after water is forced into the stomach in relation to
the amount of water excess or load (Fig. 2). When the rat
has been dehydrated by being deprived of water for various
periods of time, water is drunk as soon as allowed, and the
amount drunk is roughly proportional to the water deficit or
negative load. Excretion and ingestion are symmetrical
activities that specifically and appropriately compensate for
the disturbances of water content (Adolph, 1943). Many
tests seem to show that the accuracies of compensation by
drinking and by excreting are about equal when the water
loads are of equal magnitudes.

The relations of exchange to content shown in Fig. 2, the equilibration diagram, form a useful basis for understanding the regulation of body water, and of many other body contents. They show the specificity of the responses required for constancy, the sensitivities with which they occur, their promptness and their accuracy. A fixed set of relations, therefore, automatically keeps the rat in water balance. Similar relations have been worked out for a number of other species among mammals, other vertebrates, and some
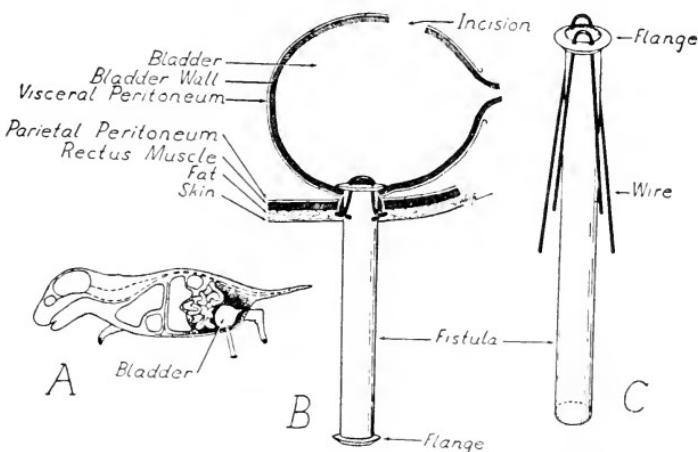
FIG. 3. Bladder cannula and its method of placement in infant rat. From Hoy and Adolph (1956).

invertebrates (Adolph, 1943). Much effort has also been expended by investigators to find through what messages and effectors the adult's automatic responses are excited and mediated; those features will be largely neglected here.

Are these relations also present in young animals, and when? Are they the same as in adults? This question we tried to answer particularly for water excretion, and first for newborn dogs (Adolph, 1943, p. 267). For rats we needed an accurate method for measuring urine flow at all ages, and eventually found it through placement of a plastic cannula in the bladder (Fig. 3). Urine is thereafter collected by exserting a capillary glass tube on the cannula, and measuring the position of the

meniscus from minute to minute as urine collects in it (Hoy
and Adolph, 1956). Quantitative collections can also be made
without the cannula, at the urinary papilla or by bladder
puncture; during rapid urine flows these collections give the
same results as with the cannula (Heller, 1947; McCance and
Wilkinson, 1947; Falk, 1955).

Water excess, administered by stomach tube, gives rise to
very little diuresis at birth (Fig. 4). In the course of several
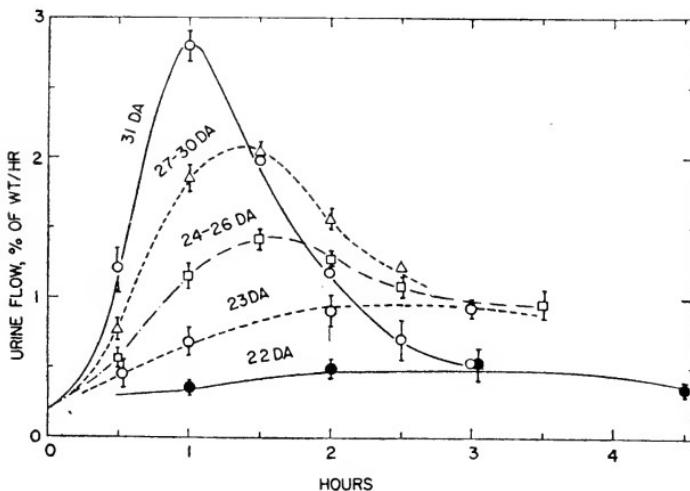
Fig. 4. Water diuresis at various ages in infant rats. Points
show mean and standard error at end of each period of urine
collection. DA = days after conception. From Falk (1955).

days the rat's response increases, until at about ten days
after birth the response per unit of body weight is of adult
size. The ages indicated on the graphs shown here are
reckoned from conception instead of from birth, the average
gestation time for rats being 21·3 days. Actually in human
infants the maturation of the diuresis was found by Ames
(1953) to be triggered by birth rather than by scheduled age,
since prematures acquired the diuretic response about as soon
after birth as postmatures did.

A familiar notion about the way in which water diuresis is
excited is to suppose that the neurohypophysis withholds its

antidiuretic hormone until the water excess is removed. This theory is widely accepted for mammals generally. In infant rats above five days of postnatal age we found that water diuresis was inhibited by injecting pitressin (Fig. 5). But at two days of postnatal age the diuresis was unabated by this
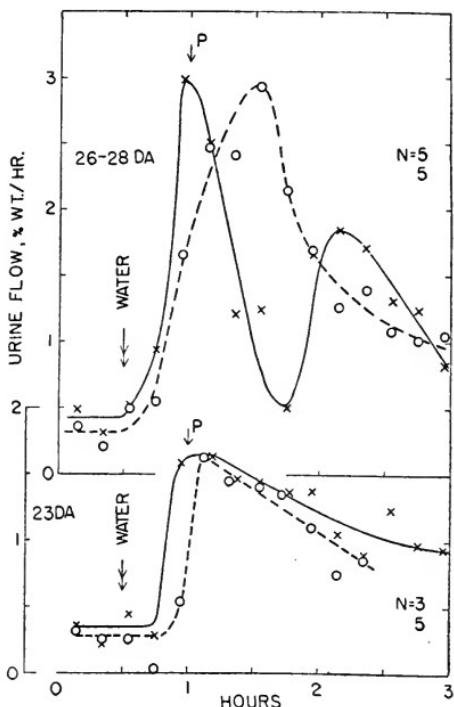
FIG. 5. Water diuresis at two different ages in infant rats (dash lines), and the effects of pitressin injections at P upon it (solid lines). DA = days after conception. From Adolph (1957).

substance. It is unlikely that the foreign pitressin is inactivated at one age and not at another, and possible that infant renal tissues are insensitive to it (Heller, 1952). But the most important conclusion is that diuresis can be aroused by some other means than the withholding of the hormone in the neurohypophysis. At this particular age of two days a

response is thus uncovered which is mediated through some other channel ordinarily masked by the known hormonal one.

The intensity of diuresis is a function of the water excess at all ages (Fig. 6), but the regression differs with age. Actually these data supply part of an equilibration diagram for infant rats, and by it one can watch the regulatory relations coming to maturity during early postnatal life. The unexcreted water has been located as excess in plasma and several other tissues. A possible theory of maturation is that some slowly developing process or structure limits the rate of water excretion.
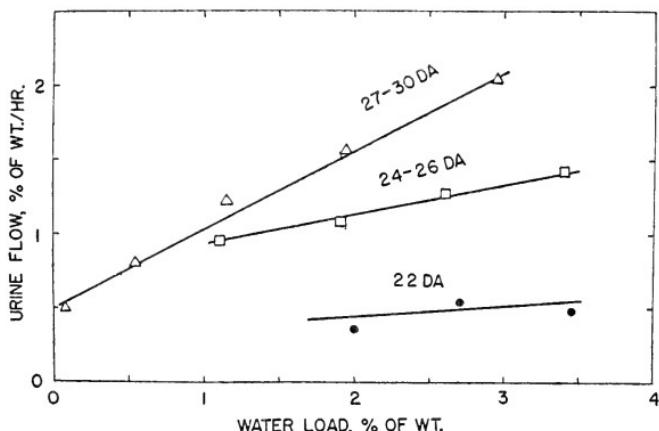
FIG. 6. Water exchange in urine in relation to body water load at each of three different ages. DA = days after conception. From Adolph (1957).

This theory is doubtful, since at every age still greater water excess arouses faster excretion. Rather, the response, expressed by the ratio between excretory rate and water load, is small at birth and becomes greater as age increases.

However, in order to see whether diuresis is impossible at birth, we tested the capacity of the infant rat to respond to several other stimuli of diuresis. To concentrated salt solutions the diuretic response is practically nil at birth, and it matures even later than the water diuresis (Fig. 7). Hypoxia arouses a primary diuresis that is small at birth and becomes greater a few days later; it also, however, arouses a secondary

diuresis that is large and sudden even a few hours after birth. Likewise, adrenaline or noradrenaline induces a full-blown diuresis on the very day of birth. Evidently the capacity for excreting water at a high rate is present, but its arousal depends on the particular form of stimulation. Consequently, any discussion of structural inadequacies or functional immaturities seems beside the present main point, which is that the specific responding system of the newborn rat is not tuned to water excesses.

Hence, we are privileged to see a physiological regulation increase in intensity in the growing individual. The regulation
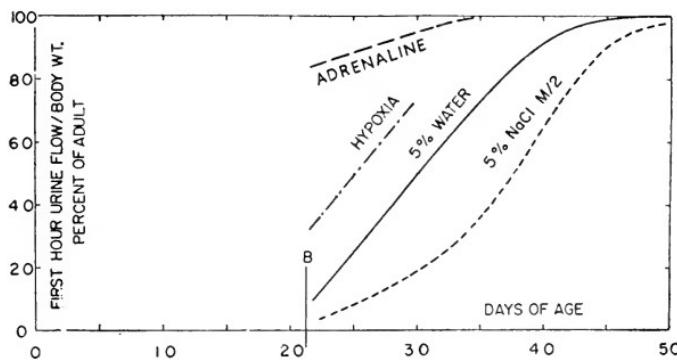
Fig. 7. Courses of development of four types of diuresis in rats. B = birth. From Hoy and Adolph (1956).

duly materializes, whether the rat has ever experienced a water excess or not; the elements necessary for it are there, some of them long before this materialization. What guides the regulation's intensity and determines its point of adult fixation is unknown. The fixation is still subject to a small degree of adaptation resulting from previous exposure to water excesses (Adolph, 1956).

The control of water intake, on the other hand, is much less understood than the control of water elimination. In early infancy, rats, like dogs (Adolph, 1943, p. 267), refuse to drink water, even after dehydration. According to Křeček, Křečková and Dlouhá (1956), as late as 28 days after birth young

rats drink more milk than water in recovering from dehydration. But in the same circumstance they drink more water than saline. Even newborn rats distinguish between milk and other fluids; at 17 postnatal days they distinguish between water and salt solutions. Such sensory discriminations are necessary before rats can link their intakes to specific deficiencies of bodily constituents. The actual tying of water drinking to water deficiency does not certainly occur until
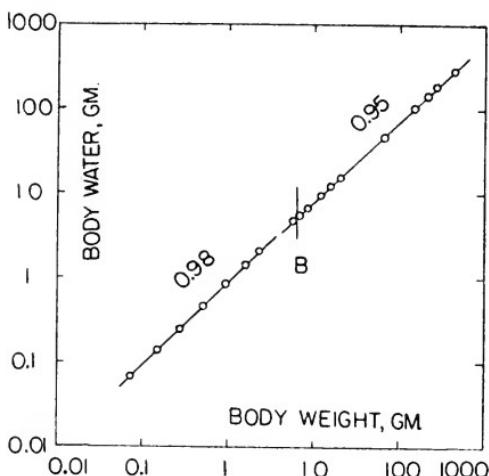
FIG. 8. Relation of log water content to log body weight in rats from foetus to adult. B = birth. Numbers represent exponents in parabolic equation relating the two quantities. Data of Hamilton and Dewar (1938), from Adolph (1957).

28 days after birth (Křeček, Křečková and Dlouhá, 1956). Already then the water intake of rats equals the water deficit imposed upon them (Adolph, Barker and Hoy, 1954, fig. 13); just as in the adults, the one-hour intake closely matches the water deficit so long as the water deficit does not exceed six per cent of the body weight.

Once the immediate regulations of water content are fixed, the adult method of maintaining water balance is persistently at work. But it is well recognized that the water content,

both absolute (body size) and relative to body solids, varies with the age of the rat (Fig. 8). What controls the absolute content of water and of each solute? The answer to this question is not available. Obviously all the items that enter the determination of growth and its correlatives participate in these controls. This is a problem that has barely been visualized, and one whose analysis may occupy many physiologists in the future.

In general, the ready corrections of water excesses and deficits result from specific response systems for diuresis and for water drinking. The systems vary between infant and adult, not only quantitatively but possibly also in the mediators and effectors used. Over a long lifetime, the regulation depends also upon detectors of body size and proportions whose characteristics and locations have not been determined.

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DISCUSSION

Shock: We have obtained some data in our laboratory on the age differences in the antidiuretic response to pitressin. Some of the results of these experiments are in accord with the concept that in many instances the senescent animal returns to a type of response and behaviour that is seen during the course of development. In these experiments we measured the concentrating ability of the kidney rather than total urine flows. Total urine flows are not useful for age comparisons since in older subjects the number of functioning units is reduced and hence there is a

lower total urine output. Our results are expressed in terms of the amount of water reabsorbed from the glomerular filtrate, that is the urine/plasma (U/P) ratio of inulin. A maximum water diuresis was induced by the oral administration of water plus an intravenous infusion of 5 per cent glucose. There were three groups of subjects—young, middle-aged and old. The young group represents nine individuals aged 26–45, the middle-aged group ten subjects from 46–65 years old, and the old group was from 66–90. Under conditions of maximum diuresis the U/P ratio was about 10 for all three groups of subjects. We gave $0 \cdot 5$ m-u./kg. body weight of pitressin, not enough to cause a rise in blood pressure, but there was a marked inhibition of the diuresis. The U/P ratio in the young group increased to 120 within 10 minutes as compared to 75 in the middle-aged and about 40 for the old. After a period of roughly 50 minutes the diuresis was again re-established in all three groups (Miller, J. H., and Shock, N. W. (1953). *J. Geront.*, 8, 446) (see Shock, Fig. 10, p. 240).

Heller: In connexion with your results, Prof. Adolph, I should like to clear up a point which has led to some misunderstanding. Some years ago (Heller, H. (1952). *J. Endocrin.*, 8, 214) we were also interested in the response of newborn and infant rats to vasopressin. Our experiments were not suitable for establishing at what time after birth the rats first responded to vasopressin. But we could determine by means of inulin U/P ratios, i.e. by the same technique as that used by Dr. Shock in man, at what postnatal age the antidiuretic response to vasopressin became quantitatively comparable to the response of adult animals. We found that this occurred only in rats older than 22 days. I would like to stress this because some workers have misinterpreted these results: they assumed that we had tried to show that a significant inhibition occurred *for the first time* after 22 days. I think that one must expect that this datum of around 20 days may change somewhat in the hands of other workers. Clearly a comparison between the antidiuretic responses of adult and infant rats depends on the choice and strictness of application of the criteria of comparison. But I think that our data agree with some work which Dr. Falk did later (1955. *Amer. J. Physiol.*, **181,** 157). She injected nicotine into infant rats and tried to find out at what postnatal age sufficient vasopressin was secreted by the pituitary gland to produce an inhibition of diuresis which would be quantitatively comparable to that in adult animals. She found that this occurred at about 17–22 days after birth.

Adolph: I think Dr. Falk (1955) got a significant inhibition considerably before 17 days. She also injected vasopressin itself, and by the method of collecting the urine which is expelled in response to perineal stimulation in the infant rat, she was able to get significant inhibition in the first week of postnatal life. There is evidence that antidiuretic hormone or something comparable which could inhibit water diuresis was then being put out by the animal.

Heller: This is precisely the misunderstanding to which I have been referring. Dr. Falk did get responses to nicotine in animals three days after birth, so you are quite right in saying that responses were obtained much earlier than after 20 days of postnatal life. But she also compared

the response of older animals with that of adults: they became comparable in quantitative terms only when the rats were 17–22 days old.

There is another point on which I should like to have your views, Prof. Adolph. We find that these responses of infant rats to vasopressin are influenced not only by the age of the animals, but also by the litter size. In other words, if there are fewer animals in the litter, they will be larger, and that may influence the development of renal functions.

Adolph: We have not tested for litter size. In general we have used the larger animals.

Black: I must apologize for introducing another hormone, but Prof. Adolph's interesting observation reminded me of some recent work on hypertonic over-hydration by McCance and Widdowson (1957. *Acta Paediat.*, (*Uppsala*), **46**, 337). We may be tacitly assuming that in these poor responses we are dealing with either renal immaturity or with this very interesting hypotension, and I wondered whether the adrenal gland came into this at all, since its histology changes very considerably from foetal to neonatal life. Could a better water diuresis be obtained in these newborn animals by giving them cortisone with the water load?

Adolph: Dr. Falk did some work on the administration of the cortical adrenal substances. At the early ages these seem to have very little effect on water diuresis and water excretion.

Swyer: I cannot speak about the rat, but so far as the human is concerned the evidence seems to be that the infant adrenal is quite effective in secreting glucocorticoids and probably aldosterone, at least in amounts relative to its own size, so that the apparently deficient response of the kidney does not appear to be due to lack of adrenal steroids. You cannot improve the renal response by giving steroids. It might be a lack of renal responsiveness to the water load rather than any insufficiency of hormonal equipment.

Heller: We have found (Heller, H. (1958). *Mschr. Kinderheilk.*, **106**, 81) that injections of cortisone into newborn or infant rats produce a significant decrease of total body water. Much the same effect is obtained with ACTH.

Adolph: I should like to make a small protest against the use of the term 'renal immaturity'. If you want the 100-day-old rat to be the criterion of everything, then everything else is either premature or postmature. But if you want to consider that every animal has an optimum for its own age, then the use of the word immaturity seems to me undesirable. The same thing applies to hypotension: what is hypotension for an adult is not hypotension for an infant.

Talbot: I should like to register a mild objection to this thesis about immaturity. For instance one might say that the parathyroid-renal phosphorus homeostatic mechanism of the human infant is at least functionally immature at birth, presumably because the mother's mechanisms have performed this homeostatic task for the infant while it was *in utero*. As a result, the infant has a very small tolerance for dietary phosphorus at birth. However, he develops the capacity to handle phosphorus satisfactorily within a few weeks.

Have you any further information about this adrenaline-induced

diuresis? Did it increase the ratio of water to solutes in the urine, or did it increase the solute output?

Adolph: Adrenaline diuresis in infant rats does involve more solute output than the water diuresis, but adrenaline diuresis is a water diuresis in that the urine is very dilute. I do not think you could blame all the adrenaline diuresis on the solute output itself.

With regard to immaturity and whether it takes experience for an animal to have a diuresis, we can point to the fact that adrenaline diuresis has no experience-factor. We have tried to see whether we could get more water diuresis in the infant animal by subjecting it to water loads on successive days. There is a considerable variation in the amount of water excretion which is produced, and we are unable to say that there is any significant change due to previous experience with water. Our provisional conclusion is that there is no adaptation apparent in the animal subjected to repeated water-loading.

CELLULAR ASPECTS OF THE ELECTROLYTES AND WATER IN BODY FLUIDS

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THE water and electrolyte contents of a complex organism are almost entirely determined by the activities of the kidneys, which operate primarily on the blood plasma and, through that, on the extracellular fluid of the organism. Casual fluctuations in the water and electrolyte contents of the organism are therefore usually the consequence of fluctuations in the composition of these two compartments of the body plasma and extracellular fluid. The electrolytes and water of the cells of the body are affected secondarily to these primary fluctuations in the composition of the extracellular fluid and plasma, and, for practical purposes at any rate, the factors that can influence them primarily are usually ignored. Nevertheless, since the cells occupy a considerable fraction of the total volume of the organism, and since there must be some reciprocity between the electrolyte and water content of cells and extracellular fluid, it is of some importance that we understand the physical and chemical factors that determine the electrolyte concentrations and volumes of the cells of the body.

The Gibbs-Donnan Equilibrium. The application of the Gibbs-Donnan equilibrium to the problem of the water and electrolyte distribution between the plasma and extracellular fluid is familiar to all who have concerned themselves with the water balance of the organism. It will be recalled that the most important consequence of the Gibbs-Donnan distribution of ions between the two fluids separated by the capillary membrane that is supposed to be impermeable to the protein molecules of plasma, is that the osmolarity of the plasma is

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significantly higher than that of the extracellular fluid. This
is illustrated by Fig. 1, and it follows that an equilibrium will
only be achieved when a counter-pressure is exerted on the
plasma equal to the colloid osmotic pressure due to the plasma
proteins. The amount of this difference of osmotic pressure is
determined by the concentration and degree of dissociation
of the proteins. Because of the high molecular weights of the
plasma proteins, their concentration, expressed as moles per
litre, is small and the difference of osmotic pressure that must
be resisted, if the system is to remain stable, is correspond-
ingly small, namely 25 mm. Hg. As a result, the organism is
able to maintain a statistical equilibrium between plasma and
extracellular fluid by virtue of the capillary pressure; at the

Plasma	Membrane	Extracellular Fluid
$Na^+ P^-$ $Na^+ Cl^-$		$Na^+ Cl^-$
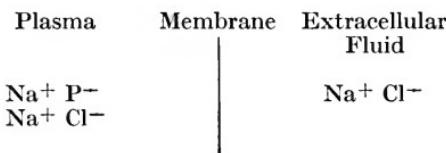
Fig. 1. The plasma-extracellular fluid
system.
(P=protein).

arterial end of the capillary the pressure is greater than this
difference of osmotic pressure so that fluid flows into the
extracellular compartment; at the venous end the reverse
holds, and fluid is absorbed.

It is worth noting that by the term "impermeability"
to a solute—here the plasma proteins—we do not necessarily
mean an absolute barrier; this is an ideal case on which cal-
culations are based, but practically it seems unlikely that a
natural membrane is completely impermeable to any of the
naturally occurring molecules in solution in the fluids, and it is
sufficient for our purposes if by "impermeability" is meant
that the rate of transport of this solute across the membrane
is negligibly small compared with that of the other molecules
that we are considering—in the particular case of plasma and
extracellular fluid, the salts and water.

The cell membrane is a more selective barrier than the

capillary endothelium, and is capable of imposing restrictions on the movements of ions that are very much smaller than the protein ions; as a result, it is conceivable that much larger differences of osmotic pressure could be established, since these smaller ions may be present in vastly higher concentrations than those of proteins with their large molecular weights. Let us consider the erythrocyte; for simplicity we may choose the cat or dog erythrocyte which shows no accumulation of potassium. The distribution of ions is indicated roughly in Fig. 2; the cell contains the protein haemoglobin which behaves as an anion, so that we may expect to be able to apply the Gibbs-Donnan equilibrium to the diffusible ions. If the Na^+, Cl^- and HCO_3^- ions could diffuse across the membrane, the position would be entirely

Cell	Membrane	Plasma
Na^+ Hb^-		Na^+ Cl^-
Na^+ Cl^-		

Fig. 2. The cat erythrocyte.
(Hb=haemoglobin).

analogous with that already considered, and the contents of the cell would have a higher osmolarity than the surrounding plasma, so that unless the membrane could resist the expansion caused by an influx of water, the cell would have to swell, and swell indefinitely since this difference of osmolarity must prevail so long as the cell contains a higher protein concentration than that in the outside medium. Cell membranes are not strong and would certainly not be able to resist the difference of osmotic pressure that would be developed, which in this case would be several times higher than in the case considered earlier, owing to the very high concentration of protein in the red cell. We know that the cat erythrocyte is stable, and we must ask: how? Theoretically, stability could be achieved by making the membrane impermeable to salts, i.e. to all the ions of the system. Alternatively, stability could be achieved by making the cell permeable to anions only and

impermeable to cations such as Na^+ and K^+. In this way the cell would be able to fulfil its function in the maintenance of the acid-base balance of the body, permitting the $Cl^- - HCO_3^-$ exchange that mediates the buffer action of haemoglobin in the cell.

It might be thought that by making the cell impermeable to cations, such as Na^+, we should be establishing conditions for a Gibbs-Donnan equilibrium leading to a large excess of osmotic pressure; however, the concentrations of impermeable cations will be equal on both sides of the membrane, so that any Donnan effect due to impermeable cations on one side of the membrane will be counterbalanced by an equal effect due to impermeable ions on the other side.

It is easy to show that an osmotic equilibrium between the inside and outside of the cell is possible, in spite of the high concentration of indiffusible protein anions within the cell; thus the impermeability of the cell membrane to cations such as Na^+ confers on it a stability that would be lacking in the presence of a permeability to this ion; in other words, the colloid osmotic pressure of the cellular proteins can only operate in the presence of a permeability to both Na^+ and anions. It is now well known, however, that cell membranes do not show an absolute impermeability to such ions as Na^+ or K^+; the use of isotopes has permitted the demonstration of an unequivocal exchange of these ions across the erythrocyte membrane. The exchanges are very slow compared with the exchanges of Cl^- and HCO_3^-, but they do occur, so that we must expect a constant movement of NaCl and $NaHCO_3$ into the cell, associated with the migration of water, unless some process prevents this. As is well known, the process that does prevent it is an active transport of Na^+ ions out of the cell; the membrane is permeable to Na^+ so that there is a continual drift of this ion into the cell because of the demands of the Gibbs-Donnan distribution, but by some process not understood, metabolic energy of the cell is employed in driving the salt out. Practically, in consequence, the cell may be described as a cell impermeable to Na^+ and therefore in stable equilibrium with its environment. The total amounts of water and electrolytes within the cell will be determined by

two main factors—the osmolarity of the plasma and the activity of this Na^+-extrusion mechanism. The passage of water across the cell membrane is very rapid, so that the cell responds to changes in osmolarity of the plasma by virtually instantaneous changes in its water content; in this way it may be said to respond passively to changes in the plasma, and its changes of water content and salt concentration may be said to be secondary to primary changes determined principally by the kidney. The operation of the second factor —the Na^+-extrusion mechanism—will influence the amount of material—salts and water—in the cell, and it would be by virtue of this mechanism that this type of cell could exert a primary influence on the water and electrolyte content of the organism. Thus, if the Na^+-extrusion mechanism operated more rapidly than the influx under the electrochemical gradient, there would be a net loss of Na^+ and of anions, namely Cl^- and HCO_3^-; this would decrease the osmolarity of the cell and water would be lost to the plasma. Such a shrinkage of cells is easily demonstrable by allowing them to recover from the effects of putting the Na^+-extrusion mechanism out of action. Thus, when the cells are cooled, the metabolic processes supplying energy can no longer work; Na^+ enters the cells accompanied by anions and they swell. When the cells are warmed, the metabolic processes begin, and the extra Na^+ is excreted until the cells return to their normal volume. The effects of agents that increase the permeability of the cell membrane are of some interest; substances like alcohol or urethane, in the appropriate concentration, can increase the permeability of the cell membrane to Na^+ and K^+ to such an extent that the Na^+-extrusion mechanism is unable to keep pace with the influx of this ion; thus, in spite of a normally functioning metabolism the cell may swell; on removing the agent it may return to its normal size.

The erythrocytes of most species contain K^+ as their principal cation, so that the cell maintains large gradients of Na^+ and K^+ (Fig. 3). The condition for an osmotically stable system could be given by an impermeability of cations,

as before, but once again studies with isotopes have shown that both Na^+ and K^+ can pass across the membrane and an active transport of Na^+ out of the cell and of K^+ into the cell must be postulated to account for the osmotic stability of the system.

It was considered at one time that a mere extrusion of Na^+ would account for the osmotic stability and high concentration of K^+ in the cell, i.e. that the extrusion of Na^+ would demand a replacement by K^+. It was pointed out, however (Davson, 1951), that this would lead simply to an excretion of NaCl and $NaHCO_3$ from the cell, with a resultant shrinkage. Extrusion of Na^+ will only lead to accumulation of K^+ if exchange of K^+ for Na^+ is obligatory on the system in order to preserve electrical neutrality. If anions can accompany the excreted Na^+ then exchange for K^+ is not obligatory. In nerve and muscle, where the concentration of non-permeable anions in the cell is very high, such a sodium-excreting mechanism would cause accumulation of K^+.

Cell	Membrane	Plasma
K^+ Hb^-		Na^+ Cl^-
K^+ Cl^-		

Fig. 3. The human erythrocyte.
(Hb=haemoglobin).

Once again, the water content of such a system will be determined by the osmolarity of the plasma and the activity of the metabolic ionic pumps; thus, over-activity of the Na^+-excreting mechanism would lead to a shrinkage; over-activity of the K^+-accumulating mechanism would lead to a swelling. It is interesting that the two processes show some degree of linkage, in that Harris (1954) has shown that accumulation of the one ion is associated with a nearly equivalent excretion of the other; the linkage is not complete, however, since on cooling erythrocytes swell as a result of gaining more Na^+ than they lose K^+; when they are re-warmed the extra Na^+ is excreted and they return to their original volume. The fact that the cell maintains its characteristic water content and proportions of Na^+ to K^+ within fairly narrow limits indicates that there is some homeostatic mechanism controlling the rates of accumulation of K^+ and

excretion of Na^+. The mechanism is not known; presumably the active transport processes are sensitive to the concentrations of Na^+ and K^+, or more probably to the relative proportions of these ions, in the cell.

The erythrocyte is a highly specialized cell, and it would not be correct to assume that all cells of the body, or even the majority, are based on a similar physiological plan so far as the maintenance of salt and water content is concerned. The striated muscle fibre has been studied very thoroughly, and it may well be that this is far nearer to being a "typical cell", so that we may now consider its main features from the present point of view. The main point of difference between the muscle cell and the erythrocyte lies in the low contents of Cl^- and HCO_3^-, these anions being replaced by organic anions

Fibre	Membrane	Extracellular Fluid
K^+ A^-		Na^+ Cl^-
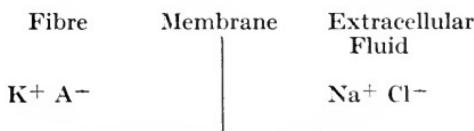
FIG. 4. The muscle fibre.
($A^- =$ indiffusible organic anions).

that apparently cannot diffuse across the plasma membrane; schematically the situation is as in Fig. 4 where A^- represents these indiffusible anions. The system would be osmotically stable were the membrane impermeable to Na^+, i.e. the rest of the ions, K^+, Cl^-, HCO_3^-, would distribute themselves across the membrane in such a way that equal osmotic activities would exist on both sides. Actually the cell membrane is permeable to Na^+, and the reason why the Na^+, K^+ and Cl^- ions do not redistribute themselves is because an active extrusion of Na^+, as fast as it penetrates, maintains an effective impermeability to Na^+. There is no need to postulate an active accumulation of K^+ in this case since, owing to the high concentration of impermeable anions in the cell, the extrusion of a Na^+ ion must be associated with the penetration of a K^+ ion, in the interests of electrical neutrality. Once again, then, the cell may maintain equilibrium with its

environment, provided that an ion-excreting mechanism is active. Loss of this, by cooling the tissue or by metabolic poisons, causes a loss of K^+ and a gain of Na^+, Cl^- and HCO_3^-, the net effect being an increase in osmolarity with a consequent swelling of the cells. Re-warming of the tissue may cause a reversal of these changes (see, for example, Steinbach, 1954).

Thus, in all of the cell types that we have considered, the system can be treated, theoretically at least, as a system that maintains an osmotic equilibrium between the interior and external fluids by virtue of an "effective impermeability" to one or more ionic types; if the membrane were truly impermeable to the ions in question, the osmotic equilibrium would be independent of metabolic processes and could be described as a true equilibrium; in practice, the effective impermeability is the result of a continuous process of active transport. For the purposes of mathematical description this is equivalent to an impermeability, at any rate under normal conditions; under abnormal conditions, on the other hand, the precariousness or instability of the equilibrium is shown by the cellular oedema that follows either the failure of the ion-excreting mechanism or such a large increase in the permeability of the membrane that the mechanism can no longer keep pace with the influx of Na^+.

If these considerations are correct, we may expect to find that by adding up the total osmolarities inside and outside the cell the two totals should be equal within the limits of experimental error. Probably the muscle fibre has been studied most carefully from this aspect, and it would seem from Conway's (1957) figures (Table I), that osmotic equilibrium does exist between the cell and its environment. The same is probably true of the erythrocyte and the nerve fibre, but it must be remembered that the analytical techniques for all the constituents of the cell are not so accurate that a difference of one or two per cent would be ascertained. Within this limit, then, it seems quite safe to affirm that these cells are in osmotic equilibrium with their environment.

Within recent years the possibility that mammalian cells

are not in osmotic equilibrium with their extracellular fluid has been seriously maintained, and an "osmotic pump", driving water continuously out of the cell, has been postulated. The experimental basis for this claim rests on the observation that mammalian tissue slices, in particular those of liver and kidney, swell when placed in "isotonic" solutions of sodium chloride, Tyrode or Krebs (Sperry and

Table I

COMPOSITION OF FROG MUSCLE AND PLASMA EXPRESSED AS M-MOLE PER kg. H₂O (after Conway, 1957)

	Fibre Concentration	Plasma Concentration
K	124	2·25
Na	10·4 (3·6)*	109
Ca	4·9	2·1
Mg	14·0	1·25
Cl	1·5	77·5
HCO₃	12·4	26·6
Phosphate	7·3	3·3
Sulphate	0·4	2·0
Phosphocreatine	35·2	—
Carnosine	14·7	—
NH₂–acids	8·8	7·2
Creatine	7·4	2·2
Lactate	3·9	3·5
Adenosine triphosphate	4·0	—
Hexose monophosphate	2·5	—
Glucose	—	4·1
Protein	0·6	2·2
Urea	2·0	2·1
Total	248·2	245·3

* Figure in brackets for sodium represents, according to Conway, the true intracellular concentration.

Brand, 1939; Opie, 1949), either at room temperature or at 0°. Robinson (1952) observed that the swelling could be prevented or reversed by maintaining the tissue at 37°; he found also that the swelling occurred in the presence of cyanide at this temperature. Since swelling was prevented by using strongly hypertonic solutions—0·55–0·60 M—he concluded that the cells were iso-osmotic with these. It will be quite clear from what has been said earlier that these facts may be explained just as easily on the assumption that the electrolyte-

excreting system fails at low temperature or in the presence of cyanide. Thus, soaking a muscle at 0° certainly leads to swelling, but this is completely accounted for by the gain of Na^+ and Cl^-; warming the muscle causes an excretion of these ions and it returns to its original volume. The same argument will apply to other tissues, and conclusive proof that this is the principal explanation for the changes taking place on cooling was provided by the elegant experiments of Deyrup (1953) who showed that if the tissues were bathed in iso-osmotic sucrose (0·3 M) they failed to swell. If the swelling in Ringer solution had been due to a failure of a water-excreting mechanism, substitution of salt for sucrose should have had no effect, whereas if the swelling had been due primarily to a penetration of NaCl, substitution of a non-penetrating substance like sucrose would have prevented it. It seems safe to conclude, then, that very large differences of osmolarity between cell contents and their environment, such as those postulated by Opie (1949) and Robinson (1952), do not occur. The detection of smaller differences, that would demand a water pump continuously excreting water from the cell to maintain an osmotic steady state between cells and their environment, must rely on very precise measurements of osmolarity.

The depression of freezing point has been employed by a number of workers with a view mainly to testing the claim that mammalian tissues were hypertonic to plasma (Conway and McCormack, 1953; Opie, 1954; Brodsky *et al.*, 1953, 1956; Conway, Geoghegan and McCormack, 1955; Itoh and Schwartz, 1956); but, as Conway's studies indicate, the interpretation of the results is not easy, since an excised tissue, when ground up at 0°, undergoes autolytic changes—in particular the breakdown of adenosine triphosphate to inosinic acid, ammonia and phosphate—that lead to a considerable increase in osmolarity. It would seem from Conway's studies that within the limits of accuracy of the cryoscopic method— probably a few per cent—the tissue cells examined—liver, kidney and muscle—are iso-osmotic with their environment.

This does not mean, however, that the maintenance of differences of osmotic pressure between cells and their environment by the excretion of water does not occur; it is well known that such fluids as urine and saliva have osmolarities that are vastly different from that of the plasma; and the elaboration of these fluids is best described by invoking an active transport of water—i.e. the functioning of a "water

Table II

CONCENTRATIONS OF IONS (M-MOLE/kg. H_2O) IN PLASMA, AQUEOUS HUMOUR AND CEREBROSPINAL FLUID OF THE RABBIT

Plasma				Aqueous Humour			
Na	151·5	Cl	108	Na	143·5	Cl	109·5
K	5·5	HCO$_3$	27·4	K	5·5	HCO$_3$	33·6
Ca	2·6	Lactate	7·9	Ca	2·3	Lactate	6·00
Mg	1·0	Phosphate	1·8	Mg	0·85	Phosphate	1·00
						Ascorbate	1·00
Total 160·6		Total	145·1	Total 152·1		Total	151·1

Cations and Anions 305·7   Cations and Anions 303·3

Cerebrospinal Fluid			
Na	151	Cl	129
K	3·5	HCO$_3$	31·4
Ca	1·3	Lactate	2·6
Mg	0·8	Phosphate	0·5
Total	156·6	Total	163·5

Cations and Anions 320·1

pump". The cerebrospinal fluid would appear to represent another example of a non-iso-osmotic fluid, and since it is in such close relationship with the nervous tissue of the brain and spinal cord, this lack of iso-osmolarity is of special interest, suggesting as it does that these tissues, too, are not in osmotic equilibrium with the blood. The results of a detailed analysis of the ionic concentrations in plasma and cerebrospinal fluid are shown in Table II; included are values for a similar

type of fluid, the aqueous humour—similar because both are specialized tissue fluids filling cavities and being virtually free from protein. By summing the cations and anions it becomes clear that the cerebrospinal fluid has a higher concentration than the plasma or the aqueous humour; allowance must be made for the lower concentrations of glucose and urea in the cerebrospinal fluid, a difference amounting to some 5 m-mole; thus the cerebrospinal fluid is hyperosmotic by some 9 m-mole. The amount is small—some 3 per cent—nevertheless it represents a difference of osmotic pressure of some 160 mm. Hg, and it is presumably because the fluid is able to drain away easily from its cavities that this pressure does not develop, i.e. the difference in osmolarity is reflected in a continuous influx of water from the blood rather than in the development of a pressure, such as would happen were the system completely closed. However, the really significant point to be made in this connexion is that the cerebrospinal fluid lies in such close relationship with the brain and cord that it seems most unlikely, having regard to the rapidity with which water may exchange between the two, that a difference of osmolarity could be maintained. That is, if the cerebrospinal fluid is, indeed, hypertonic to plasma, then so must the tissue of the brain and cord be. If this is true, then we may postulate one of two things: either a water pump that drives water out of the nerve cells into the extracellular fluid where it passes back into the blood; or alternatively the elaboration, by the capillaries of the nervous tissue, of a hyperosmotic extracellular fluid. The capillaries in this region of the body are certainly different from those in the rest of the body and are responsible, presumably, for the so-called "blood-brain barrier"; to attribute secretory activity to their endothelium is by no means an unreasonable proposition. The important point to be made here is that the difference of osmolarity is small and thus requires highly accurate analysis for its demonstration. Why the cerebrospinal fluid and nervous tissue should have this higher osmolarity is not clear; according to Flexner (1938),

the high concentration of chloride in the cerebrospinal fluid, which may be taken as a measure of this hyperosmolarity, appears at an early stage in development—at about 40 days in fact. It may be that the positive pressure of the cerebrospinal fluid depends for its maintenance on a difference of osmotic pressure between it and the blood.

The factors determining the water and electrolyte contents of connective tissue are probably simple, although they have not been studied in great detail. If a piece of collagen, or collagen plus mucoid, is placed in a saline medium, equivalent to extracellular fluid, we may expect a Gibbs-Donnan equilibrium to be established between this and the medium by

$$Na^+ Cl^- \quad \boxed{\begin{array}{c} Na^+ \ Coll^- \\ + \\ Na^+ \ Cl^- \end{array}} \quad Na^+ Cl^-$$
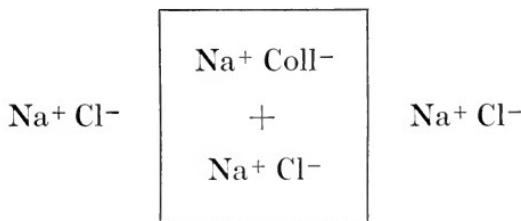
FIG. 5. Illustrating Gibbs-Donnan equilibrium between collagen and extracellular fluid. In this case there is no membrane separating the two, the collagenous gel being a separate phase.

virtue of the acidic nature of the protein and mucoid. The situation might therefore be as in Fig. 5, i.e. essentially similar to that obtaining with plasma separated by a membrane from extracellular fluid. There is no membrane separating the two, however, and separation is maintained because of a phase difference, the collagen-mucoid system being a gel, the extracellular fluid a liquid. Chemical analysis of connective tissue shows that there is, indeed, a Gibbs-Donnan distribution of ions between it and plasma and therefore, presumably, between it and extracellular fluid, the concentration of chloride being less, and that of sodium greater, in the connective tissue. There is, in consequence, a tendency for water to pass into the connective tissue phase, the salts

continuously redistributing themselves so that the osmotic pressure of this phase is greater than that of extracellular fluid and of blood. The extent to which the system will take up water will depend on the counter-pressure that can be exerted or, failing that, what is really equivalent, the mechanical rigidity of the system that will oppose distention. Presumably in such tissues as tendon and skin the structural rigidity of the system prevents an indefinite uptake of water, and the system is stabilized with a water content of about 75 per cent. In the cornea of the eye, however, the situation

Table III

COMPARISON OF EYES MAINTAINED AT NORMAL AND LOW CORNEAL TEMPERATURES

(Davson, 1955)

Expt. no.	Temp. (°)	Time (hr.)	Water Content	
			(g./100 g. tissue)	(g./g. solid)
1	7	15	82·8	4·8
	31	—	77·2	3·4
2	7	15	82·8	4·8
	31	—	77·0	3·35
3	7	17	82·1	4·65
	31	—	78·2	3·6
4	7	7	78·5	3·65
	31	—	77·8	3·5

is different; it consists, essentially, of a number of laminae of collagen-plus-mucoid, sandwiched between two cellular layers, the epithelium and endothelium. If the eye is excised and stored in the cold, say at 4°, the cornea increases in water content, due to absorption of aqueous humour. If instead of being kept at 4° the eye is maintained at about 31°—the normal temperature of the cornea—the tissue retains its normal water content (Table III). It would seem, then, that metabolic activity is preventing the collagen plus mucoid from absorbing water and salts from the aqueous humour, and this may be proved by first allowing the cornea to swell at the

low temperature and then transferring the eye to a chamber maintained at the higher temperature. In this case the absorbed water and salts are excreted back and the cornea reacquires its normal hydration (Table IV). The secretory activity that usually maintains the cornea in its normal state of hydration—about 75 per cent water—may be due to both the endothelium and epithelium, but whether it is due to an active excretion of salt, e.g. sodium, or of water, remains to be proved. The extraordinary tendency of the cornea to take up water, by contrast with tendon or sclera, is presumably

Table IV

THE EFFECT OF SUBSEQUENT WARMING ON EYES MAINTAINED FOR 15–18
HOURS AT 7°

(Davson, 1955)

Column A gives the water content after the period at 7°; column B the water
content after a further period of 6–8 hours at 31°.

Expt. no.	Water content (g./g. solid)		Change (%)
	(A)	(B)	
1	4·35	3·3	24
2	5·1	3·0	41
3	4·45	3·7	17
4	4·65	3·9	16

related to the large quantity of mucoid present as a coating over the individual collagen fibrils (Schwarz, 1953), and it seems likely that changes in hydration are really the consequence of changes in hydration of this colloid, the collagen fibrils being pushed apart by the swelling. The Gibbs-Donnan swelling of the collagen-mucoid system of skin and subcutaneous tissues may well be a factor in determining the water content and the turgescence of the tissues. Thus it would seem from McMaster's (1946) studies that the extracellular fluid may, in normal circumstances, be something of an abstraction, the space between cells and collagen fibrils being occupied by a mucoid gel; only when excessive amounts of fluid are filtered from the plasma, or under experimental

conditions of injection of fluid into the tissue, is it possible to speak of free fluid in the extracellular spaces. The nature of the collagen and mucoid in these tissues may therefore exert some effect on the water content of the tissues. In general, it would seem that acute changes in this tissue extracellular water are the result of changed factors of capillary filtration and reabsorption, but it may well be that the long-term steady-state level is influenced by the amount of mucoid in the tissue. This presumably exerts its Gibbs-Donnan difference of osmotic pressure, drawing fluid to it; the tendency is opposed by the structural rigidity of the tissue, so that a steady state is established, in contrast to the cornea where the rigidity of the system is inadequate to permit a steady state, a continuous secretory activity being necessary, and made possible by the presence of cellular membranes lining the tissue.

The possible ways in which the water compartments of the body may be altered with age become evident from this general review; thus, the activity of the ion-transporting mechanisms of the cells tends to oppose a normal tendency to cell oedema, with the result that a steady state is maintained with the cells having a characteristic ionic make-up and percentage of water. A decrease in the metabolic activity of the cells may be expected to result in the penetration of salt and water into the cells; hyperactivity, on the other hand, may cause a shrinkage of the cells, but the extent of this will be limited by the demands of electrical neutrality; excessive excretion of the Na^+ ion must be associated with excretion of some anion or with accumulation of K^+; in the latter event there will be no change in osmolarity, whilst the former process is limited by the availability of diffusible anions. It seems unlikely that a cellular dehydration could result from hyperactivity of this sort, and it seems more likely that dehydration of cells might be due to a loss of the indiffusible anions, collectively indicated as A^- in Fig. 4, but actually consisting of proteins, organic phosphates, etc. If these were replaced by such diffusible anions as Cl^- and HCO_3^-, then the process of

extrusion of Na^+ would lead to an elimination of these ions and it could well be that a new steady state would be established at a lower level of internal K^+ and Na^+ concentrations. Unfortunately, practically nothing is known of the factors that control the normal activity of the salt-excreting system of the cell.

The large differences in the amount of extracellular water that take place with age may be, to some extent, associated with differences in the amount of water per cell of the organism; thus, other things being equal, a decrease in cellular water is reflected in a rise in the extracellular water, expressed as a percentage. To prove this, however, it would be necessary to measure not so much the percentage water in the cells as the amount of water per cell, and this might be attempted by relating the water to the deoxyribonucleic acid content of the tissue. It seems more likely, however, that long-term fluctuations in the fractions of intra- and extracellular water, especially those taking place during development, will be determined by changes in the number of cells in unit weight of tissue rather than in changes of their size, and this could be achieved by (a) multiplication or reduction of the number of cells; (b) expansion or contraction of the extracellular space, by changes in the quantity of connective tissue and in the ability of this to hold fluid.

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DISCUSSION

Talbot: I was most interested, Dr. Davson, in your comments about the cellular oedema that occurs in 'sick' cells. It has been shown that animals deprived of potassium, and thereby subjected to a combination of cellular potassium insufficiency and cellular sodium intoxication, show a tendency to cellular oedema. We therefore wondered whether loss of potassium from the cell was a factor which might interfere with its sodium and water pump mechanisms.

Davson: We still do not really know what makes a cell stop accumulating. Accumulation may be a matter of the development of some anions inside the cell at the same time as the development of a process of excreting the sodium. But if you get rid of sodium, something has got to come in and it may be potassium. That eventually leads to the development of more of these ions and to a condition in which there is a high potassium concentration inside, and low sodium and chloride. When we allow the system to cool or give it poison, then we find that sodium comes in and potassium goes out; but when we warm it up again the whole thing reverses and we get back to the original state of affairs. Whether it is that the cell will stop with a given potassium concentration ratio, or a given concentration of sodium, or at a given size, we do not really know for certain. In potassium deficiency, according to the papers I read rather a long time ago, one found that potassium was substituted for by sodium.

Talbot: That is if sodium is available.

Davson: So you propose a condition where there is a sodium as well as a potassium deficiency?

Talbot: You could have simple deprivation with loss of cellular potassium, but without entrance of sodium in any appreciable amount. There you have a relatively benign situation. When you superimpose cellular sodium intoxication, things really begin to get mixed up. How do you fit that in with your very interesting observations?

Davson: It is really a matter of thinking these things out as separate problems as they arise, and there has been no systematic investigation of this. We still have no idea of the mechanism of sodium excretion, and what makes it stop. If one did know more, one would be able to fit in the results with the general physiology of the organism.

Fejfar: In clinical medicine we now accept that active sodium transport and potassium deficiency are very important factors. We assume, when we analyse a muscle biopsy specimen, that we will get a good

representative sample of what is going on in the organism. Is this a fair assumption? A second point is that most of the work has been done on kidney slices. Are kidney slices representative of the whole organism, or only of the kidney tissue?

Davson: I was thinking in terms of the tissues that I have worked with —not the kidney, but muscles and red cells. As far as I can see, the results of the work on the kidney cortex are essentially similar to those obtained on the muscle. However there might be a confusing situation if you got a lump of kidney tissue with fairly intact tubules as well as not so intact tubules; they could be accumulating sodium and indulging in their special secretory processes which are quite different from those in muscle. I have never looked with any approval on work done with slices of these specialized tissues.

Fejfar: Quite a lot of work has been done with kidney in Prague by Cort and Kleinzeller (1956. *J. Physiol.*, **133**, 287) and that is why I asked you. They support an active mechanism for sodium and passive mechanisms for potassium and chloride.

Davson: The active accumulation of potassium by most of the cells that have been studied has not had to be specifically invoked. It is almost an unnecessary hypothesis for muscle, but on the other hand one finds that the active transport of sodium is linked with that of potassium. If one is an active process, the other must be too. From the responses to changes of environment, one must say that potassium is following its gradients of electrochemical potential. On the other hand, when the matter is studied with isotopes and it is found out just how much sodium is going in, it is seen that there is a linkage between the amount of sodium crossing the membrane and the amount of potassium. It is not a rigid linkage, however.

Fejfar: Cort and Kleinzeller find that the amount of potassium crossing the membrane is usually smaller than the amount of sodium.

Davson: Yes, there is a 2 : 1 ratio. In the muscle it is a certain proportion, and in the red cell it is a different proportion. Certainly in the red cell an active accumulation of potassium as well as of sodium has to be invoked.

Fejfar: Roguski in Poland claims that one can judge general cellular metabolism from the red cells themselves. We do not agree because the red cell is not a respiring cell. Neubauer (personal communication) has made a comparison of the water and electrolyte changes between muscle biopsy specimens and red cells, and he could not find any similarity between them. He came to the conclusion that you could not judge electrolyte changes from the red cell.

Davson: That is quite true. The mammalian red cell metabolism is different; it is largely anaerobic, whereas the muscle and all the other cells are mainly aerobic.

Fejfar: I was surprised to hear you say that when cells are poisoned there is not only an influx of sodium, but also of chloride. We were taught that chloride does not usually enter cells in significant amounts and that only sodium does this, so we judge the extracellular fluid by the chloride present.

Davson: That would be a most dangerous conclusion to draw. If your chloride space altered under experimental conditions, it could very well be due to penetration of chloride into the cells.

Wallace: We have been working with tissues for some time from the standpoint of hydrogen ion gradients between cells and extracellular fluid. I have often discussed this work with investigators interested in single cells and the events that occur within the cell. One often finds that such workers are unwilling to accept the interpretations derived from analytical values for whole tissues. They point out that the interior of the cell is not homogeneous. Potassium and sodium do not appear to be evenly distributed and the hydrogen ion concentration seems to vary from locus to locus. I am certainly not ready to give up the study of ions and their distribution in tissues, but I think one must always bear in mind that membrane equilibria can only tell a part of the story. The concept of the cell, particularly the muscle cell, as an "empty bag" cannot be completely accepted.

Davson: In general I am in favour of your iconoclastic approach, but you are basing most of your argument on the findings of the electron microscopists and they are by no means above criticism themselves. They are working on fixed tissue and talk about their endoplasmic reticulum. It certainly appears as a most complicated system of canals, but one wonders how real it is. Is one to abandon all hope of applying rather elementary physical chemistry to our problems just because of these complexities? We think of the cell as being bounded by a limiting membrane with certain permeability characteristics. The electron microscopists show us the membrane which does exist, but then they find little holes or vesicles just next door to it. They say that what is happening is that the membrane is opening up, the vesicle is coming in and they have caught it just as it was coming in. It may well be that they are right. We have obviously got to be suspicious of treating things too simply—there you are absolutely right. On the other hand, I am not willing to stop applying elementary physical chemistry to problems of salt transfer just because of these complexities.

Adolph: I should like to add something to the point about swelling and shrinking with the accompanying transfers of electrolytes. When tissue slices, not only kidney slices but also liver slices, and two tissues which we did not have to slice, i.e. diaphragm and auricle, are transferred from low temperature to high, or from anoxic media to oxygen, they shrink. This shrinking in high temperature and oxygen is fully reversible any number of times; for instance, in ten-minute periods, in low temperature or in high, in nitrogen and in oxygen, we can get complete reversibility of the swelling and shrinking (Adolph, E. F., and Richmond, J. (1956). *Amer. J. Physiol.*, **187**, 437). This indicates that there is no permanent damage to these tissues from the swelling and shrinking, and it also indicates that the transfers are very rapid. It looks as though, if there is electrolyte transfer, it is as rapid as that of water. But I am not convinced that the electrolyte transfers are necessary for this swelling and shrinking. We have no method of measuring the speed of the electrolyte transfers, but we have a method of measuring that of the water transfers.

Maybe someone can furnish data which will be more convincing on whether the electrolyte transfers are equally rapid and reproducible.

Davson: I think the electrolyte transfer is very likely to be much slower and to hold up the whole process. The water transfer is very rapid in every cell, so I would say that what happens first is the movement of the electrolyte and the movement of water would not require much time. The evidence I am citing is largely based on work from Prof. Conway's laboratory.

Hingerty: One of the main experimental difficulties, of course, is in maintaining the normal condition of the cells. When you remove tissues from an animal there is a very rapid increase in molecular concentration in the cells due to breakdown of molecules such as glycogen, hexose esters, phosphocreatine and adenosine triphosphate (Conway, E. J., Geoghegan, H., and McCormack, J. (1955). *J. Physiol.*, **130**, 427). If you remove the tissue directly into liquid oxygen, grind to a frozen powder and then take a series of freezing point depressions on this frozen tissue maintained at $0°$, extrapolation back to zero time gives a value equal to that obtained for the plasma (Conway, E. J., and McCormack, J. (1953). *J. Physiol.*, **120**, 1). This certainly held for liver, kidney and muscle tissue of the rat and it would be interesting to see these techniques applied to other tissues.

The swelling of the cells in anoxic conditions cannot be due to a failure to pump out water, since the freezing point depressions of respiring and non-respiring kidney slices are the same, and the effect of anoxia may be interpreted as being due rather to cessation of the sodium pump. Breakdown of molecules may be partly responsible for the swelling but the main effect appears to be caused by sodium and chloride entering the cell (some potassium leaving), and water then entering to preserve osmotic balance (Conway, E. J., and Geoghegan, H. (1955). *J. Physiol.*, **130**, 438).

HYPERNATRAEMIA AND HYPONATRAEMIA WITH SPECIAL REFERENCE TO CEREBRAL DISTURBANCES

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Introduction

An abnormal concentration of sodium in the extracellular fluid often presents a puzzling problem for the clinician. As is well known, a change in the total amount of the sodium or of the water in the body can explain many instances—water deficiency or sodium excess producing hypernatraemia, water excess or sodium deficiency producing hyponatraemia. But many cases appear to require more than a simple account of gains and losses to explain them. Is this because a simple explanation, such as a change in the amount of water in the body, has been overlooked, or must one in such cases invoke some new mechanism, possibly under the control of the nervous system?

There have been a number of reports of "cerebral" hypernatraemia and hyponatraemia (Knowles, 1956; Edelman, 1956). With regard to hypernatraemia it seems likely that some of the contradictions in the present views (Welt *et al.*, 1952; Higgins *et al.*, 1954) might have been avoided, for in hardly any of the patients reported could a frank water deficiency confidently be excluded from the information supplied. This question is discussed in the first section. The subject of hyponatraemia seems much more difficult, but if sodium deficiency is excluded, many of the remaining cases can be accounted for by an abnormal retention of water diluting the body fluids. In the second section we present some new data on the problem, derived from a study of two patients.

Before discussing the subject in more detail it may be helpful to recall some of the factors which regulate the water content of the body.

Regulation of water

Two mechanisms, closely linked, normally guard against water depletion. One regulates the intake of water through the sensation of thirst, the other the output of water through the secretion of antidiuretic hormone. There are at least two ways in which each may be invoked: the first, a rise in the tonicity, the second, less well known, a fall in the volume of the body fluids (Smith, 1957; Strauss, 1957).

A rise in the sodium content of the extracellular fluid (ECF) is well known to produce thirst and to stimulate the release of antidiuretic hormone (ADH). The effective stimulus is not simply the rise in ECF tonicity: if the ECF tonicity is raised with a substance like urea, which diffuses freely across the cell membrane and raises the tonicity of both extracellular and cellular fluid equally, this does not stimulate thirst and antidiuresis to the same extent (Gilman, 1937). When, however, the extracellular tonicity is raised by a substance which does not diffuse into the cells, water leaves the cells until the tonicity of extracellular fluid and cells are again equal. The cells shrink. It is assumed that certain cells in the hypothalamus respond to shrinking and stimulate the sensation of thirst and the liberation of ADH.

For the release of ADH there is much evidence that there are localized receptors of this kind (Jewell and Verney, 1957; Verney, 1957). Recently Andersson (1957) has also provided additional evidence for a thirst centre. He found that when he stimulated a certain area of the hypothalamus in goats, they drank water as long as the stimulus went on, even to the point of haemolysing their own red cells. With destructive lesions in the same region, the goats would not drink water when they obviously needed it. The thirst centre and the receptors of the ADH mechanism are very close together, but probably distinct.

The position of these centres in the nervous system suggests that their control involves more than a response to changes in tonicity, and some purely nervous stimuli such as pain and emotion may initiate, or inhibit, thirst or antidiuresis.

A fall in the volume of the ECF can stimulate thirst and antidiuresis, presumably through nervous pathways (see Rosenbaum, 1957; Strauss, 1957). Smith (1957) has discussed at length where the receptors for the stimulus to antidiuresis might be: some of them may be in the left auricle of the heart (Henry and Pearce, 1956).

Hypernatraemia
Water deficiency

Normally, thirst and antidiuresis are stimulated by a very small increase in extracellular tonicity, less than two per cent (Wolf, 1950; Verney, 1957). A concentration of sodium ([Na]) in the plasma exceeding 150 m-equiv./l. may certainly be regarded as abnormal. In a study of water deficiency produced experimentally in dogs, values of 160, and in one animal that died a value of 186 m-equiv./l., were found (Elkinton and Taffel, 1942); in a man made water-deficient by Black, McCance and Young (1944) the [Na] rose to 160 m-equiv./l. In a patient from Texas reported by Gordon and Goldner (1957) a value as high as 192 m-equiv./l. was reported. He recovered.

The "dehydration reaction". The hypernatraemia of water deficiency is not simply the result of the blood becoming more concentrated, for in spite of the high blood level of sodium there may be very little sodium in the urine; it is retained in the body.

Allott (1939), who first drew attention to the problem of hypernatraemia, found the urinary [Na] ranged from 2·5 to 9 m-equiv./l. in four of his patients. It now seems most likely these low concentrations of sodium were a result of the "dehydration reaction" first described by Peters (1948, 1952). The mechanism of this reaction is not clear, though it appears to be a renal response to a fall in blood volume; in this con-

nexion it may be recalled that two of Allott's patients had had an alimentary haemorrhage.

It is partly through neglect of this phenomenon that some authors have been led to place cases of hypernatraemia with a low urinary sodium in a separate group.

Symptoms of water deficiency. There are several reasons why authors describing neurogenic or cerebral hypernatraemia may have overlooked a water deficiency. Though they often state that there is no clinical evidence of dehydration in their patients (e.g. Cooper and Crevier, 1952), this does not in fact mean very much. The word *dehydration* is used for two clinical states: one of water deficiency alone, and the other of salt deficiency which generally also entails a loss of water. This usage implies that these deficiencies produce a similar clinical picture, though it was made clear long ago that this is not so (Kerpel-Fronius, 1935; Nadal, Pedersen and Maddock, 1941). Water deficiency is not clinically obvious unless it is extreme, because the deficit is distributed throughout the body water. In salt deficiency, on the other hand, the extracellular fluid, though but a third of the total in volume, bears the whole of the deficit; it is patients with the latter who have the haggard look, the sunken eyes, the small pulse and low blood pressure of dehydration. Patients with simple water deficiency are ill, but there are no specific signs of the deficiency, the tongue may even be moist, and it is not obvious it is water they lack. If in addition, as a result of a craniotomy their faces are oedematous, it may even be mistakenly assumed that they have accumulated water in excess. The diagnostic difficulties are increased because, particularly in older patients, some of the most striking symptoms of water deficiency are cerebral rather than vascular, for instance drowsiness and confusion, and disturbances of behaviour, which can mimic a lesion of the frontal lobes. These symptoms make it more difficult to give water; but they can be completely reversed with water.

Losses of water. Abnormally large losses of water may go unrecognized. Extrarenal losses may be larger than is

generally assumed; and a good urinary output does not necessarily mean there is no deficit of water, for it may represent failure of conservation. In the unconscious or helpless patient the intake depends on the physician's instructions and the nurses' care. If the intake is less than the combined losses from the skin, the lungs and the bowels, there must be a deficit of water in the body and the plasma [Na] will eventually rise.

Some cerebral lesions are associated with a high fever, or with excessive sweating, or with an abnormally rapid respiration. With any of these the insensible losses of water may increase from the normal value of some 800 ml. They have rarely been measured, but in one patient they were thought to be as much as five litres a day (Gordon and Goldner, 1957).

One expects the volume of urine to be small in water deficiency, and its concentration high. But there are three ways in which untoward renal losses of water may contribute to water deficiency: diabetes insipidus from a failure of the pituitary-hypothalamic mechanism; defective renal function; and osmotic diuresis. Neither the first nor the second has always been excluded in cases reported as cerebral hypernatraemia. Diabetes insipidus possibly explains cases 1 and 3 of Cooper and Crevier (1952) and one case of Natelson and Alexander (1955). The force of this explanation is emphasized by a patient reported by Peters (1948), a young woman whose serum [Na] rose from 140 to 171 m-equiv./l. in 24 hours following an operation for craniopharyngioma which was complicated by diabetes insipidus. In an incontinent patient a low concentration of the urine may be the only clue to diabetes insipidus, and the effect of pitressin should be tried in all patients with hypernatraemia in whom this possibility exists.

The excretion of a large amount of solutes produces an osmotic diuresis (McCance, 1945; Hervey, McCance and Tayler 1946; Rapoport *et al.*, 1949). This happens in spite of a water deficiency (McCance, Young and Black, 1944) and may even be the cause of it.

Urea, sodium and chloride are the main osmotically active constituents of the urine. The excretion of urea may be

increased by an abnormal breakdown of body protein or by excessive protein in the diet. One hundred grams of protein contain 16 g. of nitrogen, excreted as 34 g. or 570 m-osm. of urea. Ten grams of sodium chloride provide 340 m-osm. It is not unusual for unconscious patients to receive these amounts in their feeds; and their endogenous production of urea may already be very large (Cooper *et al.*, 1951). The hypernatraemic patient of Natelson and Alexander (1955) presumably had an osmotic diuresis when he was made worse with "non-saline fluids", because these consisted partly of protein hydrolysate equivalent to 100 g. of protein. In certain neurological disturbances (Astrup, Gotzche and Neukirch, 1954; Whedon and Shorr, 1957) and in water deficiency itself (Black, McCance and Young, 1944) the breakdown of body protein may be greatly accelerated.

To detect a water deficit, the minimum data required are the estimated intake and output of water and solutes, and the volume and concentration of the urine. A water deficit is confirmed if, with the administration of water, the elevated plasma [Na] falls.

In many of the reports of cerebral hypernatraemia it is impossible to decide from the data given what the water balance was. The patients with hypernatraemia of Higgins and his co-workers (1954) seem to have begun with a deficit of water of about one litre. Subsequently their intake of water may have been as little as two litres daily. Their exogenous osmolar load was about 610 m-osm. We do not know what was the total excretion; urine volumes and specific gravities are not stated. The blood urea was high, and fell as the plasma [Na] fell, when their intake of fluid was increased. In other reports the data actually show there was a cumulative deficit of water although the fact may have been disregarded (Anthonisen, Hilden and Thomsen, 1954; Allott, 1957).

Failure of thirst. Even when losses of water do go unrecognized by the clinician, there is no danger of water depletion as long as the patient responds normally with thirst and is able to drink. For example, in uncomplicated diabetes

insipidus the plasma [Na] is not usually very much raised; in a patient of ours, a man of 28 with sarcoidosis, the plasma [Na] was at times as high as 149 m-equiv./l., but he was then very thirsty, and he would not tolerate the [Na] rising any higher. On the other hand, patients who are apathetic, weak, disorientated or unconscious may be unaware of thirst, or unable to respond to it. In these patients even normal losses of water may lead to water deficiency with hypernatraemia. It is not unusual to have elderly patients with cerebro-vascular disease who tolerate a plasma [Na] of 150 m-equiv./l. without any complaint of thirst. But when they are given water they retain it, and their clinical and biochemical responses show they had a need for it. We do not know the possible sites of the lesions which may interfere with the sensation of thirst in these people. There is, however, some evidence that in man (Leaf and Mamby, 1952; Engstrom and Liebman, 1953), as in the rat (Stevenson, Welt and Orloff, 1950) and the goat (Andersson, 1957), neurological lesions may interfere with the normal sensation of thirst.

We have had the opportunity of studying a boy of ten who had had a large suprasellar craniopharyngioma removed by Mr. C. Langmaid. There was no evidence of diabetes insipidus before the operation. After the operation, however, while he was in a stuporous state, his plasma [Na] ranged between 152 and 163 m-equiv./l. It remained high even when he recovered, and was up and about, and receiving pitressin. The boy did not complain of thirst and we think the lack of thirst led to water deficiency and hypernatraemia. These are some of the values before and after he received pitressin:—

	Date	Plasma sodium m-equiv./l.	Urine vol: ml. per 24 hours.	Specific gravity
Before pitressin	7 Nov.	161	1370	
After pitressin	21 Nov.	156	860	
	25 Nov.	156	1420	1·008

The urine volume and specific gravity while he was having pitressin suggest the treatment was inadequate, but he did

not respond, as does the ordinary case of diabetes insipidus, with thirst. (He recovered spontaneously from his diabetes insipidus, and from his hypernatraemia, after three months.) Although this type of hypernatraemia might be termed cerebral, it is in fact a water deficiency due to the breakdown of one of the mechanisms that normally ensure water balance.

Renal effects of water deficiency. Before leaving the question of hypernatraemia due to water deficiency it may be noted that in many of the reported cases the disturbance apparently produced a disorder of tubular function, manifested by oliguria with isosthenuria or by the excretion of urine with a high pH in the presence of a systemic acidosis (Cooper and Crevier, 1952 (Case 4); Gordon and Goldner, 1957; Allott, 1957). This suggests that severe water deficiency may be accompanied by tubular damage; Allott (1939) noted a tubular degeneration in two of his cases *post mortem*.

A tubular damage would help to explain the acidosis in at least one of the patients of Higgins and his co-workers (1951). It is not possible to say with any certainty whether these patients were water-deficient, but all of them had a high blood urea and in relation to this the urine volumes were certainly small. It is also possible that in some patients (e.g. Allott, 1957) polyuria with hyposthenuria represented the diuretic phase of a tubular necrosis, itself the result of dehydration.

To sum up the question of "cerebral" hypernatraemia, a failure of the thirst mechanism, with or without a diabetes insipidus, accounts for some of the cases that have been described; and, as Gordon and Goldner (1957) have ably illustrated, unrecognized renal or extrarenal losses of fluid must account for many more.

If, as we believe, cerebral hypernatraemia is the result of water deficiency then water will correct it, but only if enough is given. Unfortunately most authors have underestimated the amount of water required to correct a severe deficit. Higgins and co-workers (1954) gave up to four litres to the patients they thought were water-deficient. We give nearly

this amount routinely. Gordon and Goldner gave one of their two patients 8·24 litres in 24 hours and even this was not enough to bring down his plasma [Na] to normal. As long as the plasma [Na] remains high there can be no risk of water intoxication.

Other forms of hypernatraemia.

It is possible to produce hypernatraemia by giving an excess of salt (McCance, 1956), though more usually this produces an isotonic expansion of the extracellular fluid with oedema.

The homeostatic mechanisms may be so adjusted as to maintain the plasma [Na] at a high level. In experimental potassium deficiency the plasma [Na] was over 150 m-equiv./l., although the absorption of sodium was small and the intake of water as much as eight litres a day in one subject (Fourman, 1954). Hypernatraemia is often a feature of aldosteronism (Conn, 1956) but whether or not this is the result of the associated potassium deficiency cannot be stated. Recently Zilva and Harris-Jones (1957) have discussed the possibility of excessive adrenocortical activity producing hypernatraemia by a shift of sodium from cells to ECF.

Hyponatraemia

We may arbitrarily define hyponatraemia as a plasma [Na] lower than 130 m-equiv./l. It is obvious the concentration of sodium in the plasma may fall because of a reduction in the total amount of sodium in the ECF or because of an increase in the amount of water.

Salt deficiency

A reduction of the *total* amount of sodium in the ECF is the result of sodium deficiency.

We have already emphasized that the clinical effects of sodium deficiency are easily recognizable. Lack of salt is unlikely to arise unless, through sweating, vomiting, diarrhoea or fistulous discharge, sodium is lost from the body, because

the kidneys normally conserve sodium efficiently. For the same reason, in sodium deficiency there is virtually no sodium in the urine. To this there is one exception, namely, when the sodium deficit is actually the result of continued loss through the kidney. This happens, of course, in Addison's disease, and in "salt-losing nephritis". Furthermore, in certain patients with cerebral lesions persistent renal losses have been observed, even when the intake of sodium is much reduced (Welt *et al.*, 1952). The renal defect has been ascribed to a loss of neural impulses affecting proximal tubular function (Cort, 1954). But the patient of Merrill, Murray and Harrison (1956) with malignant hypertension was able to maintain a normal sodium balance when his own kidneys were replaced by a kidney which was transplanted from his twin brother and therefore deprived of its nerve supply. It does not seem then that a *loss* of nervous impulses is alone responsible for a failure of the kidneys to conserve salt, though the renal nerves do play a part in the response to salt deprivation (Bricker *et al.*, 1956) and to anoxia (Földi, Kovách and Takács, 1955*a*, *b*). The mechanism of the defect in "cerebral salt-wasting" remains obscure. Water excess (see below) may produce a renal loss of sodium, and some instances of so-called salt wasting may therefore be examples of water retention.

Hyponatraemia from salt deficiency can, of course, be corrected with salt.

A deficiency of sodium, producing hyponatraemia, can arise without a loss of sodium from the body. The sudden accumulation of a transudate in some part of the body produces a relative lack of salt and water. If only water is provided the [Na] falls. This state of affairs is seen most clearly after a paracentesis, when water, carrying sodium with it, may rapidly reaccumulate in the abdominal cavity. The fall in blood volume presumably stimulates thirst and the liberation of ADH; for the patient, while drinking copiously, produces only a small amount of concentrated urine containing very little sodium (Nelson, Rosenbaum and Strauss, 1951).

Water excess

Water excess is a well recognized cause of hyponatraemia when patients are given too much water while unable to excrete it at the normal rate (Wynn, 1956). This may happen in renal failure, in adrenal and pituitary insufficiency, and postoperatively, particularly after mitral valvotomy (Bruce et al., 1955). Hyponatraemia from this cause is usually obvious from the circumstances. Such patients may have no symptoms; sometimes they have the syndrome of water intoxication, with fits and other profound neurological disturbances. They may have hypertension; they certainly do not have hypotension. The face looks bloated, not drawn.

Both sodium deficiency and simple water excess respond to the administration of hypertonic saline with a rise in the plasma [Na] to normal which is subsequently maintained.

There remains for consideration a large group of cases where the hyponatraemia does not produce symptoms and its mechanism is obscure. Elkinton (1956) and McCrory and Macaulay (1957) have recently reviewed this problem. The hyponatraemia appears to be associated with an expanded volume of ECF; and the kidneys do not excrete water or retain sodium to bring back the tonicity of the plasma to normal (Leaf and Mamby, 1952).

There are at least two possible explanations. The first is that there is an abnormal stimulus to antidiuresis, say from the "volume receptors", operating through the secretion of ADH or in some other way (Kleeman et al., 1955; Ginsburg and Brown, 1957). Pitressin given experimentally to normal people leads to a retention of water, a fall in the plasma [Na] and eventually an increased renal loss of sodium in spite of the low plasma [Na] (Leaf et al., 1953; Weston et al., 1953; Wrong, 1956).

The second possibility is that an abnormal hypotonicity of the cells determines the hypotonicity of the ECF (Sims et al., 1950; Rapoport, West and Brodsky, 1951).

McCrory and Macaulay (1957) described an infant with diffuse cerebral damage and hyponatraemia. Her ECF

volume was greater than normal. The infant did not excrete a dose of water at the normal rate and the authors thought she was secreting an excess of ADH. An excessive secretion of ADH would, of course, be appropriate only to a restricted fluid intake. When her fluid intake was restricted the plasma [Na] rose to normal.

Schwartz and co-workers (1957) have recently suggested that an inappropriate secretion of ADH might account for the hyponatraemia in two patients with carcinoma of the bronchus whom they studied. They imply that there was an abnormal stimulation of the receptors for maintaining the volume of the body fluids. Their patients had normal renal and adrenal function; they excreted a normal amount of aldosterone. In one of them the plasma [Na] fell as low as 103 m-equiv./l., but the extracellular volume, far from being reduced as in sodium deficiency, was expanded and there was no evidence of peripheral vascular failure. The urine was generally hypertonic to the plasma, and this is the principal argument adduced by Schwartz and co-workers that these patients were producing too much ADH. The kidneys of these patients did not conserve sodium when their fluid intake was unrestricted, though they did so when large amounts of salt-retaining steroids were given. Schwartz and co-workers do not comment on the rate of excretion of a dose of water. But there is no doubt the kidneys did respond normally to water deprivation. Under this stimulus the urinary sodium fell and the plasma [Na] rose. Others have also described this response to water deprivation in hyponatraemia (see Edelman, 1956). It might be interpreted as the usual "dehydration reaction".

Some observations we have made on two patients with unexplained hyponatraemia are relevant.

Case reports

Albert, aged 62, was admitted on 25th May 1957 in status epilepticus accompanied by hyperpyrexia and heavy sweating. He had been up and about until then, although he had had a right hemiparesis for two years, which had become worse two months before admission. His

blood pressure was 180/80. His fits were rapidly controlled, but he then had a bilateral spastic paralysis with extensor plantar responses, and never regained consciousness. On the second day he stopped breathing and respiration had to be maintained with a Beaver respirator for 12 hours. Subsequently he had a purulent bronchopneumonia and on the fourth day a tracheotomy was done to enable a clear airway to be maintained by suction. The bladder was kept drained by a Foley catheter but the urine was not infected until the last days of his illness. He died on 11th August of bronchopneumonia.

At post-mortem there was a large area of softening in the left temporal lobe. The vessels of the circle of Willis were very atheromatous. There was evidence of an earlier hypertension; the left ventricle was hypertrophied to a thickness of 22 mm. compared to 8 mm. in the right ventricle, and the kidneys showed hypertensive changes. There was remarkably little evidence of infection in them although there was a purulent cystitis.

Albert was certainly water-deficient in the early days of his illness. His extrarenal losses of water were large, and for the first three days his total intake was only two litres. On 29th May his plasma [Na] was 137 m-equiv./l. but at the same time the volume of the packed cells in his blood was 55 per cent. He was then given six litres of water in two days; the packed cell volume fell to 41 per cent and the plasma [Na] fell to 128 m-equiv./l. Subsequently his plasma [Na] fluctuated between 130 and 110 m-equiv./l. The blood urea was 34 mg. per 100 ml. and the creatinine clearance 70 ml./min.

Ivor, aged 54, was admitted on 11th June 1957 having been ill for 18 days with acute peripheral neuropathy affecting mainly the motor nerves and accompanied by an enlargement of the liver. The plasma albumin (2nd July) was 2·9, and the total protein 6 g. per 100 ml. The cause of his illness was not discovered. In the next five days he developed a partial respiratory paralysis with bronchopneumonia. His blood pressure, which had been normal, fell to 90/60. Subsequently he was fed by tube; and his purulent bronchial secretion was aspirated through a tracheostomy. At the end of June he began slowly to recover and was taking some food by mouth on 4th July, but almost immediately had a severe relapse. Tube feeding continued until the end of July, by which time he was able to move his limbs, though they were still very weak. He subsequently had three relapses and died in December. We have not the details of the latter stages of his illness.

Before he was fed by tube his intake of water was inadequate to cover his losses, which were augmented by copious sweating associated with his chest infection, and he must have sustained a considerable deficit of water and probably of salt. The water deficiency was corrected on 17th and 18th June by the administration of a total of 8·9 litres of water, of which he excreted only 3·5 litres during those two days. Consistent with a "dehydration reaction", on 17th June his urine contained only 2 m-equiv. sodium in 24 hours. With the correction of his water deficit his plasma [Na] fell from 133 to 120 m-equiv./l. in 24 hours. In spite

of the low plasma [Na], on 19th June he excreted 210 m-equiv. of sodium in three litres of urine. The plasma sodium remained low, ranging from 109 to 123 m-equiv./l. until August, when it gradually rose to 133 m-equiv./l. Except on two occasions, both early in his illness, one associated with salt deficiency and both with lung infections, he did not have peripheral vascular failure. His blood urea was 26 mg. per 100 ml. and the endogenous creatinine clearance 85 ml./min.

The daily feed in these patients consisted of protein, 90 g., fat, 120 g., carbohydrates, 120 g., in four litres of fluid. Until 6th July it contained 170 m-equiv. of sodium and thereafter 68 m-equiv., of sodium; the urinary excretion of sodium fell correspondingly in both patients.

Muscle analysis

The question whether the total sodium content of the body was low, or normal, but diluted by an excess of water in the ECF could be settled by an analysis of muscle.

Table I

ANALYSES OF MUSCLE FROM THE TWO PATIENTS, COMPARED WITH "NORMAL" VALUES

	Water per cent	m-equiv./kg. fat-free tissue		
		Cl	Na	K
Albert	74·4	31·8	45·5	91·2
Ivor	79·1	27·2	47·9	89·3
Talso, Spafford and Blaw (1953)	77·6±0·6	19·1±3·9	33·7±6·4	94·0±5·9
Wilson (1955)	77·5	25·6±5·1	40·6±6·0	92·3±7·6
Barnes, Gordon and Cope (1957)	80·3±1·6	23·1±6·5	43·6±11	91·3±8·3

ANALYSES OF PLASMA TAKEN FROM THE TWO PATIENTS AT THE TIME OF THE MUSCLE BIOPSY

	m-equiv./l.		
	Cl	Na	K
Albert	89·6	124	5·5
Ivor	87·6	124	3·7

The specimens were taken from paralysed muscles in both patients. The electrolyte contents are shown in Table I, with "normal" values for specimens taken from anaesthetized

patients. The potassium content was normal. The sodium content, far from being lower than normal, was in fact at the upper limits of the normal. The chloride content was similarly high. For this to happen with a low concentration of sodium in ECF, the amount of ECF in the muscle samples must have been larger than normal.

Hypertonic saline

The infusion of hypertonic saline produced only a transient increase in the plasma [Na].

The response was studied in detail in Albert. He had 500 ml. of 5 per cent sodium chloride (436 m-equiv.) infused over about three hours on 15th June when his plasma [Na] was initially 127 m-equiv./l. (Fig. 1).

The immediate response to this infusion was an osmotic diuresis with an output of 7·3 ml./min. of urine containing 330 m-osm. and 155 m-equiv. of sodium per litre. During the infusion he excreted 80 m-equiv. of sodium. The plasma [Na] increased to 143 m-equiv./l. during the infusion and was 138 m-equiv./l. at the end. In the following 21 hours he responded quite differently. He excreted only 55 m-equiv. of sodium and his urine flow fell to 0·2 ml./min. with a concentration of 696 m-osm./l. He was thus retaining water and diluting the sodium he had retained. Three days later his plasma [Na] was again only 130 m-equiv./l.

Ivor had infusions of 300 ml. of 5 per cent sodium chloride on 22nd June and 540 ml. on 24th June. We did not make very detailed studies of his response, but the plasma [Na] before and after the second infusion was 113 and 115 m-equiv./l. During the first three hours of this infusion when he had received 190 m-equiv. he excreted only 30 m-equiv. Both the infusions were followed by a retention of water.

These are not the responses one would expect from salt-depleted patients (Black, Platt and Stanbury, 1950). They imply that the osmolality of the body water was being maintained even at the expense of increasing the volume of the extracellular fluid. This is the normal response to hyper-

tonic saline (Crawford and Ludemann, 1951; Birchard, Rosenbaum and Strauss, 1953; Papper *et al.*, 1956), and depends, of course, on the liberation of ADH (Holland and Stead, 1951).
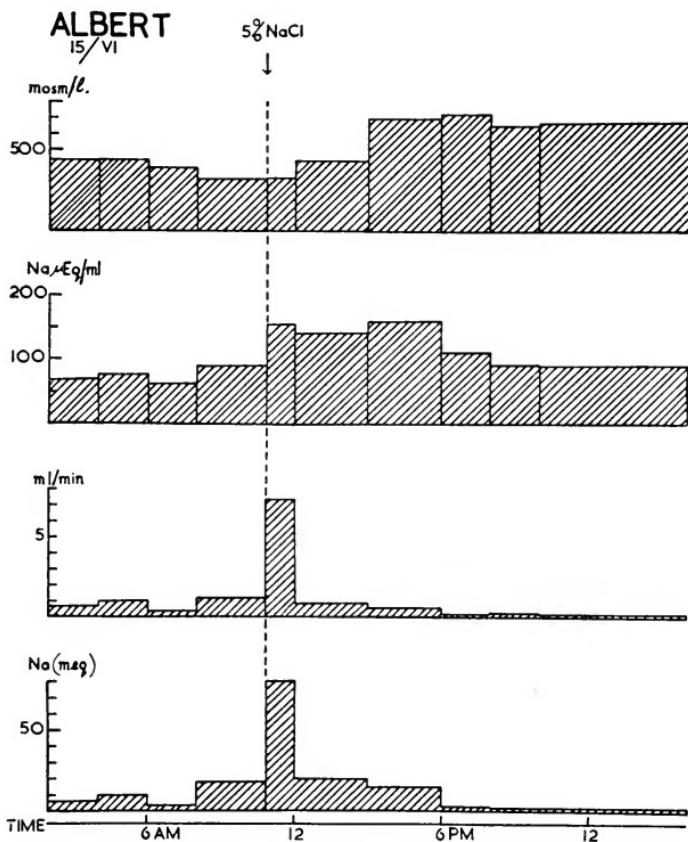

FIG. 1. The changes in total sodium excretion, urine flow, sodium concentration, and osmotic concentration of the urine after the infusion of 500 ml. of 5 per cent sodium chloride (436 m-equiv.).

Water deprivation

When fluid was withheld for 19 hours both patients produced a urine of small volume and high osmolality (Table II). The osmolality was not as high as might be expected in normal people; but the osmolality of the plasma of both patients was

low. The ratio of urine to plasma osmolalities, which can normally rise to about 4 with water deprivation, was $3 \cdot 7$ in Albert and $3 \cdot 3$ in Ivor. The deprivation of water was associ-

Table II

EFFECTS OF DEPRIVING THE TWO PATIENTS OF WATER FOR 19 HOURS

Changes in urine and plasma	Albert	Ivor
Maximum urine concentration (m-osm/l.)	904	870
Flow at maximum concentration (ml./min.)	$0 \cdot 27$	$0 \cdot 11$
[Na] (μ-equiv./ml. of urine)	$22 \cdot 6$	$14 \cdot 6$
Plasma (m-osm./l.)	243	267
Change in plasma ([Na] m-equiv./l.)	112–117	112–123

ated with a great reduction in the renal excretion of sodium, and the increases in the plasma [Na] were unexpectedly large. They were not maintained however, for the plasma [Na] had returned to the original levels after 48 hours.

Effect of water

The effect of a water load was adequately tested only in Albert, who on 15th July received one litre of water in 30 minutes, by stomach tube. He excreted all of this water in less than three hours, achieving a diuresis of $7 \cdot 3$ ml./min., with an osmolal concentration of 54 m-osm./l., and a sodium concentration of 4 m-equiv./l. These low concentrations are similar to the minimum values obtained in normal persons (Schoen, 1957). The values for the plasma sodium before and after the test were 115 and 112 m-equiv./l. Remarkably low osmolal concentrations were found twice in the 24-hour collections of urine from Albert. The values, 153 and 168 m-osm./l., were lower than in the plasma, in spite of the fact that at these times the plasma [Na] was exceptionally low, 104 m-equiv./l.; these values were obtained on the days immediately following administration of pitressin (see below).

We did not find any very low urinary concentrations in eight 24-hour collections from Ivor that were tested. In one specimen an osmolal concentration of 245 m-osm./l. was the same as that of the plasma taken at that time.

Effect of potassium chloride

In view of Laragh's (1954) findings of a rise in plasma [Na] with the administration of potassium chloride in patients with hyponatraemia, we gave 100 m-equiv. of potassium chloride on two successive days to both the patients. There was no increase in the plasma [Na] and only a slight rise in the plasma [K].

The data so far reported show that the renal excretion of sodium could be made to vary from very small to very large amounts, and, in particular, although sodium continued to be excreted while the plasma concentration was low, the kidneys were able to conserve sodium during the dehydration reaction. But a rise in the plasma [Na] produced by hypertonic saline was followed by retention of water which restored the osmolality of the plasma to its original level.

Effect of pitressin

All these results might be taken to show that these patients had an intact antidiuretic mechanism which operated to maintain their plasma osmolality at a lower level than normal. Their response, however, to exogenous ADH given as pitressin was quite unexpected.

After one litre of water by intragastric drip the patients received 100 m-u. of pitressin intravenously and 5 i.u. of pitressin in oil intramuscularly. Urine was collected in hourly periods for the following five hours; the gastric drip was running throughout, but the amounts given after the initial load were unfortunately not recorded. The results are shown in Table III. In Fig. 2 they are compared with the results of water deprivation. Ivor began with a concentrated urine, but after the first hour the maximum osmolality achieved after pitressin was some 500 or 600 m-osm. less than after dehydration. The effect of pitressin was tested a second time in Albert, and he then passed urine with a concentration of 215 m-osm./l., that is, lower even than his own hypotonic plasma. The low concentration of urine in these tests depended on the comparatively high urine flow, and not on a

reduced excretion of solutes. The rate of excretion of sodium and of solutes was actually higher than with dehydration, though lower than immediately before the pitressin was

Table III

EFFECTS OF PITRESSIN IN THE TWO PATIENTS WHILE THEIR
HYDRATION WAS MAINTAINED

Changes in urine and plasma	Albert	Ivor
Maximum urine concentration (m-osm./l.)	280	387*
Flow at maximum concentration (ml./min.)	1·5	2·4
[Na] (μ-equiv./ml. of urine)	33·2	60·2
Plasma (m-osm./l.)	233	243

* The results on the first collection (see Fig. 2) have been neglected.

given. Glomerular filtration rates were not measured. The same batch of pitressin was shown to have normal activity in other subjects.
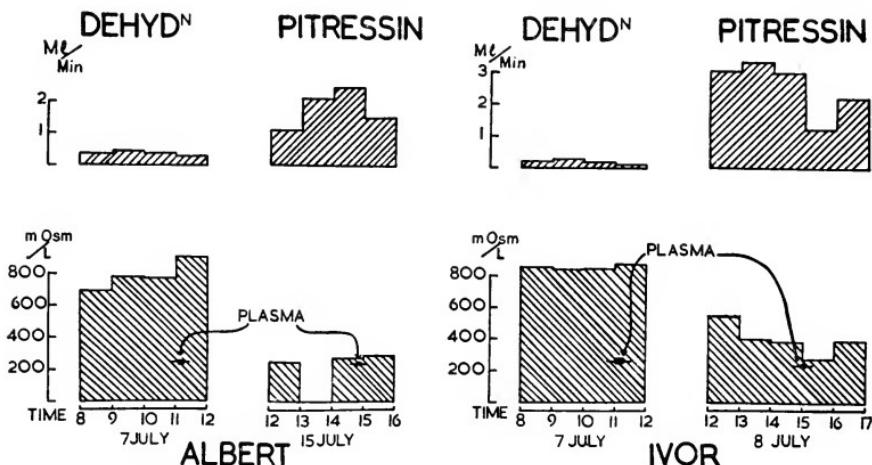

FIG. 2. Comparison of the changes in the flow and concentration of urine following deprivation of water and following pitressin and a water load.

The difference between the effects of water deprivation and pitressin is far greater than anything observed in normal people (Jones and de Wardener, 1956), and indeed indicates an almost complete failure to respond to pitressin in the

presence of a water load, while the response to water depriva-
tion was nearly normal. Pitressin was not entirely without
effect on the urine flow since this diminished.

The failure of Albert and Ivor to respond to pitressin might
represent the human counterpart of the experiments of
Wesson and co-workers (1950). Their dogs with an isotonic
expansion of the ECF did not respond to pitressin.

We have mentioned that the failure of response was not a
complete one, and it therefore remains possible that the
original expansion of the ECF represented an effect of the
patients' own ADH, as Schwartz and co-workers (1957)
postulated for their two cases. Although Schwartz and co-
workers do not remark on it, there were occasions when their
patient W. A., like Albert, produced a hypotonic urine
following an additional expansion of the ECF. These observa-
tions would be consistent with the suggestion that when the
ECF is expanded beyond a certain point the kidneys become
refractory to the action of ADH.

If we assume that an overproduction of ADH was reponsible
for the hypotonicity of the ECF in Albert and Ivor, the
alternatives previously suggested still remain, whether the
stimulus to ADH production represented a homeostatic
mechanism for maintaining a hypotonic ECF in two people
who might have had "hypotonic" cells; or whether it repre-
sented a response to an abnormal stimulation of some un-
identified receptor.

Summary

The problem of hypernatraemia seems in general to be one
of water deficiency. That of hyponatraemia is sometimes
one of salt deficiency, but often one of excessive dilution of
the ECF with water. The latter seems to have been the fault
in the two patients we studied. Muscle biopsies revealed
normal or high sodium contents. In their responses to hyper-
tonic saline, water deprivation, and water loading their
homeostatic mechanisms were adjusted to maintain an
abnormally large volume of ECF with low tonicity. Though

they produced a hypertonic urine of low volume when deprived of water, they did not always produce a hypertonic urine with pitressin and water. Under certain circumstances, therefore, the kidney can excrete a hypotonic urine in the presence of pitressin while retaining its ability to respond normally to dehydration.

Acknowledgements

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DISCUSSION

Wallace: Hypernatraemia is seen very frequently in young infants with dehydration secondary to diarrhoea. I think that there are two points worth noting here. The first is that infants can lose large amounts of water in their stools without losing physiologically equivalent amounts of sodium. The sodium content of stool water can be very low. It is almost as though the gut contents had been passed over an exchange resin. The second item is that, in infants at least, the hypernatraemia is accompanied by an ever greater degree of hyperchloraemia. Since the flame photometer came into the laboratory chloride has been a neglected ion. We have wondered whether or not chloride might not be an ion with much more autonomy than it is generally given credit for. In the children we have studied, gain of water and loss of chloride have been the primary measurable events occurring during clinical recovery.

Davson: Does the gut remove the sodium from the normal faeces?

Wallace: In normal faeces there is very little sodium.

Davson: It may be that the active accumulation mechanism is set to take up any sodium that is in the gut.

Wallace: A few stools from infants with the salt-losing type of adreno-genital syndrome and with concurrent diarrhoea have been examined. The sodium in stool water from these infants has been found to be much

higher than we have found in the child with hypernatraemia. The urine of the infant with hypernatraemia is also low in sodium. One finds both the gut and kidney strongly retaining sodium beyond what might seem an optimal degree. I wonder what this means?

Young: This hanging on to sodium without any excess excretion in the urine is just what happens in experimental dehydration. If you are not putting sodium into the body either by mouth or intravenously, there is never a high output of sodium in the urine, even if the serum sodium is rising. There is nothing extraordinary about that in the baby. Why the kidneys function that way, I do not know, but they did so under conditions of experimental dehydration in the normal adults studied by Dr. Black, Prof. McCance, and myself (1944. *J. Physiol.*. **102**, 406).

Desaulles: Is there any possibility of making chromatograms of blood and urine steroids in the kind of case you have just described, Prof. Wallace? The aldosterone content was very high, wasn't it?

Wallace: We can obtain such chromatograms but I am always told that close to a litre of blood or urine is required, and these are tiny children.

Desaulles: For aldosterone determination 100 ml. is enough. The condition fits so well with the picture of a very high aldosterone output that I wonder if those cases cannot be explained by the very high aldosterone levels. In these all the sodium is retained without changes in the water content. In the recovery period you have water retention and a decrease in aldosterone. After that you reach a steady state, i.e. a new form of equilibrium, though it is perhaps not the true equilibrium. That is only a hypothesis for the moment, until we have more precise values.

Davson: Does aldosterone influence the absorption of water by the intestine?

Desaulles: I have no precise data.

Black: I want to express agreement with Dr. Fourman, because I think that none of the alleged clinical tests for water depletion, such as the 'fingerprint' test, are any good. There is also another possible cause of so-called cerebral salt-wasting. We had a patient in with hemiplegia and a period of hypotension. Ten days later he was mopping up about six litres of saline fluid a day and losing it through his urine. The only suggestion I can make is that during the period of hypotension he sustained tubular damage and that later he was in a renal salt-losing state, in which the cerebral part was just an accident. I have seen this before and I think it is particularly liable to happen in older people who have a smaller renal reserve.

Fourman: I think that is a very interesting comment. The very severe dehydrations probably do produce renal lesions and we have been wondering whether that accounts for the systemic acidosis, which is so often a prominent feature.

Wallace: Chloride acidosis always occurs.

Fourman: What is the plasma bicarbonate?

Wallace: In our experience it is always low. Chloride is making bicarbonate forfeit its place in serum.

Desaulles: Dr. Fourman, was it possible to make steroid determinations in your case?

We have made an observation on animals that is not identical but may point in the same direction as the observation you have made. If adrenalectomized rats are given a very high salt load, hypernatraemia is produced in a relatively short time. Firstly, then, the sensitivity to ADH and pitressin decreases considerably. We did not get any serum values but in the urine there is a strong dilution due to the greater urinary output. Secondly, treatment with aldosterone in relatively high doses for four or five days causes sensitivity to pitressin to disappear completely.

Fourman: With high aldosterone dosage there is certainly an expansion of the extracellular fluid, and it may be that this expansion diminishes the sensitivity to pitressin.

As regards the steroid assays, I do know that Schwartz and Bartter's cases, which were analogous in many ways, were not salt-deficient; they had an expanded extracellular volume and the aldosterone output in the urine was normal. A. Gowenlock in Manchester measured the aldosterone output in one of our patients and it was normal. We also did 17-ketosteroid assays as a crude measure of their corticoid output, and the results were normal. It is obvious that the hyponatraemia does not lead to a stimulation of the aldosterone output of the adrenal.

Desaulles: Could this be given the same interpretation as the findings of Prader, Spahr and Neher (1955. *Schweiz. med. Wschr.*, **85**, 1085)? There may be some form of sodium-losing syndrome.

Adolph: It seems to me, Dr. Fourman, that in order to show that there is something more to one of these syndromes than a lack of drinking behaviour or drinking response on the part of the individual, you have to perform your tests in a certain order; you have to be sure that the patient has plenty of water when you do the salt test and plenty of salt when you do the water test. Could you have switched the tests around and still obtained the same results?

Fourman: The saline load was done three weeks before the dehydration. The dehydration preceded the pitressin by one day in one of the patients, by a week in the other patient. The pitressin test was accompanied by a load of water at the time. I do agree that one test can influence another but I do not think that they did in this instance.

Borst: When a high or a low sodium concentration in the blood plasma is maintained we believe that this is almost always due to an insufficient circulation. This insufficiency often results from dehydration, but it may have other causes such as cardiac failure or hypoproteinaemia. We found a high blood sodium in anaemic patients who had had recurrent haemorrhages from peptic ulcer. They had no free access to water and had been treated with abundant saline infusions; they had substantial oedema. During several days the urine contained less sodium than tap water. After a large transfusion of blood the sodium excretion started and the blood sodium fell to a normal level. Simultaneously, the output of water increased and the urea concentration of the urine, which had been very high, decreased. The counterpart was observed in cachectic patients with anaemia and hypalbuminaemia who adhered to a salt-free diet and who had a liberal intake of water. They maintained a low blood sodium concentration in the presence of oedema.

A large blood transfusion elicited a considerable water diuresis and the blood sodium rose to normal, while the oedema fluid was excreted.

With both the high and the low sodium concentrations the circulation was inadequate. In the first instance the excess of sodium and a less considerable excess of water was excreted as soon as the normal blood volume was restored. In the second the rise in blood volume led to the elimination of the excess of water and of a less considerable excess of salt.

The interesting observations of Dr. Schwartz and Dr. Fourman show that variations in circulation may not always be the primary factor in the excretion of sodium and water. It is, however, difficult to distinguish renal responses to variations in the circulation from other reactions on the part of the kidneys. Moreover any considerable loss or retention of salt and water has an effect on the circulation. The problem is that an excess or an inadequacy of the circulation in patients cannot be measured in a satisfactory way. Since this factor cannot be disregarded we have to estimate it on the basis of indirect evidence.

GLANDULAR SECRETION OF ELECTROLYTES

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The ducts or tubules of glands with external secretion are usually quite complex in structure and morphologically they differ to a considerable extent from gland to gland. It is, therefore, reasonable to assume that the ducts do not merely serve as pathways for the secretion formed in the acini, but that they contribute somehow to the elaboration of the final secretory product. This possibility has already been considered in the past century by Merkel (1883), mainly on morphological grounds, and by Werther (1886), who made a comparative investigation of the concentration of salt in various types of saliva. The results of these experiments were, however, inconclusive, and in 1950 Babkin restated the need for a study of the physiology of the glandular ducts. Since then, certain advances have been made through comparative work, by the application of concepts from modern renal physiology and with the use of electrophysiological methods, relating changes in membrane potentials to ionic transport. It is the purpose of the present paper to review this work and to present a theory of the mechanism of glandular electrolyte secretion based on the available data.

Fig. 1 shows a comparison between the concentrations of the main electrolytes in sweat, parotid saliva, tears and pancreatic juice in relation to secretory rate, calculated in milligrams per gram gland per minute. The following similarities and differences between the four secretory products are apparent from Fig. 1:

The Excretion of Sodium:

In sweat and in parotid saliva the concentration of sodium is smaller than the concentration of sodium in plasma and

varies with the rate of secretion. With increasing secretory rate the concentration of sodium rises to about 60 m-equiv./l. in the sweat and to about 90 m-equiv./l. in the parotid saliva, but no definite maximum is reached in either secretion. This finding conforms with the old work of Heidenhain (1868), Langley and Fletcher (1889), Kittsteiner (1911, 1913), and Hancock, Whitehouse and Haldane (1929).
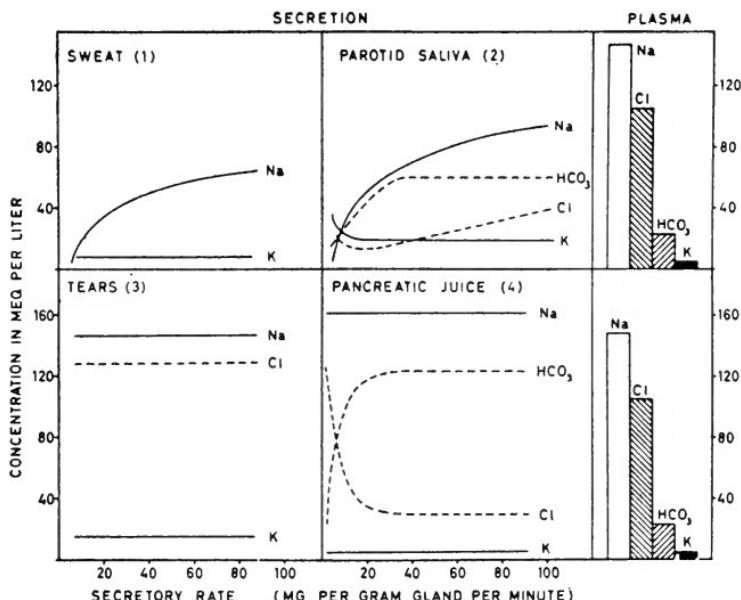

Fig. 1. The concentration of the main electrolytes in sweat, parotid saliva, tears and pancreatic juice in relation to secretory rate (in milligrams per gram gland per minute). From the data of 1: Schwartz and Thaysen (1956); 2: Thaysen, Thorn and Schwartz (1954); 3: Thaysen and Thorn (1954); and 4: Bro-Rasmussen, Killmann and Thaysen (1956).

In tears and in pancreatic juice the concentration of sodium in secretion water is about equal to the concentration of sodium in plasma water and is independent of the rate of secretion.

The Excretion of Potassium:

The concentration of potassium in all four secretions is independent of wide ranges of variation in secretory rate.

In parotid saliva, however, a definite rise in potassium concentration is noted at rates smaller than 15 mg. per gram gland per minute. This finding is in agreement with the results of Langstroth, McRae and Stavraky (1938) and Burgen (1956). A similar rise in potassium concentration possibly occurs at low rates of sweat secretion (Kuno, 1956), but could not be demonstrated with the experimental technique employed by Schwartz and Thaysen (1956). In the two other secretions a rise in potassium concentration at low secretory rates has never been observed.

The Excretion of Anions:

The main anion of sweat and tears is chloride. This anion accounts for about 80 per cent of the sum of the concentrations of sodium and potassium in the tear fluid. Chloride concentration of sweat is not depicted in Fig. 1, but Locke and his co-workers (1951) found the following relation: sodium $= 1 \cdot 12$ chloride $+3$ m-equiv./l.

The chief anion of parotid saliva and pancreatic juice is bicarbonate. With increasing secretory rate the concentration of bicarbonate rises in both secretions and reaches a maximum of about 60 m-equiv./l. in parotid saliva and about 90–130 m-equiv./l. in pancreatic juice. When this maximum concentration (which is subject to individual variation) has been arrived at, the concentration of bicarbonate remains independent of further increases in the rate of secretion. The concentration of chloride varies inversely with that of bicarbonate. In both secretions and at all rates the sums of the concentrations of the two anions equal about 80–90 per cent of the sums of the concentrations of sodium and potassium.

The following hypothesis has been put forward to explain the demonstrated differences in the excretion of the main cations. In all four glands a precursor solution is formed in which the concentration of sodium is independent of the rate of precursor formation. In the sweat and parotid glands, but not in the other two glands, sodium is consequently reabsorbed by a process of a limited maximal capacity (Thaysen,

Thorn and Schwartz, 1954; Thaysen, 1955; Schwartz and Thaysen, 1956; Bulmer and Forwell, 1956; Bro-Rasmussen, Killmann and Thaysen, 1956). Like sodium, potassium is transferred into the precursor at a constant concentration, but it is not reabsorbed in any of the glands. The rise in potassium concentration at the low secretory rates in parotid saliva (and in sweat?) may be secondary to reabsorption of water from the precursor as indicated by Langstroth, McRae and Stavraky (1938) and by Thaysen, Thorn and Schwartz (1954), and/or to an exchange between sodium and potassium ions during the process of sodium reabsorption.

Table I

COMPARISON BETWEEN THE CALCULATED CONCENTRATIONS OF SODIUM AND POTASSIUM IN THE PRECURSOR SECRETIONS OF FOUR SECRETORY PRODUCTS AND THE CONCENTRATIONS OF THE SAME IONS IN PLASMA WATER

	SWEAT	PAROTID	LACRYMAL	PANCREATIC	PLASMA WATER
Na	79	112	146	161	160
K	9	19	15	5	5
SUM	88	131	161	166	165

Fig. 2 shows a linear regression of the rate of sodium excretion in parotid saliva on the rate of secretion. According to the above hypothesis the values for slope and intercept in Fig. 2 can be interpreted to mean that sodium is transferred into the precursor solution at the rate of $0 \cdot 112$ microequivalents per mg. of saliva discharged and that $2 \cdot 4$ microequivalents are subsequently reabsorbed per gram gland per minute. The sodium concentration of the sweat precursor has been calculated in a similar manner from the data of Schwartz and Thaysen (1956) and the values are compared to those of the other secretions and to plasma water in Table I. According to Table I the sums of the concentrations of sodium and potassium in the presecretions of saliva and sweat are lower than the sums of the concentrations of the same ions in the two other secretions and in plasma water. No other cations

are present in parotid saliva and in sweat in sufficiently large concentration to make up for this difference. Judging from the results of Table I, the production of sweat and parotid saliva should therefore involve secretion of hypotonic precursor solutions, a process which *a priori* does not appear very likely.
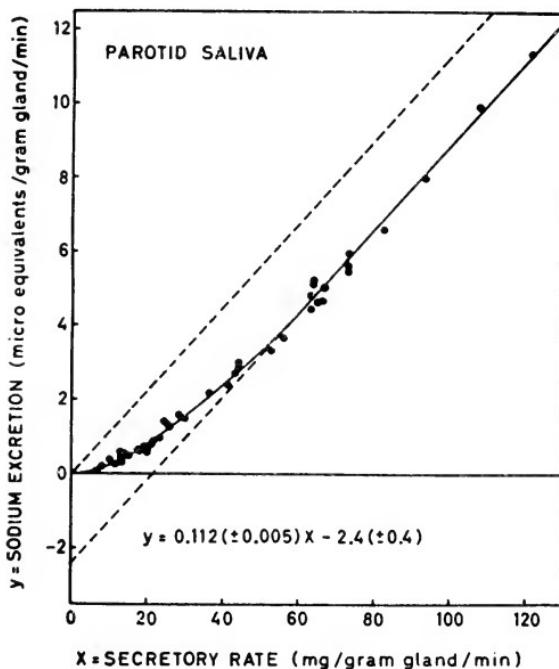

FIG. 2. The relation between the rate of sodium excretion in parotid saliva (in microequivalents per gram gland per minute) and secretory rate (in milligrams per gram gland per minute). The linear regression has been calculated for all data at or above a secretory rate of 60 milligrams per gram gland per minute.

It must be emphasized, however, that the calculated figures for precursor sodium concentration (and sodium reabsorption) in the sweat and parotid glands underestimate actual values, since the regressions for sodium excretion on secretory rate have been fitted to points which approach, but do not reach, a rectilinear relationship within the observed range (cf. Fig. 2). One explanation for this considerable splay in

the observed values from the asymptote could be that there is a certain back-diffusion of water in the sequence of active sodium reabsorption. As demonstrated below there is reasonable qualitative evidence to suggest that water is, in fact, reabsorbed from the precursors of sweat and parotid saliva.

Fig. 3 illustrates that the concentration of urea in sweat, tears, and parotid saliva remains proportional to the con-
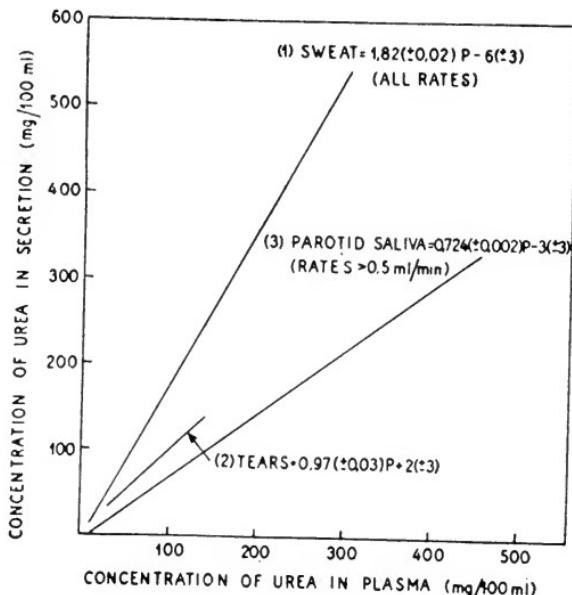

FIG. 3. The relation between the concentration of urea in the plasma (P) and the concentration of urea in sweat, parotid saliva and tears. From the data of 1: Schwartz, Thaysen and Dole (1953); 2: Albrectsen and Thaysen (1955); and 3: Thaysen and Thorn (1954).

centration of urea in the plasma within a wide range of variation in the latter. This finding indicates that urea is excreted in these secretions by a process of simple diffusion and not via a specific secretory mechanism which might become saturated by increasing load. Potentially urea may, therefore, be used as a tracer for the movement of water within the secreting glands in a similar manner as in the glomerular nephron.

Fig. 4 shows the relationship between the S/P (secretion/plasma) concentration ratio for urea and the rate of secretion of sweat, parotid saliva, tears and pancreatic juice.

In tears and in pancreatic juice there is apparently diffusion equilibrium between the secretion and the plasma at all
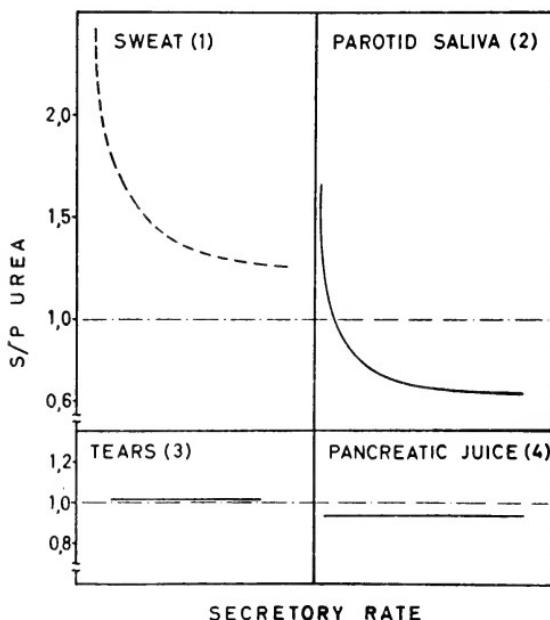

FIG. 4. The relation between the S/P ratio for urea and secretory rate in sweat, parotid saliva, tears and pancreatic juice. From the data of 1: Araki and Ando (1953) (the curve is shown as a broken line because it represents the approximate mean of two determinations and because secretory rate cannot be directly compared to that of the other glands); 2: Albrectsen and Thaysen (1955); 3: Thaysen and Thorn (1954); 4: Bro-Rasmussen, Killmann and Thaysen (1956).

rates of glandular activity. On the basis of these findings no statement can be made about the existence or non-existence of an internal circulation of water in these glands.

In sweat and in parotid saliva S/P urea varies with the rate of secretion. In the sweat S/P urea decreases from 2 or 3 at the low secretory rates to about 1 when sweating

becomes profuse (Araki and Ando, 1953; Bulmer, 1957). In parotid saliva the ratio decreases from about $1 \cdot 6$ at low rates of secretion to about $0 \cdot 6$ when the flow of saliva is brisk (Albrectsen and Thaysen, 1955). Since no specific secretory mechanism for urea exists in either gland, it is reasonable to conclude that urea, which is diffusing into the gland with some precursor solution, is raised to a concentration greater than that of the plasma by reabsorption of water from the precursor in a region of the gland which is less permeable to urea than the site of precursor formation. The rate of change in S/P urea with secretory rate suggests that water reabsorption represents a relatively constant quantity at all rates of precursor formation, and it is not unreasonable to assume that the reabsorption of water occurs as a mere passive sequence of active sodium reabsorption.

Quantitative information about precursor formation and water reabsorption can, however, hardly be gained from these results or from similar "clearance" studies with other solutes. Morphological and physiological evidence strongly argues against the possibility that the secretion precursor represents an ultrafiltrate of the plasma like the urine precursor of the glomerular nephron. A "glandular inulin" probably does not exist, and it is quite possible that exact knowledge about the composition of the precursor secretions and about the manner in which they are modified as they flow down the glandular ducts can only be obtained by micropuncture techniques.

However, Lundberg (1955, 1957a,b,c), working on the electrophysiology of the submaxillary and sublingual glands of the cat, has obtained results which provide indirect support in favour of the hypothesis that sodium is reabsorbed from a precursor secretion in some of the duct-possessing glands.

In the submaxillary gland, which produces a secretion in which sodium concentration varies with secretory rate in about the same manner as in parotid saliva and sweat, Lundberg (1955) demonstrated that the lumen of the (striated?) ducts becomes negative as compared to the hilus, when the

gland is activated by stimulation of the chorda. A similar internal duct negativity could not be demonstrated in the sublingual gland (Lundberg, 1957a), which (like the lachrymal and pancreatic glands) produces a secretion that is isotonic with the plasma and has a sodium concentration of about 150 m-equiv./l. Provided that the potential changes on stimulation can be regarded as the electrical signal of ionic transport, Lundberg (1957a) concludes that there is a net transport of cation from the lumen to the blood side in the ducts of the submaxillary gland, but not in the sublingual gland. Although the composition of the submaxillary secretion was not measured simultaneously with the duct potential, the latter appears large enough for it be to accepted that the reabsorption of anion is merely a passive sequence of active cation transport.

With one microelectrode inserted into acinous cells and the other electrode on the gland surface, Lundberg (1955, 1957a) detected a considerable increase in the negativity of the acinous cells on stimulation of the submaxillary as well as of the sublingual gland. The lumen of the acini, likewise, becomes negative as compared to the morphological interior, but this negativity decreases slightly with continued stimulation of the gland. These potential changes may be due to a net transport of anion from the blood side into the glandular lumen. In another paper Lundberg (1957c) directly demonstrated this anionic dependence of secretion and secretory potentials in the perfused sublingual gland. Substitution of sodium chloride with sodium nitrate or sodium thiocyanate caused the secretion to stop almost entirely and decreased the potential changes. The secretory response and the potentials reverted to normal when sodium chloride was again added to the perfusate.

On the basis of the experiments quoted in the present report, it appears reasonable to suggest the following mechanism for the secretion of electrolytes and water by the duct-possessing glands. Active outward transport of anions is a main factor in the formation of the secretory products of all

glands. In some glands the chief anion transported is chloride (sweat, tears, sublingual saliva); in others bicarbonate ions are added in varying proportion, possibly due to the presence of carbonic anhydrase in the cells (pancreatic juice, parotid saliva, submaxillary saliva). It is reasonable to assume that water moves in a merely passive sequence of ionic transport from the blood side into the glandular lumen, and that the presecretions of all glands are isotonic or nearly isotonic.

In certain glands (sweat, parotid and submaxillary) sodium is reabsorbed from the precursor secretion as it flows down the glandular duct system, and it is likely that anions move from duct lumen to the blood side in a passive sequence of the active sodium reabsorption. The chief anion reabsorbed in this manner appears to be chloride, independently of whether the primary secretion contains primarily chloride or primarily bicarbonate ions. It can be seen from a glance at Fig. 1 that the parotid and the pancreatic glands apparently form pre-secretions of qualitatively similar composition, and that the main difference in the anionic pattern of the final secretory products is that chloride ions have been removed from the saliva precursor. As a consequence of active sodium re-absorption a certain quantity of water is, moreover, diffusing back into the blood stream, although it is obvious that water reabsorption does not occur isotonically as in the proximal renal tubule.

It is only possible to speculate on the morphological sites of the different ionic transports in the duct-possessing glands. According to Fig. 5 it is, however, not unreasonable to suggest that sodium reabsorption is located in the striated intralobular ducts. Striated epithelium is present in the parotid and submaxillary glands, which apparently reabsorb sodium, but it is absent in the sublingual, pancreatic and lachrymal glands, which show no evidence of sodium re-absorption. The precursor secretions are probably formed by the acini as well as by the cuboidal epithelium of the intercalary ducts, the former producing a viscous secretion with a high concentration of organic material, the latter a

watery secretion with a low concentration of organic material (cf. Babkin, 1950). With respect to the sweat gland it is

| SWEAT | PAROTIS | SUBMAX. | SUBLING. | PANCREAS | LACRYMAL |

$$S_{Na} < E.C._{Na} \qquad\qquad S_{Na} = E.C._{Na}$$

S_{Na} varies with secretory rate S_{Na} independent of secretory rate
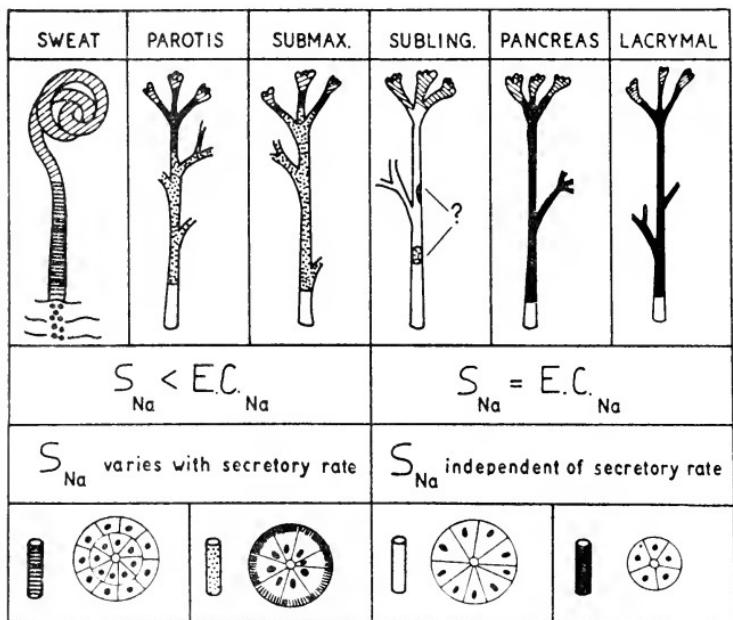
FIG. 5. Comparison between the histological structure of the six main duct-possessing glands and their secretion of sodium ions. S_{Na} = concentration of sodium in the secretion. $E.C._{Na}$ = concentration of sodium in the extracellular fluid. The coil of the sweat gland and the acini of the other glands are cross-hatched. The epithelia of the ducts are illustrated by different symbols, which refer to the schematic cross-sections at the bottom of the figure. The cross-sections are (from left to right): double-layered epithelium of sweat duct; striated epithelium of intralobular ducts; high cylindrical epithelium of excretory ducts; low cuboidal epithelium of intercalary ducts.

suggested that precursor formation is located in the coil, whereas reabsorption of sodium takes place in the duct.

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DISCUSSION

Davson: As far as I can make out, Dr. Thaysen, you postulate that there is a region through which the urea can pass quite easily, and later on in the ducts there is a relative impermeability to urea. This is rather in conflict with what people have thought in the past, because, on the assumption that it penetrates into all cells very rapidly, urea has been used to determine cell water. Your view certainly does fit in with what is found with the cerebrospinal fluid and the aqueous humour; urea does not penetrate those barriers easily. If one confined oneself to these instances, then, one would say that urea did not penetrate cells easily at all.

Thaysen: Yes, I believe that the cells in the region of water reabsorption are less permeable to urea than the cells at the site of precursor formation. In all probability the difference in permeability is, however, relative rather than absolute. In other words, I do not think that the cells at the site of precursor formation are so freely permeable to urea that the concentration of urea in the precursor is equal to that of the plasma at all

rates of secretion. Certainly this is not the case in the parotid gland (Fig. 4, p. 68). Conversely, I do not venture to claim that the cells in the duct are impermeable to an extent that would completely prevent urea from diffusing back into the blood stream along the concentration gradient created by water reabsorption. But the amount of urea diffusing back through the relatively impermeable duct epithelium is limited by the short span of time during which the secretion remains in the duct. Urea may equilibrate rapidly over some cellular membranes, more slowly over others. This difference is not important when one measures total body water as the volume of distribution of urea, because one waits until complete equilibrium has been established before the measurement is made. But the difference is important in the rate-dependent process of secretion, where the time available for diffusion becomes limiting.

Karvonen: In prolonged sweating the potassium concentration is higher to start with and then gradually decreases. There is no similar change in sodium or chloride and that would agree quite well with the reabsorption and consequent storing of potassium in the tubule, whereas sodium and chloride are not stored (Ahlman *et al.* (1953) *Acta endocr., Copenhagen,* **12,** 140).

Thaysen: Yes, the first sample of sweat obtained after stimulation may have a higher potassium concentration than the following ones. One reason for this may be that the first sample is contaminated with cellular debris, sebum and sweat residues on the skin surface.

Karvonen: It is not just the rinsing factor, because we paid quite a lot of attention to rinsing the skin and we still get this difference; the potassium is probably stored in the gland or at least in the tubule.

Thaysen: In that case it cannot be contamination. Your finding is very interesting to me, because we found exactly the same thing with the parotid secretion. The first sample of saliva obtained after stimulation invariably had a higher potassium concentration (and a higher urea concentration) than the following ones. This phenomenon occurred independently of the rate at which the first sample was produced. We speculated that the vigorous flow of saliva, caused by stimulation, "pushed out" first a small amount of secretion, which had been produced at the low secretory rates prior to stimulation, and which consequently had a high concentration of potassium and urea and a relatively low sodium concentration (1954. *Amer. J. Physiol.,* **178,** 155; 1955, *Scand. J. clin. Lab. Invest.,* **7,** 231). Burgen (1956) also observed a high potassium concentration in the first samples of saliva obtained after stimulation.

Wallace: I have kept quiet here because a baby usually does not sweat until the age of 3–4 months, nor does he shed tears—he only learns to do that later.

Karvonen: In Finland babies have hot sauna baths quite young and Dr. Eila Kassila of the Children's Clinic, Helsinki, has made an investigation on the composition of the sweat they produce during the saunas. I do not think that much difference was found between baby and adult.

Wallace: It is of interest that there is a very specific disease in children, cystic fibrosis of the pancreas, in which the ability of the sweat glands to reduce the sodium concentration in sweat seems to be lost. It is of

theoretical interest that this is a disease which manifests itself primarily in the lungs and pancreas with gross pathology, and yet has this very subtle physiological pathology in the sweat glands. Have you done anything with that type of patient?

Thaysen: Yes, but I never did much with them. We did find a very high sodium concentration in their sweat.

Wallace: A high sodium concentration in sweat is found in nephrosis, and Dr. Warming-Larsen of Copenhagen has studied this problem. The nephrotic child gaining oedema has a high sodium concentration in the sweat yet a very small sweat volume; but overnight, as he diureses, he puts out an increased volume of sweat yet at the same time the sweat sodium concentration falls. The net amount of sodium lost from the sweating skin is the same whether he is oedematous or not. I would like to know about the relation of ADH to sweat volume; does ADH control the sweat glands as well as the kidney?

Thaysen: That is interesting. Off-hand one would have guessed that the sodium concentration of the sweat would have been low during the phase of oedema formation and high when the patient started to diurese. That would agree with what we know about the action of aldosterone on the glands and with the results of sweat and saliva analyses in other oedamatous states. Since the quantity of sodium excreted per unit area of the skin per unit time remained constant, whereas the volume of sweat increased when the child diuresed, an ADH effect might be a possibility worth considering. However, as far as I am aware, it has been shown that ADH has no effect on the volume of sweat produced (Amatruda, T. T., Jr., and Welt, L. G. (1953). *J. appl. Physiol.*, 5, 759; Pearcy *et al.* (1956). *J. appl. Physiol.*, 8, 621).

Adolph: Can somebody clarify the reports that tears are very hypertonic when they are formed?

Davson: I did some analyses a long time ago, and we discovered that the chloride concentration was equal to that of the blood. It is a very difficult problem obtaining tears, because you have got to make the person cry very hard to get enough to do an analysis.

Thaysen: In 1889 Massart (*Arch. Biol., Paris*, 9, 537) applied sodium chloride solutions of varying concentration to the conjunctival sac of a few test subjects. He never analysed the tear fluid, but from the reactions of the test subjects to the different solutions he concluded that a 1·3 per cent solution of sodium chloride was isotonic with the tears. According to Krogh and co-workers (1945. *Acta physiol. scand.*, 10, 88) this experiment forms the only basis for the rather widespread statement in physiological and pharmacological textbooks that tears are hypertonic as compared to the plasma. In 1945 Krogh measured the osmotic pressure of tears and found them to be isotonic. The finding was confirmed by Giardini and Roberts in 1950 (*Brit. J. Ophthal.*, 34, 737).

Black: If you inject ^{42}K intravenously and then collect serial samples of saliva you find that the specific activity of potassium in the saliva is several times that of the specific activity of the potassium in plasma at the same time. This behaviour is analogous to that in urine and suggests to us that the potassium in saliva is, like that in urine, secreted by cells,

and not just filtered from the plasma. One then wonders whether epithelium does not similarly push out potassium in exchange for the sodium which is being reabsorbed—the sort of mechanism that is possibly under aldosterone control.

I believe that although Conn has concentrated mainly on sweat in his tests for aldosterone activity, he has also used saliva in a similar way. With a rice diet we did not get any falling off in the sodium concentration in the saliva, as far as we could determine.

Thaysen: An exchange mechanism between sodium and potassium ions at the site of sodium reabsorption is certainly a very likely possibility. This may be one factor causing the potassium concentration of the final secretory product to exceed that of the plasma. However, glands which apparently possess no sodium-reabsorbing mechanism may also have a potassium concentration in their secretions, exceeding the plasma potassium concentration. This applies, for example, to the lachrymal (Fig.1, p. 63) and the sublingual glands (Lundberg, 1957b). Therefore I believe that two factors may be at stake. First, the presecretion is frequently formed with a potassium concentration which exceeds that of the plasma (and a correspondingly lower sodium concentration). Second, in some glands additional potassium ions are added to the presecretion in exchange for reabsorbed sodium ions. Similarly, the adrenal steroids may have a dual site of action in the glands. In contradistinction to the situation in the glomerular nephron, adrenal steroids may act on the gland cells forming the presecretion and thus alter the Na/K ratio of the precursor, and they may act on the cells in the ducts which reabsorb sodium ions from the presecretion in exchange for potassium ions. There is some evidence indicating such a dual site of action of aldosterone on the glands (Thorn *et al.* (1954). *Fed. Proc.*, **13**, 310), but I do not know of any conclusive experiments. One way of approaching the problem may be to compare the effect of aldosterone on glands with and without a sodium-reabsorbing mechanism, e.g. on the sweat or parotid gland as contrasted with the lachrymal or pancreatic.

As regards your comment about the rice diet, sodium depletion, induced by a low sodium diet, causes the concentration of sodium to decrease and the concentration of potassium to increase in sweat as well as in saliva (McCance, R. A. (1938). *J. Physiol.*, **92**, 208). However, the response of the glandular epithelium to sodium depletion is both delayed and incomplete as compared to that of the kidney tubule (Robinson *et al.* (1955). *J. appl. Physiol.*, **8**, 159; Thorn *et al.* (1956). *J. appl. Physiol.*, **9**, 477).

Karvonen: Can anyone comment on the statistical finding that men have lower sodium and potassium than women in their sweat, and that the Na/K ratio in women is significantly lower than in men (Ahlman *et al.* (1953). *J. clin. Endocrin. Metab.*, **13**, 773)?

Desaulles: That is a very interesting challenge. We have similar findings in animals, not in sweat but in urine, but I have absolutely no explanation for it. It is just an observed fact.

Talbot: I wonder if those who are commenting on the sodium, chloride and potassium concentrations in sweat all have in mind the relationship

between rate of sweating and the concentration, because it varies enormously.

Thaysen: Yes, that is very important. Secretory rate must be controlled in all work on electrolyte composition of secretions, and comparative studies can only be made on the basis of standard rates. This is of course equally important whether one states the result in absolute concentrations of sodium and potassium or as the Na/K ratio. When the developing organism is under study, secretory rate must be expressed per unit weight of gland or some other parameter allowing for the influence of growth. I believe that negligence of these important factors is the main reason why the literature on variations with sex and age in the secretion of electrolytes is, largely speaking, inconclusive and frequently mutually conflicting.

With respect to Dr. Karvonen's remark I should like to add this comment. I take it that the sweat tests have been done in the usual way, i.e. with collection of sweat from a smaller or larger area of the skin, not from individual glands, and that the difference between the men and women is stated on the basis of comparable sweating rates per unit area of the skin. It does tell us, then, that there is a difference between sweating of men and women, but it does not tell us anything about the reason for the difference. As is well known, the number of functioning sweat glands per unit area of the skin varies between individuals, between the sexes and with age, as well as between different skin regions in the same person. Comparable rates per unit area of the skin are therefore not necessarily the same as comparable rates per gland. Physiologically it is of course the rate per gland that matters and not the rate per unit skin area. Let us take it that women have half the number of glands per unit skin area that men have. Since the rate per unit skin area was comparable, the mean flow per gland in the women would then be twice that in the men. A higher sodium concentration in the sweat of the women might therefore merely be due to the fact that secretory rate per gland was larger. Let us take it that the men and women had an equal number of glands per unit skin area. In that case the difference between the sexes could not be due to a difference in the rate per gland, but might well be due to hormonal or other factors. What I mean is that in comparative work it is a prerequisite to determine not only secretory rate, but also the number of functioning glands, if you want to make deductions from your findings. A method for determination of the number of functioning glands within the area of sweat collection has been published by Dole and Thaysen (1953. *J. exp. Med.*, **98**, 129).

HORMONAL ASPECTS OF WATER AND ELECTROLYTE METABOLISM IN RELATION TO AGE AND SEX

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NEARLY all the hormones may have some influence on water and electrolyte metabolism. However, for most of them, this effect is indirect and occurs only under highly abnormal circumstances. Thus, the dehydration which exists in uncontrolled diabetes mellitus or in hyperparathyroidism is the result, respectively, of gross deficiency of insulin or excess of parathormone, and certainly does not point to any physiological rôle of these hormones in water metabolism. The same is essentially true of thyroid hormone and, though perhaps with reservations, of the sex hormones and gonadotrophins. Only posterior pituitary antidiuretic hormone (ADH) and certain of the adrenocortical steroids are directly concerned with the day-to-day and minute-to-minute adjustments needed to maintain fluid and electrolyte homeostasis in mammals. The major details of this hormonal control are well known and it is not necessary to relate them here. It is proposed, on the other hand, to examine how the influence of hormones on fluid and electrolyte balance differs at various ages and in the two sexes. In general, it is fair to say that little attention has been paid to considerations such as these, and for the most part knowledge is meagre.

In Infancy

Fluid and electrolyte control is notoriously inefficient at birth and during the first few weeks or so of life. The late development of the loop of Henle is generally considered to be responsible for this (Hubble, 1957), the infant kidney being unable, in consequence, to vary tubular reabsorption of water

and salt. There does not appear to be any inability to secrete ADH or adrenocortical hormones, though it is possible that the infant does lack the power to adjust the amounts secreted with any precision. The endocrinological situation, therefore, is essentially one of target-organ insensitivity due to immaturity.

An interesting hypothesis relating to neonatal weight loss has been put forward by Gans and Thompson (1957). These workers measured the urine output and its content of oestrogens and 17-hydroxycorticosteroids in six normal male neonates during the first few days of life. The findings were similar in all the infants. Large amounts of oestriol (up to a milligram or more) were excreted on the first post-partum day, the quantity falling rapidly during the next two or three days to the order of 1 or 2 μg. by the sixth day. Oestrone and oestradiol were found to the extent of 1–2 μg. during the first and second days and then disappeared. There was a decreasing excretion of urine during the first three to five days, and by the end of this time, postnatal weight loss had ceased. The excretion of 17-hydroxycorticosteroids showed only minor fluctuations throughout. The specific gravity of the urine was low at first but became more concentrated as the excess of water was excreted, in spite of the fact that fluid intake was increasing during this time.

Gans and Thompson suggest that part at least of the hydraemia of the newborn infant is due to water retention caused by the high circulating oestrogen level—the oestrogens being, of course, of maternal origin. As the oestrogens are excreted, the fluid excess is eliminated.

Adrenal hyperplasia

Adrenal hyperplasia is a disorder with a definite predilection for the female sex. In Wilkins' series (Wilkins, 1957) the ratio was 62 females to 19 males. The clinical manifestations of this disorder and its pathogenesis need not concern us here. It is, however, relevant to observe that about one-fourth of these patients have a tendency to loss of sodium and

to elevation of the plasma potassium, as a result of which early death may occur from dehydration and circulatory collapse, or from cardiac arrest due to hyperkalaemia. Once again, the number of females affected is some three times that of males.

The mechanism for this sodium loss is not understood. Very likely there is a defect in aldosterone synthesis, but it is also possible that some of the abnormal steroids produced by the hyperplastic adrenals may actually cause sodium loss. It is well known that surprisingly large amounts of sodium chloride and cortexone acetate (DOCA) may be needed to remedy the electrolyte defects in these infants, suggesting that more than mere replacement of deficient hormone is necessary. However, the response to 9α-fluorohydrocortisone, together with cortisone, may be far more satisfactory. In a $1\frac{1}{2}$-year-old patient of the writer's, a female pseudohermaphrodite with the salt-losing disorder, 10 mg. daily of DOCA intramuscularly, together with large sodium supplements, was necessary to maintain electrolyte balance. With only 0·25 mg. of 9α-fluorohydrocortisone daily by mouth, it was possible to maintain balance with no sodium supplement at all.

A small proportion of patients with adrenal hyperplasia (about 6 per cent) may show hypertension. It is possible that in these there is actually sodium retention. Bongiovanni and Eberlein (1955) have demonstrated in such a patient a defect in the synthesis of cortisol different from that usually found in adrenal hyperplasia. This patient was producing increased amounts of cortexone and 17-hydroxycortexone; it is thought probable that these steroids were responsible for the hypertension.

Changes in Relation to Adolescence

Knowledge of endocrine changes in relation to adolescence is rather sketchy. It is ably summarized by Tanner (1955). The impact of these changes on fluid and electrolyte metabolism is somewhat obscure. Certain morphological changes of

possible significance occur. Thus, a considerable growth spurt in the weight of the adrenal gland, more in boys than in girls, has been observed. It is almost entirely due to growth of the cortex. The weight of the thyroid also shows an adolescent spurt, but without any sex difference. Scanty data on hormone excretion indicate a slow increase in the excretion of oestrogen in both boys and girls during childhood, with a marked increase at puberty in the case of girls, while in boys the rate of increase hitherto manifested is merely maintained. Androgen excretion is similar in the two sexes before puberty; after puberty there is a marked rise in the case of boys, but a not unimportant rise also occurs in girls, no doubt as a result of increased adrenocortical activity. There is a gradual rise in the rate of secretion of adrenal corticoids, without sex difference, from birth to maturity. The increase appears to be proportionate to body size, without any adolescent spurt. The blood level of 17-hydroxycorticosteroids is much the same at all ages, and the responsiveness of the adrenals to stimulation by adrenocorticotrophic hormone is also unaffected by age, except, of course, in so far as the adrenal glands are smaller in children than in adults. A steady fall in the serum protein-bound iodine over the years six to 15 parallels the fall in basal metabolic rate, and the precise significance of this is obscure.

The sum total of these changes does not seem to have any striking impact on fluid and electrolyte metabolism.

Effects of the Menstrual Cycle

An important sex difference is introduced by the cyclic variations in hypothalamic-pituitary-ovarian (and perhaps adrenocortical) function which determine the menstrual cycle in females. It might well be expected that these would lead to important fluctuations in fluid and electrolyte balance.

Variations in body fluid during the menstrual cycle have been recognized for a long time, but the first full description of "premenstrual oedema" was given by Thomas (1933) who

reported weight gains of up to 14 lbs. at or during menstrua-
tion in two women. Several other writers (see Chesley and
Hellman, 1957) have concluded that approximately 30 per
cent of women have weight gains associated with menstrua-
tion. The suggestion that premenstrual weight gain is due to
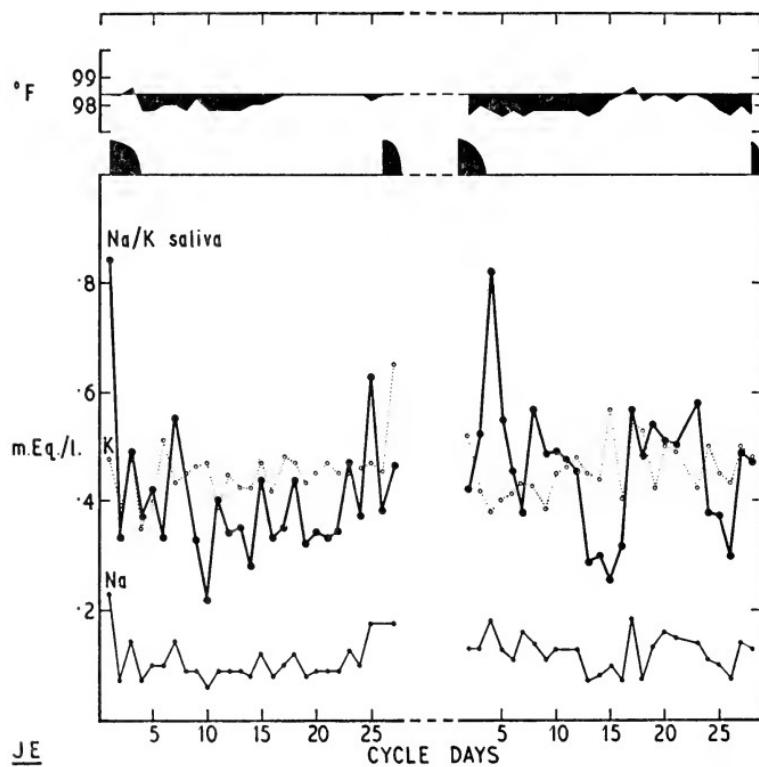
Fig. 1. Salivary sodium and potassium concentrations and Na/K
ratios in two cycles from a normal woman. In this and other figures
the upper curve is of the basal body temperature in °F. The black
shapes represent menstrual periods.

water and salt retention, mediated by oestrogens, is due to
Thorn, Nelson and Thorn (1938). Long and Zuckerman
(1937) postulated a rôle of adrenal salt-retaining hormones
in the electrolyte imbalance causing premenstrual fluid
retention.

In a recent investigation, Chesley and Hellman (1957)

studied 23 normal young women and found that in one-third of them the weight was maximal during the premenstrual eight days—in accordance with earlier writers. Closer analysis, however, failed to substantiate the physiological basis of such weight gains, since, when they did occur, they
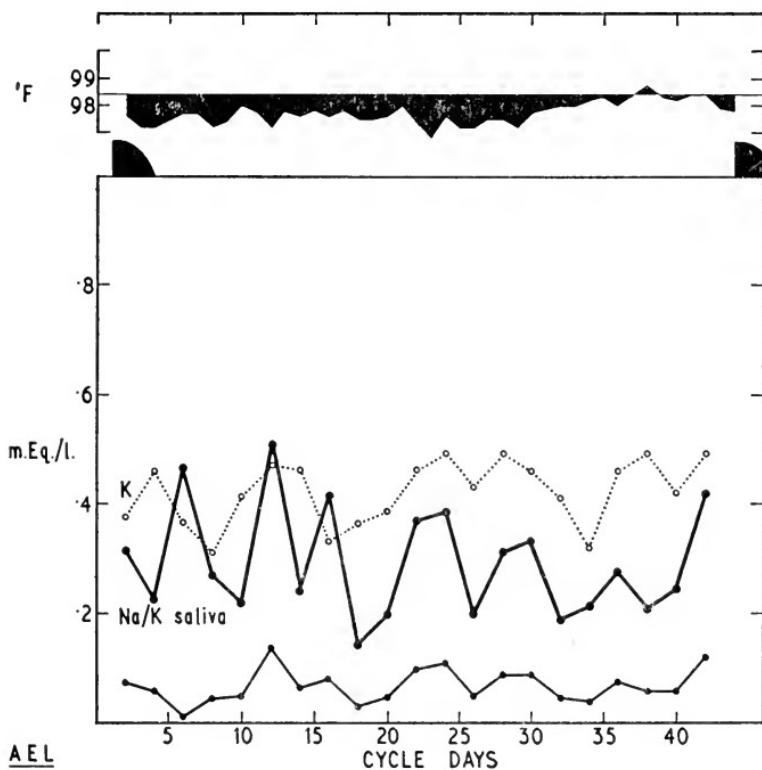
FIG. 2. A long, but ovular, cycle in a normal woman.

were slight and were not repeated from one cycle to the next. It was further shown that the incidence of premenstrual weight gain was the same as would be expected on a purely random distribution of weight gains throughout the menstrual cycle. These workers also studied the salivary sodium and Na/K ratios throughout the cycle; they were unable to find any consistent pattern of variations such as would have been

compatible with increased adrenal salt-retaining hormone
secretion during the premenstrual phase.

The present author's own limited studies on salivary and
urinary Na/K ratios in the menstrual cycle have been directed
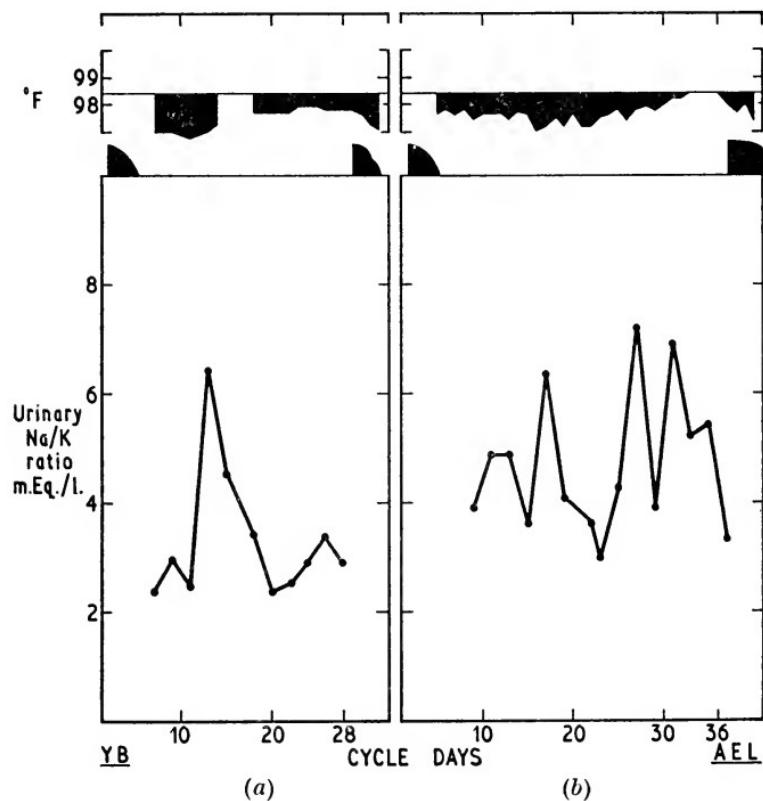
Fig. 3. Urinary Na/K ratios in two normal women. In (a) there
appears to be a peak at about the time of ovulation. In (b) the
ratio appears to be higher during the second half of the cycle.

mainly towards an attempt at elucidating the basis for so-
called premenstrual tension which is widely supposed to
depend upon premenstrual salt and fluid retention (see, for
example, Greene and Dalton, 1953, who consider an increased
oestradiol/progesterone ratio to be largely responsible).
The findings are in agreement with those of Chesley and

Hellman (1957) in that no precise pattern of variation in salivary or urinary sodium and potassium concentrations or Na/K ratios, either in normal women or in those complaining of premenstrual tension, has been discovered.

Fig. 1 shows two cycles from a normal woman: the Na/K
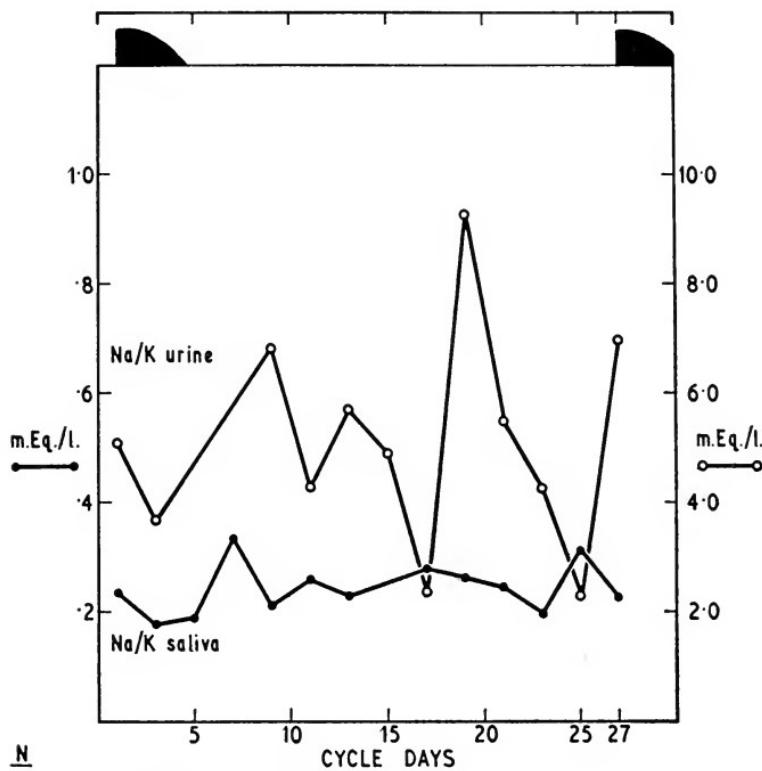
FIG. 4. Urinary and salivary Na/K ratios compared in a woman who experienced premenstrual tension.

ratio appears to be high at the start of both cycles and there is a distinct fall (mainly due to increased potassium secretion) at what may be judged from the basal temperature record to be the time of ovulation in the second cycle.

FIG. 2 shows a long but ovular cycle in another normal patient (A.E.L.) No convincing pattern is discernible. Fig. 3b shows the urinary Na/K ratios in another cycle from

patient A.E.L. If anything, the ratio is higher in the second
half of the cycle—i.e. sodium retention is less premenstrually.
In Fig. 3a, the urinary Na/K ratio appears to rise sharply just
at the time of ovulation—i.e. at the time of an oestrogen
peak, when, according to the usual view, the tendency should
be towards sodium retention.
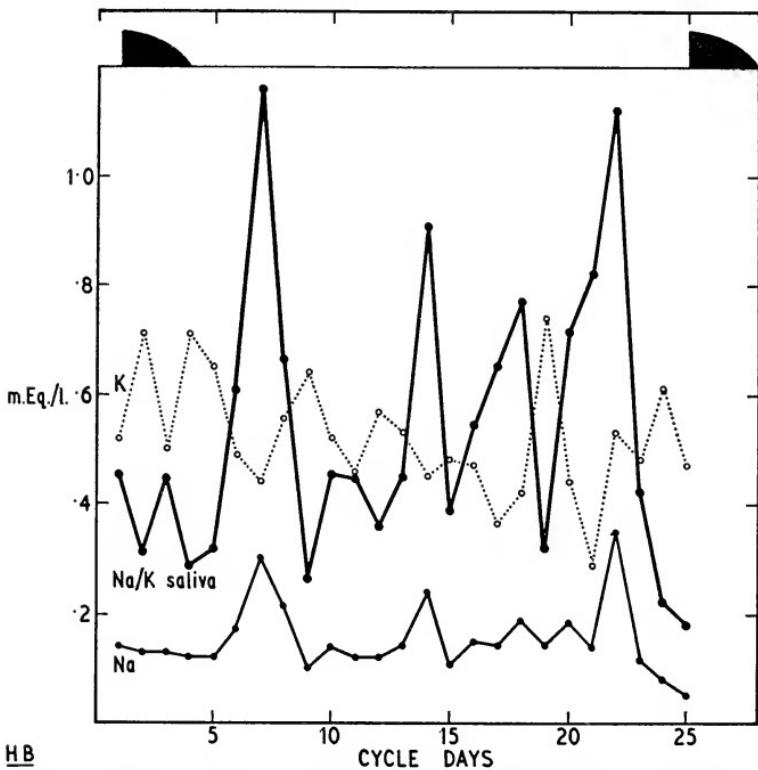
FIG. 5. Salivary sodium and potassium concentrations and ratios
in a woman who experienced premenstrual tension.

Figs. 4–6 relate to women who experienced definite pre-
menstrual tension. In Fig. 4 the salivary and urinary Na/K
ratios are compared. The latter (note that its scale is ten
times that of the salivary Na/K ratio) is much more variable
than the former, and neither shows any definite pattern.
Certainly there is no evidence of sodium retention premen-
strually. Fig. 5 shows the salivary Na/K ratios in another

patient; they fluctuate violently but show no evidence of premenstrual sodium retention.

Fig. 6 shows three consecutive cycles in a patient who experienced quite severe premenstrual tension. In the first
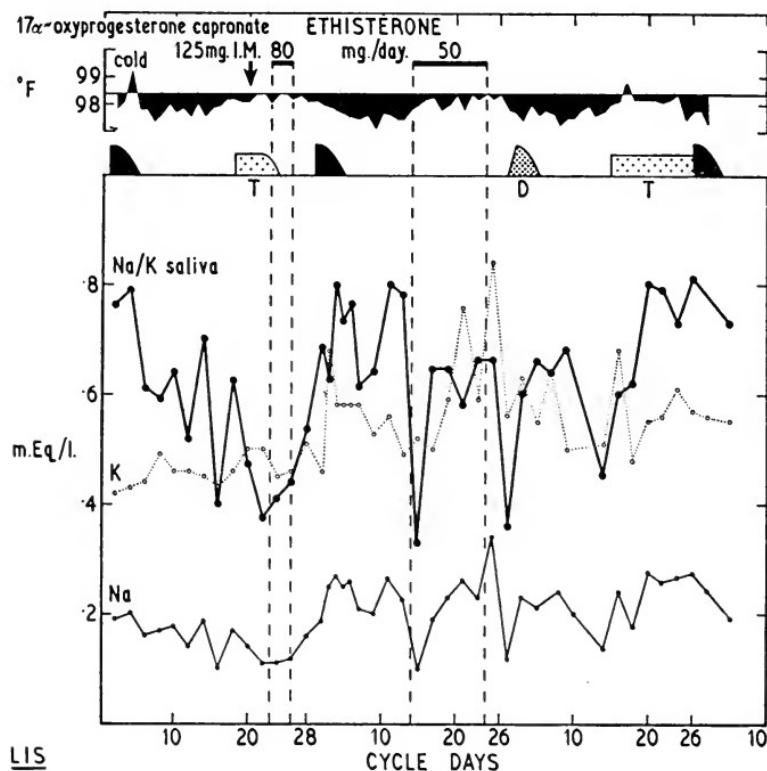
Fig. 6. Three cycles in a woman who experienced premenstrual tension. For explanation see text.

T = tension. D = dysmenorrhoea. 17α-Oxyprogesterone capronate was injected at the point marked ↓ ; ethisterone was administered orally in doses of 80 and 50 mg. per day where indicated.

cycle, the Na/K ratio in the saliva was definitely lower, due to a lower sodium concentration, in the second half of the cycle. An injection of 125 mg. of 17α-oxyprogesterone capronate intramuscularly failed to affect the symptoms, but when ethisterone, 80 mg. daily by mouth, was started three days later the tension disappeared, in spite of the Na/K ratio

remaining low. In the next cycle, 50 mg. ethisterone daily was given from the 14th day of the cycle. There was no tension (though the succeeding period was painful). Yet again the Na/K ratio appears to have been on the whole lower in the second half of the cycle. In the third cycle, no treatment was given; the usual premenstrual tension appeared but this time the premenstrual Na/K ratios were the highest in the cycle.

It must be confessed that the writer does not know how to interpret these findings, beyond concluding that they do not provide evidence for theories currently held to account for premenstrual tension and its relief (which, in the writer's experience, is by no means invariable) with progesterone or its analogues.

Pregnancy

In no physiological circumstances do such profound hormonal changes occur as in pregnancy. The output of oestrogens rises some thousandfold, of progesterone ten to twentyfold, and of adrenocortical and thyroid hormones to less impressive, but still significantly increased levels. A new hormone, chorionic gonadotrophin, found only in pregnancy, of foetal, and therefore partly paternal origin—a "foreign protein", to some extent—appears in the circulation immediately after implantation, rises to striking levels by about the 60th day of gestation and then as rapidly falls to about one-quarter the maximum level during the remainder of pregnancy. The sum total of these changes is to produce a substantial degree of fluid and sodium retention in all pregnant patients. Oedema is of course common; its association with hypertension, with or without albuminuria to give pre-eclamptic toxaemia, is also not uncommon. Toxaemia is, for the obstetrician, one of the remaining major problems he has to face. Its pathogenesis continues in obscurity, in spite of extensive research.

Only one or two aspects of this vast problem will be dealt with here.

That water and sodium are retained in considerable

quantity during pregnancy has been shown by numerous balance studies (see Rinsler and Rigby, 1957 for references). Chesley and Boog (1943) found an increased thiocyanate space in normal pregnancy, the increase being still greater in pre-eclamptic toxaemia. From this it was concluded that much of the sodium retention was due to expansion of the extracellular fluid (ECF) compartment. However, Gray and Plentl (1954), using a sodium isotope dilution technique, found little change in the sodium space and total exchangeable sodium in normal pregnancy. They observed a total gain of some 500 m-equiv. of sodium during the last six months of pregnancy, which they felt could be accounted for by the products of gestation and the expanded maternal blood volume. The maintenance of an essentially unchanged non-pregnant sodium space during normal pregnancy, despite the rise in plasma volume, suggests that there is little change in ECF.

The gain of sodium and water, with maintenance of a normal total-exchangeable sodium value and with an increased thiocyanate space, provides indirect evidence that in normal pregnancy there is an alteration of cell permeability with an increased maternal storage of intracellular sodium and water. The increased intracellular storage of sodium, together with the foetal requirements, are a drain on the salt content of the ECF, which, if uncorrected, would lead to diminution of the ECF and plasma volumes. It has been demonstrated by Bartter and co-workers (1956) that a fall in ECF volume without change in tonicity leads to a rise in aldosterone excretion. Such a rise in aldosterone excretion occurs in pregnancy (Venning and Dyrenfurth, 1956; Venning *et al.*, 1957; Rinsler and Rigby, 1957) and may form part of a homeostatic mechanism for maintaining the ECF volume and meeting the loss of sodium from the ECF into the maternal cells and foetal tissues by increased renal reabsorption.

In pre-eclamptic toxaemia, clinical examination alone is sufficient to demonstrate the expanded ECF compartment. Expansion of this compartment was shown by Bartter and

co-workers (1956) to cause a fall in urinary aldosterone excretion in normal persons. In the pre-eclamptic patients studied by Rinsler and Rigby (1957), the aldosterone outputs were considerably less than those at the same stage of normal pregnancy and it was concluded that this was because of the expanded ECF compartment. The output of aldosterone in these toxaemic patients is less, for a given urinary Na/K ratio, than in the normal group; yet despite the low aldosterone output, sodium retention is maintained or increased. This suggests that a mechanism other than that of aldosterone secretion may be responsible for the sodium retention of pre-eclamptic toxaemia.

Labour, especially if prolonged, is another aspect of pregnancy in which electrolyte disturbance may assume importance. Hawkins and Nixon (1957) have demonstrated a consistent loss of plasma water and increase in plasma specific gravity after only 20 hours of labour, indicating a state of dehydration long before the appearance of clinical signs. In addition, they found an increase in plasma sodium and a decrease in chloride and potassium. This, they suggest, is due to increased renal excretion of chlorides necessitated by the disturbance of acid-base balance due to ketosis resulting from shortage of available glycogen. After 48 hours of labour, a striking fall in plasma potassium and in circulating eosinophils was seen. This is consistent with increased adrenocortical activity, such as is known to occur after surgical operations (MacPhee, 1953). In labour, this fall in plasma potassium may be particularly important because of its influence on uterine contraction. It is very probable that potassium depletion in long labours materially adds to the inefficiency of an already inert uterus.

Changes in Steroid Metabolism in Ageing Men and Women

The most extensive study of this subject has been made by the Worcester group (Pincus *et al.*, 1955). Certain of their

conclusions, of possible relevance to our main theme, are as follows:

Oestrogens. In men, the output of oestrogens remains relatively constant with increasing age; in women, on the other hand, the output declines between the ages of 40 and 60 years, reaching a level somewhat below that of men and thereafter remaining constant. Of the separate fractions, oestrone and oestradiol decline slowly in men, accompanied by an increase in oestriol which makes the total oestrogen output appear constant; in women the most marked decline in earlier decades is in oestriol output, the least marked in that of oestrone, while in the later decades further small declines in oestrone and oestradiol are accompanied by an apparent increase in oestriol. Oestriol is a metabolite, not a secretory product as the other two may be; its increase with advancing age may therefore be due to lesser destruction of secreted oestrogen.

Neutral Steroids. The rate of decline of 17-ketosteroids is similar in both sexes. The urinary ketonic androgens are higher in men than in women and decline more steeply in the former, particularly during the earlier decades. During these decades, the decline of androgens is steeper than that of 17-ketosteroids, so that with advancing age the ratio of 17-ketosteroids to androgens increases, albeit somewhat irregularly. Since the androgenic activity of the 17-ketosteroids is to be attributed chiefly to androsterone, it follows that the rate of production of androsterone (and presumably of its precursors) declines more rapidly than that of the less androgenically active 17-ketosteroids. Though this might have been expected for men, as a result of declining testicular function, it is perhaps more surprising in women and suggests a decrease in output of either adrenal or ovarian androgens, or both.

The ratio of androgens to oestrogens is higher for men than for women at all decades until the ninth.

The output of adrenal corticosteroids is rather higher in men than in women at all ages and varies but little with age. In contrast, the non-ketonic steroids, a mixture of substances

of doubtful origin, part adrenocortical and part perhaps gonadal, decline with age much as do the 17-ketosteroids. Thus the outputs of the various classes of neutral steroids change with age in dissimilar fashion. Close study of the data suggests that the steroids of adrenal origin are less affected by age than are those derived from the gonads, but that adrenal steroids are not uniform in behaviour in this respect.

This differential behaviour is clearly shown by the various α-ketosteroids. The 11-deoxy steroids, androsterone and aetiocholanolone, decrease regularly and markedly with advancing age, in both men and women. In contrast, the 11-oxygenated 17-ketosteroids decrease much less markedly with increasing age in both sexes. The 11-oxyaetiocholanolones decrease least of all; these substances derive chiefly from cortisol and its metabolites.

To evaluate the significance for fluid and electrolyte control of these hormonal changes in ageing men and women is none too easy. The most important of the above-mentioned hormones from this point of view are the adrenal corticosteroids, the output of which changes least. Beyond that simple statement it is unsafe to venture.

Nothing has hitherto been said about the rôle of antidiuretic hormone of the posterior pituitary in the control of electrolyte and fluid metabolism under the various circumstances discussed above. Though it is true that numerous reports have appeared in the literature implicating ADH in a variety of pathological states characterized by oliguria and oedema, it is the opinion of van Dyke, Adamsons and Engel (1955) that "the assays used to support this belief are so grossly inaccurate as to make valueless any conclusions that have been reached." If we may accept that opinion, nothing further need be said.

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DISCUSSION

Milne: Dr. Swyer rightly stressed the difficulties of showing the cyclical changes in electrolytes in the menstrual cycle. But there is one change which has been found by all those who investigated it, and that is the cyclical changes in organic acid excretion in urine. There is both high citrate and high α-ketoglutarate excretion at the time of ovulation and an abrupt fall immediately premenstrual. That is very constant indeed. The easiest way to produce changes in these organic acids experimentally is by variation in the systemic acid-base balance. Body alkalosis, not the pH of the urine, tends to cause a rise in excretion and acidosis a fall. It struck me that the previous investigations of acid-base balance in the menstrual cycle had been rather contradictory. Some workers claim there is a cyclical change in serum bicarbonate and others suggest a change in pCO_2, but this has been contradicted in other papers. Dr. Swyer, have you any data in your metabolic studies which relate to acid-base balance in normal menstrual periods?

Swyer: No, but I am very interested to hear of these changes.

Scribner: Dr. Swyer, were your studies made with constant intake?

Swyer: No, we did not attempt that because our subjects were ordinary ambulant persons and it was rather difficult to restrict them much. It was hoped that if the changes were going to be sufficiently distinctive,

with people who were keeping themselves on an ordinary kind of régime they would show up in spite of any day-to-day variations. That is certainly a deficiency in our studies, but I do not think it entirely invalidates them.

Adolph: I would like to go further and suggest that if the balances or the outputs reflect variations of intake, they might be just as valuable as variations of output would be on a constant intake.

Thaysen: How were the Na/K ratios in the saliva done?

Swyer: They were obtained by collecting saliva first thing in the morning, as nearly as possible at the same time each day, for a fixed length of time (five minutes). In one series, the first five minutes was collected, and in another series the first five minutes was discarded and the second five minutes collected, as I understand there is something significant in that. We were unable to see any difference at all when done in these two ways. The saliva was collected just by spitting into a bottle, and the only stimulation was that the patients were chewing paraffin.

Thaysen: Your Na/K ratios showed very great fluctuations and it was difficult for me to assess any cyclical change. The Na/K ratio may vary considerably just because of changes in the rate, and these variations are quite independent of hormonal or other influences. I do not think that the estimation of the Na/K ratio permits one to dispense with the necessity for measuring secretory rate.

Swyer: Another thing we did was to measure the volumes which were produced in this fixed time, and try to correct for the variations in volume. It did not seem to make any difference at all, but I do agree that some of the variables might have been inadequately controlled.

Thaysen: I believe that you might find the ratio very reproducible when you use a standard secretory rate.

Talbot: Dr. Swyer, you mentioned something about a will-o'-the-wisp, sodium diuretic hormone of adrenal origin. Do you believe in its existence, and if so, have you or any of those here a solid notion as to the nature of the beast?

Swyer: I certainly have no solid notion. It is an idea that has been mooted to account for the apparent inability of normal amounts of sodium-retaining hormone to counteract the sodium loss. I know there have been very active searches for it, and that large amounts are found in the salt-losing type of adrenal hyperplasia.

Desaulles: We have worked quite a lot on this problem and we have got something which is derived from the adrenal, but what it is we do not really know. Dr. Wettstein (1958. *Iva.*, **29**, in press) has just described how he found it and how he is working on it, but that is as far as we have got.

Adolph: I would like to raise a general problem which Dr. Swyer brought up. How is one to judge whether in labour there is dehydration? All the criteria by which we can judge of the existence of dehydration would be in a very fluctuating state at such a moment, and I realise that Dr. Swyer was not making any positive statements about it. Is there any way in which we can judge hydration, dehydration or super-hydration, as transitory states of the organism?

McCance: It is a question of definition. Do you mean by dehydration a rise in the tonicity of the extracellular fluid due to an increase in the quantity of sodium there, or do you mean by dehydration a decrease in the total amount of water in the body?

Adolph: I think one type of dehydration would exist if we are satisfied that there is no change in the concentration, but a decrease in the volume.

Fourman: In the patients with hypernatraemia who have an increased volume of the extracellular fluid, is this increase appropriate or inappropriate to the requirements of the cells? Is the hypotonicity something determined by the cells or something imposed upon them? Water intoxication with its characteristic symptoms (Weir, J. F., Larsen, E. E., and Rowntree, L. G. (1922). *Arch. intern. Med.*, **29**, 306) exemplifies an inappropriate imposition; here the hypotonicity of the extracellular fluid is accompanied by a swelling of the cells. An "appropriate" fall in tonicity and increase in volume of the extracellular fluid is not associated with these symptoms. The patients I mentioned earlier do not have evidence of water intoxication.

After every stress, these patients with hyponatraemia returned to their original low concentration of extracellular fluid. One feels that the concentration is determined by the cells—a new steady state. We do believe that this low concentration of the extracellular fluid must be the result of a low osmotic pressure of the cells. There are obviously different kinds of hypotonicity of the extracellular fluid, with and without symptoms of water intoxication. When there are symptoms, the cells are swollen. Miss Leeson and I have been wondering whether a lack of symptoms means the cells are not swollen, but merely hypotonic.

Swyer: I was referring to the opposite problem, namely that in labour dehydration is accompanied by lack of potassium. Presumably this increase in specific gravity of the plasma and the apparent loss of plasma potassium would not be consistent with normal functioning of the cells. It might therefore complicate still further the prolongation of labour.

Fourman: I do need convincing that any case of high serum sodium, which these patients have, is not a case of dehydration.

You made another very fascinating statement which I would be very glad to have amplified. Not being a paediatrician I do not see very much of these adrenogenital syndromes. In Addison's disease, on the other hand, I think it would be exceptional to find that the patient with a high plasma potassium as a leading feature would die of a cardiac arrest as a result. The general experience is to find that there is depletion of sodium and, incidentally, but not clinically important, a raised serum potassium. I wonder whether there is a different abnormality in a simple lack of sodium-retaining hormone, which would account for the—to me rather surprising—predominance of changes in the serum potassium. We have one patient who is being maintained after adrenalectomy with cortisone but because she has heart failure we are not giving her any sodium-retaining hormone. To my astonishment our problems in her are those of transient paralysis with very high plasma potassium (9 m-equiv./l.).

Swyer: High plasma potassium is one of the outstanding features, as I think Dr. Talbot will also agree, in adrenal hyperplasia. In the salt-losing

variety you get figures up to 16 m-equiv./l. or more with survival, though not for long. I have not personally encountered that, but it has been seen in the Hospital for Sick Children, Great Ormond Street.

Young: I think there is a much simpler explanation for the young infant's rapid rise in serum potassium under conditions of stress. The babies with the adrenogenital syndrome feel poorly and vomit; therefore they take in very little water and become dehydrated. Their blood urea goes sky high at the same time as the serum potassium, and I think that both are due to a rapid rate of cellular breakdown secondary to the dehydration. I have no real proof of this, but all neonates becoming dehydrated very quickly show a high serum potassium level.

Milne: In these cases in babies with high serum potassium, is the myocardium less sensitive to the hyperkalaemia? This could be inferred from the work of Widdowson and McCance (1956. *Clin. Sci.,* **15,** 361) on serum potassium in foetal pigs. Anyone with experience of hyperkalaemia in acute renal failure in adults would find very severe ECG changes long before the serum potassium reached 10 m-equiv./l., and death usually occurs very shortly after the potassium reaches 10 m-equiv./l. These high figures rather startle me; I would like to know what is happening to the ECG during the period of hyperkalaemia.

Davson: The effect of potassium on the heart is linked with that of calcium. It may be that over long periods the calcium might rise too and tend to compensate for the raised potassium.

Scribner: We have made some studies on dogs and we could not greatly increase the tolerance of the dog to hyperkalaemia by giving calcium. Large doses of calcium increased tolerance no more than 1 m-equiv./l.

Adolph: This unanswered problem may leave us with an age difference in the susceptibility of the heart to potassium.

Young: Once the potassium goes up towards 10 m-equiv./l. in babies they become desperately ill and they sometimes die. I do not think they have any better tolerance to these very high serum potassium levels than adults. If you take infants with the adrenogenital syndrome off all their treatment in order to confirm the diagnosis, which may be difficult in young males, it is a very frightening experience to see the heart misbehaving with both the clinical effects and the ECG changes of hyperkalaemia.

McCance: You seem to have found something in which the infant appears to react in the same way as the adult.

Young: Kerpel-Fronius has over-emphasized, perhaps, the differences in the physiology of the infant, but his points are all intended to underline the differences in the effect of stress on the infant. In the treatment of infants, sometimes the physiologist's point of view has made the clinician oversensitive. He is frightened to give infants the treatment that would be appropriate for adults because of the differences in the physiology of the infant—whereas the clinical condition must and can be treated effectively as long as the relatively minor differences in physiology are borne in mind.

There is one point I should like to make which refers to the papers by Prof. Adolph and Dr. Swyer. It seems to me, since the baby tends to

retain water before birth and still excretes high amounts of oestrogens after birth, that the persisting influence of the mother's oestrogens in the early days of life might be the explanation of the infant's poor response to a water load.

Adolph: This oestrogenic influence seems to me a very interesting possibility. Has anyone any data on the influence of the maternal hormones upon water balances or exchanges?

Fourman: Another question is whether oestrogens do inhibit the diuretic response to an overdose of water in adults.

Heller: We have been injecting sex hormones of various kinds into newborn rats to see whether we could influence the amount of total body water or whether we could retard its decrease as the animals get older. These are only preliminary experiments, but so far neither oestrogens nor progesterone have produced any effect.

Swyer: Gans and Thompson (1957) produced evidence that the hydraemic neonate might retain fluid as a result of maternal oestrogens, and that as maternal oestrogens were excreted, the weight fell and then remained reasonably constant. So it does look very much as though at least some of the fluid retention in the newborn infant is due to maternal oestrogen. I do not think that that can be held to account for the poor handling of the water load since that extends for the best part of the first year, or so I understand.

McCance: No, only about 14 days, I believe.

Adolph: I think I can clarify this contradiction of ages to some extent. If you read the literature up to 1923 you learn that in the first year the human infant excretes water very slowly. Such conclusions were reported by Lasch (1922. *Z. Kinderheilk.*, **36**, 42) and others, with inadequate methods of collecting urine. The problem was clarified when Ames (1953. *Pediatrics, Springfield,* **12**, 272) did some well-controlled studies on the excretion of a water load in infants of 1, 3, 7, and 14 days of age. She showed that within 14 days the excretion of a water load becomes 63 per cent as great, and within 90 days even as great as in the adult human, if one bases water load and excretion on unit body weight.

Swyer: Does this also apply to resistance to dehydration and handling of electrolytes?

Adolph: I do not think we have any good data on the resistance to dehydration. We know much less about hydropaenia than we do about superhydration.

Widdowson: Dr. Talbot, if a newborn baby and an adult were deprived of all water, which would live longer, and why?

Talbot: The minimum daily water expenditure of the small infant relative to his body water stores is ordinarily about twice as great as it is in the adult. For this reason, the infant usually tends to become dehydrated when deprived of water about twice as fast as the adult. If only this relationship is taken into account, one would expect the adult to outlive the infant. However, the infant is born with a "surplus" of water, equivalent to about one day's water requirements, which he is meant to shed during the first few days of life. The shedding of this surplus fluid

tends to delay the development of serious dehydration during the neo-natal period. This process coupled with other attributes might enable some newborn infants to survive total thirsting as long as an adult.

Heller: I seem to remember that what Gans and Thompson showed was that there was a decrease of body water in the infant which was corre-lated with the excretion of maternal oestrogens, but this does not establish a causal relationship.

Swyer: I think the point they were trying to make was that there was this parallel fall in oestrogen and in body water with no change in adrenal steroid output. They put two and two together and thought one was due to the other.

Wallace: Dr. Swyer, what about the situation of a diabetic woman and her baby? In a great number of instances there is a very intense water retention.

Swyer: I can counter that by saying what about the baby of a pre-diabetic mother? It shows just the same changes before the mother has diabetes. I do not think we know why the prediabetic mother has a large baby—there have been suggestions that it is due to excess growth hormone secretion by the mother, but there is no very convincing evidence.

Wallace: This kind of baby generally seems to have a great deal of water in him—more water than in equivalent weight normal babies.

Swyer: That is very true. The baby is large but it is not postmature—indeed, it behaves more like a premature.

Wallace: Is that an oestrogen effect?

Swyer: I do not think we know.

Wallace: Very often during these discussions the words "inefficient" and "immature" have been used to describe the newborn infant. Mr. Peter Rickham in his book, "The Metabolic Response to Neonatal Surgery" (1957. Harvard University Press), develops the point of view that the newborn infant is tolerant of adverse experiences such as fasting, thirsting and surgical trauma. Despite the fact that the new-born has an extra load of water in his body and a low metabolic rate he does seem to have a certain toughness that at a later date is not so evident. "Immaturity" and "inefficiency" may not be synonymous.

Bull: I should like to support that observation. We see enough burnt children and adults to be able to assess their comparative mortality in given degrees of burning. Although it is widely stated that children react badly to burning—and burning largely involves the problems of fluid and salt management that we are talking about today—we failed to find any evidence that the small children react any worse than their elder brothers and sisters (Bull, J. P. and Fisher, A. J. (1954). *Ann. Surg.*, **139**, 269). The prognosis falls steadily from about 30 years to old age. We do not frequently see babies during their first 14 days, but at least in the first year there is no evidence that they react worse than older children and adults.

GENERAL DISCUSSION

Richet: Dr. Thaysen, mercury poisoning is supposed to inhibit some enzymic actions and possibly reabsorption by tubular cells. I should like to know something about the secretion of sweat during mercury poisoning and whether you found any differences due to that substance?

Thaysen: I have not done any experiments of this kind myself, but studies on mercurial diuretics have been performed, not on the sweat glands but on the salivary glands, by White and co-workers (1955. *J. clin. Invest.*, **34**, 246). White showed that there was no significant effect of mercurial diuretics on salivary sodium, potassium or chloride excretion.

Richet: Dr. Desaulles has reminded me that during chronic mercuric poisoning, acrodynia for instance, there is an increase in sweating.

Thaysen: That might be due to a cerebral effect of chronic mercury poisoning rather than to a local effect of the mercury directly on the glands.

Davson: It is rather a fortunate accident that the mercurials are diuretics and that they have that specific action on the kidney tubules. If you were to try and raise the mercury concentration in the blood so as to put some specific mechanism apart from the kidney out of action, you would kill the person anyway, because mercury would interfere with so many other metabolic reactions if you really could get a reasonable blood level of it for any length of time. So I think investigation of it is out of the question.

Hingerty: Is there any evidence that plasma magnesium goes up at the same time as plasma potassium? In hypersecretion of aldosterone, plasma magnesium has been reported as being decreased in a few cases. We found some years ago (Conway, E. J., and Hingerty, D. J. (1946). *Biochem. J.*, **40**, 561) that when plasma potassium went up in adrenalectomized rats it was accompanied by an almost parallel increase in the plasma magnesium; cellular magnesium also went up but rather less.

Richet: We have made determinations of plasma magnesium in more than 200 patients during acute and chronic renal failure. During acute renal failure there is always an increase in plasma magnesium concentration. Our technique with yellow titanium gives normal values of $1 \cdot 5 - 1 \cdot 7$ m-equiv./l. In acute renal failure we sometimes get $3 \cdot 0 - 3 \cdot 5$ m-equiv./l. serum magnesium. In contrast, serum

potassium is increased in only 20 per cent of our patients. We have noticed that the serum magnesium increases more rapidly and more frequently than serum potassium. During chronic renal failure we have found exactly the same thing. The serum magnesium begins to increase when the urea clearance is below 15 ml./min., even if serum potassium remains normal for a long time.

McCance: That agrees with observations Miss Watchorn and I made in 1932 (*Biochem. J.*, **26**, 54). We generally found that the serum magnesium was high in chronic renal failure and indeed searched for such cases when we wanted high values for our ultrafiltration experiments.

Scribner: I want to bring to your attention the work done by Dr. Konrad Buettner, professor in the Division of Climatology at the University of Washington, Seattle (1953. *J. appl. Physiol.*, **6**, 229). His observations bear on the sweating data that we have heard and also on considerations of cellular tonicity. If you study water transfer through skin and exclude sweating, the normal human skin will absorb water into the skin against an osmotic gradient that is five times isotonic. In other words if you expose it to increasing concentrations of sodium chloride solution, the skin will take up water until a concentration which is five times isotonic is reached. The mechanism of absorption is not known and there has been no work to elucidate why this occurs. The rate of absorption in an adult human is about 20 ml./hr. for the total skin, and is correspondingly less for smaller areas of skin. Such factors as the storage phenomenon etc. have been excluded by the methods of undertaking this study. The practical implications of this are perhaps of interest. For example, at low rates of sweating, data on electrolytes in sweat may be abnormally high throughout due to this absorption, and there is some chance that by the proper control of conditions you may be able to absorb water in survival experiments at sea, since sea water is only three times isotonic.

Davson: What happens to the water? Is it immediately carried away by the capillaries?

Scribner: Yes. Deuterium studies have shown that. Ten—twenty ml./m.^{2}/hr. are the actual figures for the absorption.

Talbot: In the last war in survival ration studies we immersed some very dehydrated volunteer subjects in the equivalent of sea water for an hour or so, and were unable to detect any absorption of water through the skin, using changes in total body weight as an index; so this is very interesting.

Scribner: The problem of controlling sweating during these studies is a difficult one and this investigator has gone to great lengths to control this variable.

Davson: Was there also a control on whether salts were being absorbed, when they say that five time isotonicity would have stopped it? Just the fact that the skin absorbs water does not mean that salts are not absorbed as well.

Scribner: The concentration of salts goes up in the outside fluid. Also water is absorbed from capsules containing crystallized salts such as sodium and calcium chloride which are separated from the skin by a layer of air. The type of salt used determines the amount of water vapour in the air. The results by this technique agree with the hypertonic solution studies.

Hingerty: What salts have been investigated?

Scribner: Sucrose, potassium chloride, sodium chloride. The phenomenon is believed to be purely an osmotic effect.

Borst: Before the war Viennese clinicians reported on considerable absorption of water by the skin in heart failure. The prognosis could even be determined by studying the rate of absorption. Dutch workers repeated the experiments but could not demonstrate any absorption at all. However an absorption of 20 ml./m.^{2}/hr. is less than was expected according to the Viennese papers and it is possible that a more exact technique would have given positive results.

BODY WATER COMPARTMENTS
THROUGHOUT THE LIFESPAN*

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OUR first knowledge of the composition of the body was acquired during the last decades of the nineteenth century. The methods used were desiccation and chemical analysis which allowed the determination of the contents of water and electrolytes in carcasses or in single organs. With the recent introduction of the dilution methods a new field of study has grown up based on the *in vivo* measurements of the total quantities of body water and its partitions. Direct dilution methods are now available for the measurement of total body water and of the extracellular water. The intracellular water is calculated as the difference between total body water and extracellular water and is thus a derived value (Moore *et al.*, 1956).

A few comments should be made about the methods and the evaluation of the measurements. In the material presented the total body water has been determined as the volume of dilution of deuterium oxide. In the children the extracellular water has been measured as the volume of dilution of thiosulphate and in the adult groups as the volume of distribution of radioactive bromide corrected for red cell bromide, for the relative water contents of plasma and interstitial water, and for the Donnan effect. As the volume of dilution of thio-

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sulphate is smaller than the corrected volume of dilution of radiobromide the values for extracellular volumes will not be directly comparable for the children and the adults. The same will apply to the calculated intracellular water. All the methods used are reproducible within the 5 per cent range. As the absolute quantities measured are difficult to compare from one individual to another it has become customary to express the results as relative values. The standard of reference used is the body weight as this standard in our experience has been the most simple. In the interpretation it is important to realize that a rather large biological variation appears within groups of the same age and sex.

Although the study of the body water compartments throughout the lifespan is still fragmentary, certain trends in relation to age and sex have appeared. It will be the purpose of this paper to outline these features in a description of the body water compartments during the three main phases of life: growth, maturity and ageing.

Growth

Growth implies a variety of fundamental processes: cell multiplication, increase in cell size, accumulation of extracellular material, increase in fat and minerals.

The alterations in the body water compartments during growth have been studied by Friis-Hansen (1956). From a series of 93 normal children studied with deuterium oxide, with thiosulphate or with both, a series of 31 individuals with simultaneous measurements of all three water compartments will be presented.

It appears from Table I that the absolute amounts of total body water, of extracellular water, and of intracellular water demonstrate an increase throughout infancy and childhood. It is seen that the intracellular water rises more markedly than the extracellular water.

In Table II the three measurements are given as percentages of body weight. The total body water shows a relative decrease throughout infancy and childhood with a most marked

decrease during the first two years of life. The relative
decrease in extracellular water is more marked than the
decrease in total body water. The intracellular water demons-
trates about the same relative value throughout childhood.
It should be mentioned that no sex difference appeared in this

Table I

BODY WATER COMPARTMENTS IN CHILDREN. ABSOLUTE VALUES

Age	*Water compartments in litres*			*Number of subjects*
	TBW	*ECW*	*ICW*	
0–11 days	2·65	1·45	1·20	5
11–180 ,,	3·10	1·42	1·68	9
$\frac{1}{2}$–2 years	5·40	2·36	3·04	7
2–7 ,,	8·96	3·40	5·56	9
7–14 ,,	27·62	7·52	20·10	1

series. The tendencies found in this group are similar to the
findings in the larger group including cases with single measure-
ments of total body water or of extracellular volume. A
statistical analysis of the larger group has shown that most of
the differences between the age groups are significant.

Table II

BODY WATER COMPARTMENTS IN CHILDREN. RELATIVE VALUES

Age	*Values in per cent of body weight*			*Number of subjects*
	TBW	*ECW*	*ICW*	
0–11 days	76·4	41·6	34·8	5
11–180 ,,	72·8	34·9	37·9	9
$\frac{1}{2}$–2 years	62·2	27·5	34·7	7
2–7 ,,	65·5	25·6	36·9	9
7–14 ,,	64·2	17·5	46·7	1

The relative decrease in total body water with advancing
age indicates a relative increase in total body solids, i.e. cell
solids, mineral solids and body fat. The total body solids
thus represent the fraction of the body which demonstrates
the highest degree of absolute increase during growth. This
increase in total body solids represents one important facet
in the alterations in body composition with advancing age.

Within the body water compartments the measurements with thiosulphate demonstrated a relative decrease of extracellular water during growth. A similar degree of decrement in extracellular space with advancing age has been reported by Ely and Sutow (1952) using the thiocyanate method, and by Cheek (1954) using the corrected bromide space. The relative values for intracellular water in the series presented stayed about the same throughout infancy and childhood. No similar investigations are available in the literature, but it is interesting that Corsa and co-workers (1956) found that the total exchangeable potassium as related to body weight stayed the same throughout infancy and childhood. As about 95–98 per cent of the exchangeable potassium must be present within the cells their results can be taken as corroborative evidence for Friis-Hansen's (1956) findings of the relative constancy of the intracellular water.

An alteration in the interrelationship between the extracellular and intracellular water during growth thus appears. When the extracellular water is expressed as a percentage of total body water the extracellular compartment decreases from 55 per cent in the youngest group to 38 per cent and 28 per cent in the two oldest groups, again reflecting the relative decrease of the extracellular water. This altered relationship between the extra- and intracellular water is another important facet in the body compositional changes during growth.

The alterations during growth could be produced in two ways: (1) They could be due to a proportional alteration in the composition of all tissues, or (2) they could be caused by an intracellular increase in some tissues whereas other areas would develop in a different way.

Histochemical studies are helpful in the interpretation of this problem. Kerpel-Fronius (1937) found in studies of muscular tissue from human newborn babies and from adults a relative increase in intracellular phase during growth, whereas such a change did not appear in the skin or in the central nervous tissue. Kerpel-Fronius also drew attention to the fact that the total muscle water had increased from

29 per cent of total body water in the newborn baby to 51 per cent of total body water in the adult and he stressed that an increase in total muscular tissue rich in intracellular phase was a prominent feature in the alterations in body composition during growth.

Yannet and Darrow (1938) found in their studies of cats a relative increase in intracellular phase during growth in muscles, whereas only very small alterations appeared in liver tissue or in brain tissue. In studies of growing chickens Barlow and Manery (1954) reported a similar relative increase in the intracellular phase in muscular tissue.

It appears from these studies that the alterations measured with the dilution methods must be results of a development varying quantitatively and qualitatively from one tissue to another.

In conclusion the alterations in body composition during growth can be described as a disproportional increase in total body solids, total body water, extracellular water, and intracellular water. When the values are related to body weight the following trends are seen during growth: a decrease in total body water, an increase in total body solids, a decrease in extracellular water, and a relative constancy in intracellular water. When the water compartments are related to total body water the trend is for a relative decrease in extracellular water and a relative increase in intracellular water.

Maturity

The body water compartments in adults will be described with particular reference to the sex difference.

The material presented comprises ten normal males and ten normal females at ages from 23 to 54 years, average age in the middle thirties. The series was studied by H. V. Parker in Dr. Francis D. Moore's laboratory, Peter Bent Brigham Hospital, Boston (McMurrey *et al.*, 1958). The methods applied were: total body water was determined with deuterium oxide; the extracellular water was measured as the radiobromide space, which was corrected for red cell bromide,

for the relative water contents of plasma and interstitial water, and for the Donnan effect. As the extracellular water according to the method applied here shows a higher normal value than is obtained with the thiosulphate method the results for extracellular and intracellular water in this series will not be directly comparable to the findings in the group of children.

Table III

BODY WATER COMPARTMENTS IN ADULTS. ABSOLUTE VALUES

Sex	Age range	Body weight kg.	TBW	ECW	ICW
			Water compartments in litres		
Males	23–54	72·5	38·9	16·8	22·1
Females	23–51	59·3	28·7	13·3	15·4

The absolute average values for total body water, extracellular water and intracellular water appear in Table III. As expected all values are higher in the males than in the females, corresponding to the higher average weight in the male group. Most of the difference in total body water is accounted for by the difference in intracellular water.

Table IV

BODY WATER COMPARTMENTS IN ADULTS.   RELATIVE VALUES

Sex	Age	Weight kg.	TBW	ECW	ICW
			Water compartments in per cent of body weight with standard error of the mean		
Males	23–54	72·5	54·3	23·4	30·9
			±1·39	±0·64	±0·89
Females	23–51	59·3	48·6	22·7	25·9
			±1·47	±0·54	±0·96

In Table IV the average values are given in per cent of body weight. The males contain 54·3 per cent of total body water whereas the females contain 48·6 per cent. This difference is statistically significant $(P=0·01)$. The relative values for the extracellular water are very close to one another. The intracellular water amounts to 30·9 per cent in the males and to 25·9 per cent in the females. This difference is statistically significant $(0·01 > P > 0·001)$.

The similarity of the relative values for the extracellular water and dissimilarity of the relative intracellular water volumes in the two sexes gains further support from other parts of the same study. As is seen in Table V, simultaneous studies of total exchangeable sodium and potassium were carried out in these patients according to the method described by Moore and co-workers (1956). The total exchangeable sodium which was determined through an independent measurement demonstrates relative values very similar in the two sexes. As about 85 per cent of the total exchangeable sodium can be accounted for in the extracellular space the findings can be taken as supportive evidence for the correctness of the very close relative values for the extracellular

Table V

BODY WATER COMPARTMENTS AND TOTAL EXCHANGEABLE
ELECTROLYTES IN ADULTS

| Sex | *Values related to body weight with standard error of the mean* | | | | |
	ECW (%)	Cl_e (m-equiv./kg.)	Na_e (m-equiv./kg.)	ICW (%)	K_e (m-equiv./kg.)
Males	23·4	29·3	39·5	30·9	48·0
	±0·64	±0·71	±1·06	±0·89	±1·38
Females	22·7	28·6	38·3	25·9	39·4
	±0·54	±0·92	±1·09	±0·96	±1·40

water in the two sexes. The relative values for the total exchangeable potassium which was determined independently of the intracellular water demonstrate a pattern very similar to the findings of the intracellular water. In both measurements the females have a relative value about 20 per cent below the males. As 97 per cent of the total exchangeable potassium must be within the cells this finding can be taken as evidence for the correctness of the measurements of the intracellular water. It is worth mentioning that a calculation of the average intracellular potassium concentration in the two sexes results in very similar values: 152 m-equiv. per litre intracellular water in the males and 149 m-equiv. per litre intracellular water in the females, and thus indicates that no difference in cellular composition exists in the two sexes.

It appears from the series that males have a higher relative content of body water than females, confirming the results with the deuterium oxide method reported by Edelman and co-workers (1952a) and Ljunggren, Ikkos and Luft (1957).

This sex difference in body composition does not appear to be due to a difference in the relative amounts of extracellular water in the series presented. The extracellular water represented 22·7 per cent of body weight in the females and 23·4 per cent in the males. This similarity in the relative values for extracellular water is in agreement with the findings of Cheek (1953), of Reid and co-workers (1956) and of Ljunggren, Ikkos and Luft (1957) using the corrected bromide space, of Ljunggren, Ikkos and Luft (1957) using the thiosulphate method, and of Griffin and co-workers (1945) using the thiocyanate method.

The lower relative content of total body water in females as compared to males in the series presented is due to a relatively lower content of intracellular water in the females. A similar difference in the content of intracellular water appears in the series studied by Ljunggren, Ikkos and Luft (1957) in which the intracellular water was calculated on the basis of an extracellular space measured with radiobromide as well as with thiosulphate. Further evidence of the relatively lower content of intracellular water in females compared to males is present in the consistent findings of a lower relative amount of total exchangeable potassium in females as reported by Edelman and co-workers (1952b), Arons, Vanderlinde and Solomon (1954), Blainey and co-workers (1954), Sagild (1956), and Ljunggren, Ikkos and Luft (1957).

The lower relative body water in females indicates a higher relative content of total body solids in females than in males. As the relative amount of intracellular solids, as judged by the relative values for intracellular water and total exchangeable potassium, must be assumed to be lower in females than in males, it seems justified to conclude that females must have a higher relative amount of fat (or other non-cellular solids) than males.

When the body water compartments are related to total body water as a standard of reference another sex difference appears. In males the extracellular water accounts for 43 per cent of total body water and in females for 47 per cent, whereas the intracellular water amounts to 57 per cent of total body water in the males and 53 per cent in the females. The difference between these ratios is statistically significant $(P < 0.001)$. This difference in the distribution of the total body water between the extracellular and intracellular compartments can be explained as the result of a higher development of tissues rich in intracellular material and relatively poor in extracellular phase, such as muscle tissue, in the males.

In conclusion: the sex difference in body composition is outlined as a higher relative content of total body water, a higher relative content of intracellular water and a lower relative amount of total body solids and especially of body fat, in males than in females. The total body water is distributed with a lower extracellular fraction and a higher intracellular fraction in males than in females.

Ageing

Our experiences in the old age group are based upon the investigations carried out in seven apparently normal males with an average age of 75 years and seven apparently normal females with an average age of 68 years. This group was studied in Dr. Francis Moore's laboratory (Parker, Olesen and Moore, 1958). The methods used were the same as those applied to the younger adults.

The essential findings in the old age group are presented in Table VI.

A comparison between younger and older adults reveals the following findings: total body water decreases from $54 \cdot 3$ per cent to $50 \cdot 8$ per cent in males and from $48 \cdot 6$ per cent to $43 \cdot 4$ per cent in females. The extracellular water rises slightly in males and decreases slightly in females. The intracellular water decreases from $30 \cdot 9$ per cent to $25 \cdot 4$ per cent in males

and from 25·9 per cent to 22·4 per cent in females.   The differences mentioned are not statistically significant except for the decrease in intracellular water in males $(0·01 > P > 0·001)$.

The tendency to a decrease in the relative values for total body water found in both sexes is mostly due to a decrease in intracellular water. From an unpublished study of Dr. N. W. Shock (1957), in which the antipyrine space and the thiocyanate space were measured in a larger group of males, the following data are of interest. A comparison of 23 subjects

Table VI

BODY WATER COMPARTMENTS IN YOUNGER AND IN OLDER ADULTS.
RELATIVE VALUES

Sex (Number)	Age	Weight kg.	Water compartments in per cent of body weight with standard error of the mean		
			TBW	ECW	ICW
Males	23–54	72·5	54·3	23·4	30·9
(10)			±1·39	±0·64	±0·89
Males	71–84	68·1	50·8	25·4	25·4
(7)			±1·55	±1·36	±0·58
Females	23–51	59·3	48·6	22·7	25·9
(10)			±1·47	±0·54	±0·96
Females	61–74	63·9	43·4	21·4	22·4
(7)			±1·32	±0·45	±0·97

aged 40–49 and 32 subjects aged 70–79 showed that the values for total body water related to body weight decreased from 54·8 per cent to 50·9 per cent, and those for the calculated intracellular water decreased from 30·5 per cent to 25·1 per cent. The extracellular water changed from 24·3 per cent to 25·8 per cent only. The same pattern of a slight decrease in total body water and in intracellular water related to body weight was seen in a male series studied by Olbrich and Woodford-Williams (1956). Sagild's findings of a decrease in total exchangeable potassium in the old age groups of both sexes can also be interpreted as evidence of a decrease in the intracellular phase related to body weight (Sagild, 1956).

From the uniform tendencies in these materials it seems reasonable to conclude that the slight decrease in total body water and in intracellular water related to body weight reflects real alterations in the body composition with advancing age. With the decrease in the relative value for total body water there is a relative increase in total body solids. As the intracellular phase shows a relative decrease the increase in total body solids must be assumed to be caused by a relative increase in non-cellular solids, most probably body fat.

The alterations in the extracellular water related to body weight are not quite uniform and the changes are small. It is of interest that extracellular water expressed as per cent of total body water in both sexes shows a rise from younger to older subjects, in the males from 43 per cent to 50 per cent, in the females from 47 per cent to 49 per cent. This tendency is also seen in Shock's and in Olbrich and Woodford-Williams' series and indicates an altered relationship between the extracellular and intracellular water.

In conclusion: the alterations in body composition in the old age group as compared to younger adults were rather small. A tendency to a relative decrease in total body water and in intracellular water and a relative increase in total body solids, most probably body fat, was found. The extracellular water stayed essentially the same in values related to body weight, but demonstrated a tendency to increase in per cent of total body water.

Acknowledgement

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DISCUSSION

Hingerty: Are these differences in the intracellular water related to the proportion of functional muscular tissue? Have you any comparative data for women athletes, for example?

Olesen: We have no measurements on muscle mass, but we assume that there may be differences due to variations in muscle mass.

Black: Have you analysed your subjects in terms of their occupation?

Olesen: We have not investigated that, but it could probably be done. I have the impression that muscular females have higher exchangeable potassium relative to body weight than the fat ones.

Křeček: Babies of six months have the highest total body water. Have you seen any relationship to the weaning of these babies at this period?

Olesen: I have no data on this question.

Widdowson: Dr. Olesen, can you tell us approximately at what age the fat-free body tissue of the baby becomes adult, or chemically mature, as regards its intracellular-extracellular relationships?

Olesen: It appears from Dr. Friis-Hansen's material that chemical maturity occurs about the age of twelve months.

Widdowson: Have you made any calculations of the body fat at different ages?

Olesen: I have tried to compare the different groups and it seems that there is a relative increase in body fat throughout childhood. It is a slight one but it does exist if we accept that all the non-cellular solid changes are changes in body fat. This calculation is quite apart from possible changes in body minerals and I do not know to what extent these would interfere.

Borst: Is there any relationship between the creatinine output and the intracellular fluid?

Olesen: In the original description of the method of determination of total exchangeable potassium from Dr. Moore's laboratory (Corsa *et al.* (1950). *J. clin. Invest.*, **29**, 1289), a relationship was found between creatinine excretion and the amount of total exchangeable potassium. This has not been studied in this particular series.

Heller: How far is it justifiable to take mean figures from ten young adult females without considering the rôle of the menstrual cycle? Have you had enough cases to pay attention to this point?

Olesen: No, but it would appear from what Dr. Swyer mentioned yesterday that it would not mean very much, as the latest view is that these body weight changes are randomly distributed throughout the menstrual cycle.

Shock: It seems to me that we have two possible interpretations of this age reduction in intracellular water. The interpretation I favour is that the reduction in total intracellular water is a reflection of the loss of functional cells or the loss of protoplasm, rather than a change in the water concentration of the remaining protoplasm. Have we any other evidence that would make one interpretation more probable than the other?

Davson: I think that is a very sound point, because a cell can change in size without there being a change in the relative value of the water or solid contents of the organism. Is there any change in the histological appearance of old tissue that would indicate whether the cells had become smaller or larger?

Shock: I cannot answer this question and must refer it to the pathologist or histologist. In our own work we have been looking for indices of the total amount of man left functioning at a given age. Surface area leaves much to be desired as a criterion, but one can account very nicely for the age reduction in basal metabolism in terms of cellular loss if body water is used as the index. In other words, although the basal metabolism per unit of surface area goes down with age, the basal oxygen consumption per unit of intracellular water does not change at all with age. When you try this with renal function data, renal plasma flow per unit of body water goes down just as much as the renal plasma flow per unit of surface area.

Scribner: Total exchangeable potassium might possibly be a good parameter for this measurement of protoplasm.

Dr. MacIntyre of Hammersmith has made an interesting study (to be published), in which he finds a direct correlation between either body weight or body fat and the extracellular space as measured by bromide. The implication of this correlation is that fat tissue has an extracellular space relationship to its weight which is the same as that of non-fat tissue. This relationship is consistent with the data presented by Dr. Olesen.

Bull: This is in contradistinction, for instance, to the blood volume, which is a poor function of total body weight or of fat, and is closely related to lean body mass. I would suggest that blood volume and metabolic rate are related to intracellular water and possibly to exchangeable potassium rather than to extracellular water.

McCance: Do those who see many old people professionally get the impression that they are fatter than middle-aged people? There are often indications that in old age man is rather wasted and has not much fat; but perhaps his shrinkage is more in protoplasm than in fat.

Swyer: One possible interpretation is that fat people do not live so long; most of the really old people are pretty thin.

Fejfar: My experience is that older people usually eat more than they did when they were middle-aged—they eat more than they need to.

Shock: I have no information on what they eat, shall I say, spontaneously. But I do know that on many metabolic balance studies that we carried out on middle-aged and older people, one of our primary problems was to get our older people to consume the diets which were eaten by the middle-aged control group without much difficulty. The variations were usually in the protein intake, particularly when we tried to increase it by adding meat three times a day. A great deal of coaxing was needed to get our older people to consume diets of this kind.

Fourman: Dr. Olesen, Dr. Shock and others suggest from their data that, in adults, the percentage of total body water that is extracellular water increases with age. I would like to try to visualize what this means. One should not think of the extracellular fluid as a bag of water. Obviously about a quarter of it is accounted for by the plasma volume, and perhaps a fifth by the lymphatic fluid; but what about the rest? The rest is a film of fluid which surrounds the cells and the fluid of the collagenous tissue of the body. If the cells, the muscle cells in particular, without changing in number, shrink with age, then one would get a change in the relation between the volume of the muscle cells and the amount of fluid bathing them, since a single cell when it shrinks increases its ratio of surface area to volume. I wonder whether this is the explanation of the increase in ratio of extracellular to intracellular water with age: a shrinkage in each cell without change in the total number of the cells, but each cell still having to have its film of fluid surrounding it.

THE EFFECT OF VARIABLE PROTEIN AND MINERAL INTAKE UPON THE BODY COMPOSITION OF THE GROWING ANIMAL *

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THE quantities of various nutritive substances in the growing body at any given point represent the metabolic integration of the daily additions to the body from the diet from the time of conception. Measurement of the rate or quantity of addition may or may not measure the nutritional requirement for a given substance. Whether it does or not will depend upon the requirement for synthesis and metabolic transformation and upon the possibility of the body being able to store the substance. Thus, the day-by-day accretion of fat or glycogen cannot measure a requirement but the accretion of protein and mineral may do so, once any capacity for storage is exceeded. Information concerning requirements for growth is usually obtained by measurements of external balance for variable periods of time. The information acquired concerning the requirements for growth and the composition of growth by this method is often strangely contradictory and always incomplete. Much of the data so obtained indicate that extensive storage of dietary components occurs, or that the composition of the body tissues is variable and dependent upon quantity and quality of the intake.

That body tissues can vary significantly in composition except under extreme conditions is difficult to reconcile with present-day knowledge of tissue composition.

The experiments to be described here were undertaken in an attempt to characterize the effects of high and low mineral and protein intakes, in various combinations, upon the body composition of the growing albino rat as determined by direct whole body analysis. Previous work using this method of approach has been concerned with single constituents and not with the interrelationships of all of the components. The data indicate little variability in composition for the collective soft tissues of the body. The only intake-dependent relationship that seems of significance is in the relative proportions of skeleton to soft tissues.

Experimental Methods

A. Animals and Diets

Male weanling Sprague-Dawley strain rats were used in all feeding experiments. Two groups of animals were used to measure food consumption on the high and low protein diets. In these experiments spill-proof feeding tunnels were used, and the animals caged singly. The remaining groups of animals were housed in units of four in steel wire cages with open-mesh bottoms. Continuous access to unlimited quantities of food in open containers was allowed. Distilled water was similarly offered from dropping bottles. All groups of animals were allowed to grow for a period of 20–25 days. This period of time was chosen as it allowed approximate doubling of weight for the most slowly growing groups.

The experimental diets were compounded using powdered fat-free cow's milk (Starlac, The Borden Company), electrolyte and vitamin-free casein (Nutritional Biochemicals Corporation, Cleveland), dextrose, a fat mixture composed of equal parts of corn oil (Mazola Corn Oil, Corn Products Refining Co., Argo, Illinois) and hydrogenated vegetable oil (Crisco, Proctor and Gamble, Cincinnati, Ohio), and a salt

mixture (NaHCO₃, 7·4 g.; KCl, 12·0 g.; CaCO₃, 12·0 g.; (NH₄)₂HPO₄, 14·9 g.; MgSO₄, 2·5 g.; KI, 0·001 g.) to produce the compositions shown in Table I. The salt mixture was compounded to imitate the ion ratios found in fat-free cow's milk. Ferrous sulphate, 2·0 g., copper sulphate, 0·22 g. and aureomycin, 0·25 g. per kg. of diet were incorporated in the mixtures. A vitamin mixture (Vitamin Diet Fortification, Nutritional Biochemicals Corporation) in quantities calculated to make all diets equal in this respect was added to the mixtures.

Table I

Analysis of diets

| | | g./100 g. *Diet* | | | | m-mole/100 g. *Diet* | | | | |
Diet	Protein	Fat	Carbo-hydrate	Ash	Other*	Na	K	Cl	Ca	P
HPHE	23·4	30·0	35·5	6·02	5·1	16·90	32·4	28·3	23·1	21·3
HPLE	23·4	30·0	35·5	3·08	5·1	8·64	16·6	14·5	11·8	10·9
LPHE	12·0	32·0	50·5	6·02	2·4	16·90	32·4	28·4	23·1	21·3
LPLE	12·0	32·0	50·5	3·08	2·4	8·64	16·6	14·5	11·8	10·9
Friskies	26·8	6·5	51·4	11·60	3·7	15·10	15·3	13·9	84·0	56·8

* Moisture+Fibre (calculated by difference)

Prior to the beginning of the feeding experiments, all animals had been weaned to a commercially produced small animal feed (Friskies, The Carnation Milk Company) known to produce excellent growth, general health and reproduction in the albino rat. Preliminary feeding trials with the high protein experimental diets in comparison with the Friskie diet indicated equal effectiveness as measured by weight gain, general appearance, activity, gentleness and lack of morbidity.

Eight groups of animals were studied, namely:

1. Weanling group (WEAN) 70–80 g. rats weaned to Friskies.
2. High Protein–High Electrolyte (HPHE), see Table I
3. High Protein–Low Electrolyte (HPLE), see Table I.
4. Low Protein–High Electrolyte (LPHE), see Table I.
5. Low Protein–Low Electrolyte (LPLE), see Table I.

6. Rats fed Friskies by way of control. See Table I for composition of this ration.
7. A high protein, high electrolyte group fed to measure food consumption.
8. A similar group to No. 7 but fed the low protein, low electrolyte diet.

At the end of the allotted period of growth (20–25 days) the animals were etherized and 2 ml. of blood removed for analysis either by heart puncture or tail incision. Killing was accomplished by further ether exposure. The dead weight was obtained and the abdominal cavity, thorax and skull opened with heavy shears.* The whole body was then dried in an oven at 85°–95° C. until a constant weight was reached (4–5 days). During the drying process, the carcass was further broken up with heavy shears. The disintegrated carcass was extracted repeatedly with a cold mixture of equal parts ethyl and petroleum ether and re-dried to constant weight. The dried extracted carcass was then homogenized in a Waring Blendor with 5 volumes of anhydrous acetone and the solvent evaporated off and the material re-dried. This process produces a fine homogeneous powder suitable for quantitative analysis. The powder was stored in a desiccator.

B. Chemical Methods

Water. Calculated from weight loss after desiccation.

Fat. During the course of the analytical work, the fat extraction method used as applied to tissues by Hastings and Eichelberger (1937) was examined for completeness of fat extraction when applied to whole carcass. Powdered carcass was exhaustively extracted in the Soxhlet apparatus serially using ether, alcohol and chloroform. This process increased the degree of fat extraction to the extent of 1·5–4 g. per animal. Analysis of the material subjected to such extraction

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* Intestinal contents were not removed. Analysis of the total gastro-intestinal tract and contents of similarly fed animals for water and fat-free solids indicated that their inclusion does not appreciably alter the interpretation of the data.

indicated that its nitrogen content multiplied by $6 \cdot 25$ plus the weight of its ash very closely approximated 100 per cent of the material. Consequently, fat has been calculated in all of the data by the relation: Fat=dead weight—water weight—(nitrogen $\times 6 \cdot 25$+ash weight). All of the constituents shown in Table II have been calculated as g., m-mole or m-equiv. per 100 g. of protein plus ash (i.e. fat-free dry solids).

Ash. A sample of carcass powder was weighed after incineration at 600° in platinum.

Nitrogen. Determined by macro-Kjeldahl analysis using selenium as a catalyst.

Chloride. A micro modification of the method of Lowry and Hastings (1942) was used with cold nitric acid filtrates. Samples of the homogenized powder were also analysed polarographically for chloride, using sulphuric acid filtrates, with excellent agreement between the two methods.

Sodium, Potassium and *Calcium.* These were determined on the ash after separation of calcium using methods previously described (Bergstrom and Wallace, 1954).

Magnesium. Determinations were done on the ash using the method of Fister (1950).

Phosphorus. This was determined on the ash by the method of Fiske and Subbarow (1925).

All electrolyte and nitrogen analyses were in duplicate.

Results

The analytical data obtained in the experiments are shown in Table II. For comparative purposes the whole body analyses on the albino rat of Light and co-workers (1934) and of Cheek and West (1956) are included. Also shown are the average data of Widdowson and Spray (1951*B*) for six normal human newborn babies and the data for single whole adult human bodies of Widdowson, McCance and Spray (1951*A*), Forbes, Cooper and Mitchell (1953) and Mitchell and co-workers (1945). The data for water, protein and ash have been calculated per kilogram of fat-free body weight. The water and electrolytes are also shown using as a reference standard

Table II

COMPOSITION OF WHOLE BODIES AND SERUM

Group	RATS Wean	HPHE	HPLE	LPHE	LPLE	Friskies	Light 1934	Cheek 1956	Infant Widdowson 1951B	Adult 1951A	Adult Forbes 1953	Adult Mitchell 1945
No. of Animals	10	8	12	12	8	10	7	(2)	6	1 (L)	1	1
Body Wt. g. S. D.	71·4 ±3·38	170·6 ±7·18	171·6 ±11·4	111·9 ±8·95	121·8 ±17·1	165·4 ±8·92	274·6 (1)	166·0 (2)	3500·0 (4)	45·0 kg	53·9 kg	70·55 kg
Fat-Free (F-F) Body Wt. (5) S. D.	64·9 ±3·18	151·8 ±7·28	149·7 ±10·05	97·7 ±7·15	102·9 ±12·37	151·9 ±9·04	230·0 (1)	150·0 (2)	2930·0 (4)	34·4 kg	42·8 kg	61·42 kg
H₂O g./kg. F-F Body Wt. (5) S. D.	795·0 ±0·68	762·0 ±2·39	774·0 ±0·78	771·0 ±0·72	755·0 ±1·95	766·0 ±0·49	734·0 (1)	756·0 (2)	821·0 (4)	732·0	694·0	779·0
Protein g./kg. F-F Body Wt. (5) S. D.	170·7 ±6·00	202·2 ±20·3	194·3 ±7·21	185·5 ±7·62	203·0 ±15·4	192·5 ±5·2	224·5 (1)	203·4 (2)	148·0 (4)	192·0	235·3	165·0
Ash g./kg. F-F Body Wt. (5) S. D.	34·6 ±1·11	35·2 ±5·8	31·1 ±0·88	40·9 ±3·0	40·9 ±4·8	41·0 ±0·85	40·6 (1)	41·1 (2)	31·2 (4)	75·8	68·6	55·9
H₂O/100 g. Prot+Ash S. D.	387·0 ±42·4	323·0 ±15·8	344·0 ±14·6	342·0 ±15·0	312·0 ±31·5	328·0 ±0·87	277·0 (1)	309·0 (2)	458·0 (4)	273·5	229·0	353·0
Na m-equiv./100 g. Prot+Ash S. D.	26·2 ±0·66	20·9 ±1·22	22·2 ±0·89	24·1 ±1·72	23·2 ±1·56	24·6 ±0·17	21·3 (1)	21·4 (2)	55·0 (4)	48·2	—	—
K m-equiv./100 g. Prot+Ash S. D.	35·0 ±0·69	30·0 ±1·21	35·9 ±1·30	34·9 ±1·10	33·4 ±0·61	33·7 ±0·67	22·0 (1)	29·3 (2)	28·4 (4)	36·5	—	—
Ca m-equiv./100 g. Prot+Ash S. D.	215·0 ±11·5	198·0 ±20·5	167·0 ±9·5	252·0 ±21·0	222·0 ±19·8	243·0 ±7·6	238·0 (1)	215·0 (2)	270·0 (4)	620·0	396·0	415·0
Mg m-equiv./100 g. Prot+Ash S. D.	15·1 ±0·55	11·1 ±0·73	11·9 ±0·82	14·0 ±0·82	12·8 ±0·95	14·1 ±1·49	9·8 (1)	11·7 (2)	12·2 (4)	9·3	—	—
Cl m-equiv./100 g. Prot+Ash S. D.	21·5 ±0·81	16·6 ±0·56	18·2 ±0·70	18·5 ±0·96	18·9 ±1·28	17·6 ±1·05	13·6 (1)	15·9 (2)	—	—	—	—
P m-mole/100 g. Prot+Ash S. D.	95·0 ±2·49	82·0 ±5·9	77·7 ±2·95	96·3 ±7·72	92·9 ±3·89	85·4 ±4·1	95·5 (1)	—	98·6 (4)	208·6	124·2	129·0
Na Serum m-equiv./l. S. D.	—	143·8 ±3·03	143·7 ±2·49	143·4 ±5·08	146·0 ±3·26	150·2 ±4·7	133·7 (1)	145·0 (3)				
K Serum m-equiv./l. S. D.	—	6·6 ±0·73	5·3 ±0·51	6·2 ±0·42	5·8 ±0·59	6·77 ±0·57	5·11 (1)	4·7 (3)				
Cl Serum m-equiv./l. S. D.	—	97·2 ±2·39	104·1 ±2·42	104·5 ±2·71	99·8 ±3·06	99·8 ±2·31	102·2 (1)	110·0 (3)				
Serum Prot g./l. S. D.	—	5·61 ±0·18	6·14 ±0·22	5·29 ±0·26	5·09 ±0·34	6·39 ±0·45	6·28 (1)	—				

(1) See original data for min. and max. values.
(2) Derived from regression equations.
(3) Mean data only 70–410 g. animals.
(4) No measure of variation available.
(5) Calculated as ash plus protein plus water.

Data of Cheek and of Widdowson calculated on basis of fat-free dry solids. All other whole body data calculated as m-equiv, m-mole, or g. per 100 g. protein plus ash (see text).

100 g. of protein plus ash. This is equivalent to the commonly used reference standard of fat-free dry tissue (*vide supra*).

Fig. 1 graphically presents the currently obtained data in terms of grams of ash, protein and water per kilogram of fat-free body. The grams of fat per kilogram of fat-free body are shown to the right of the columns. It is evident that the compositions of the fat-free bodies are essentially similar. The relative proportions of water, ash and protein have not been greatly modified by variation of the diet producing the growth
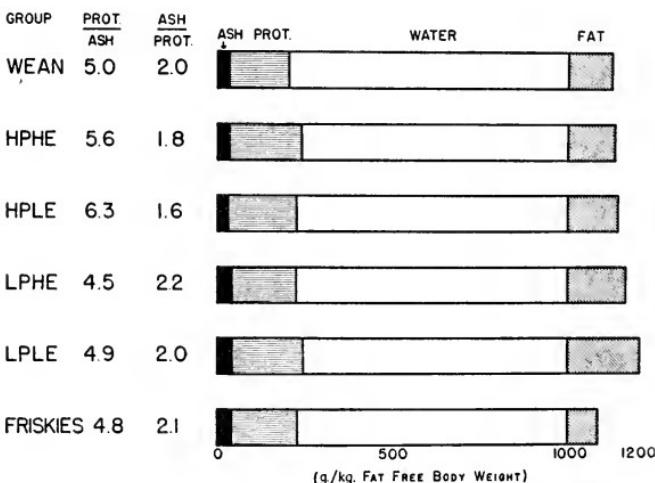
Fig. 1. Ash, protein and water content calculated per kilogram of body weight for the six groups. Fat per kilogram of fat-free body is shown at the right.

increment. Only if body fat were included would gross variation occur. The young animals (WEAN) are relatively low in ash and protein and high in water; with growth the bodies acquired relatively more ash and protein than they did water. The fat contents of the animals on the low protein diets are significantly higher than they are on the high protein.

In Fig. 2 the absolute values for total fat-free body weight, water, protein and ash for the five groups are shown as contrasted against the Friskie group as an arbitrary reference

standard of growth. The high protein groups are very closely equivalent in weight, protein and water content to the standard. The two low protein groups reach two-thirds of the high protein groups with regard to weight, water and protein. The degree of mineral accretion in the high protein animals is significantly different, the high electrolyte group accreting
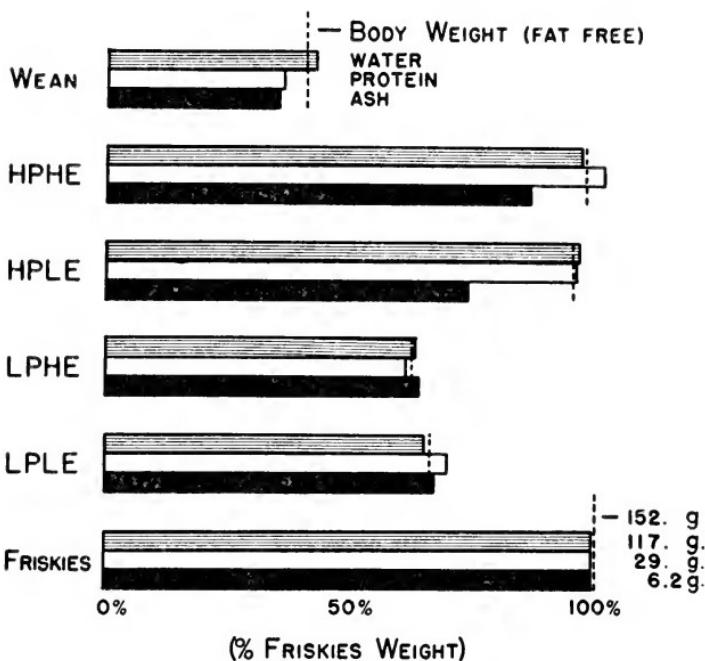
FIG. 2. Absolute quantity of gain of water, protein and ash calculated as per cent of the Friskie or control group. The dashed, vertical lines indicate the body weights as a percentage of the Friskies.

much more than the low, but less than the Friskie group which was on an equivalent protein but higher ash-containing ration. In the low protein groups, whether on high or low electrolyte intake, the gain of ash is not significantly different. It is evident that protein intake is a limiting factor allowing exploitation of a high ash intake only on a high protein diet. The low protein, low electrolyte animals show a greater relative and absolute accretion of protein than do the low

protein, high electrolyte group. This is significant at the 1 per cent level.

The protein to ash ratios shown in Fig. 1 and evident in Fig. 2 indicate the main significance for body composition resulting from diets of variable protein and electrolyte content. The high-protein-fed animals have more protein in relation to
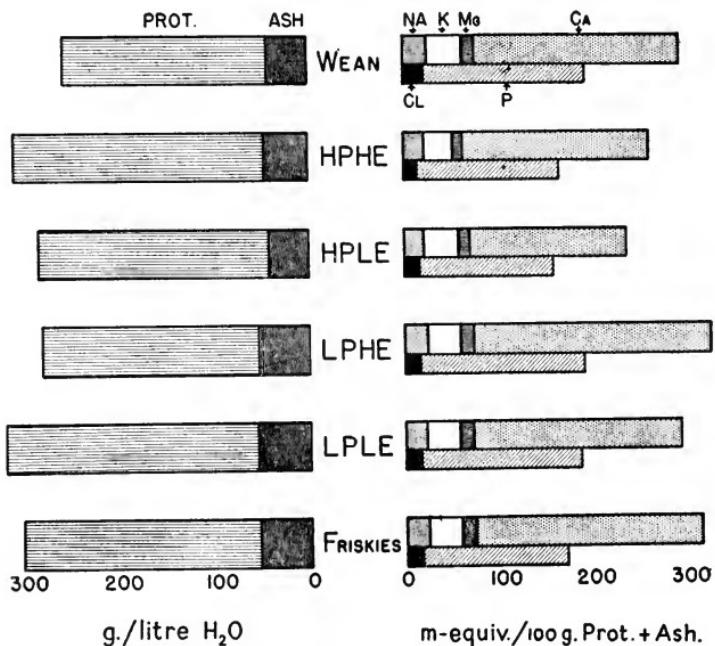
Fig. 3. Diagrammatic representation on the left is of the ash and protein content calculated on a kilogram of water basis. On the right the individual elements composing the ash and their relationship to the sum of protein plus ash (fat-free dry weight) are shown.

ash than do the low-protein-fed animals. Since bone contributes 90 per cent of the ash, the ratios represent the soft tissue to bone proportions in a very general yet valid way. It seems evident that only on a high protein intake can the growing body lay down maximal bony tissue. In the Friskie group where the ash of the intake is very high and composed chiefly of calcium salts, an even greater accumulation of ash

occurs at the relative expense of soft tissue. Where this relationship stops is not answered by the present data.

While all animals are grossly similar in body composition, as shown in Fig. 1, certain significant differences can be found upon more detailed examination of the data. The concentration of ash and protein in the body water and the nature of the composition of the ash are shown in Fig. 3. It is evident, as has been noted, that only in the weanlings and in the low protein, high electrolyte group does a significantly different amount of protein per unit of water appear.

All of the experimental data for individual constituents of the body have been calculated using four reference parameters: i.e. grams or m-mole per whole body, per kilogram of fat-free whole body, per kilogram of water and per 100 g. of protein plus ash (fat-free dry tissue). All of these calculated individual values have been compared among the four groups. The following statements can be made:

I. The Effects on the Protein Content of the Body.

A. By Protein Intake.

Only in those animals on the high electrolyte diets did increased protein intake result in increased protein content of the body on any of the enumerated bases.

B. By Electrolyte Intake.

In the animals on the high protein intakes, the electrolyte effect was variable depending upon the reference base used for calculation. In the low-protein-fed animals a high electrolyte intake reduced the protein content of the body calculated on any basis.

II. The Effects on the Mineral Content of the Body.

A. By Protein Intake.

On any basis of calculation, other than absolute body size, the bodies of the animals fed a low protein intake, whether with high or low electrolyte, contained more ash, calcium,

magnesium, sodium, chloride and phosphorus than those fed a high protein intake.

B. By Electrolyte Intake.

The high electrolyte diets led to increased calcium and decreased chloride in all groups calculated on any basis.

In the high protein groups the high electrolyte intakes also resulted in more ash and less potassium when calculated on any basis.

In Table II the serum concentrations of sodium, potassium, chloride and total protein are shown for the four experimental groups. The only consistent significant difference is for the concentration of total serum protein. Serum protein concentrations are higher in the high-protein-fed groups. The lower protein concentration may indicate protein deficiency in the low protein group and other evidence for such deficiency is given below. The validity of serum protein concentrations as a reliable index of protein malnutrition can be questioned. In this connexion it is of interest that the serum protein concentration of the breastfed infant is lower than that of the infant fed cow's milk (Tudvad, Birch-Andersen and Marmer, 1957).

Animals in experimental groups No. 7 and No. 8 were fed in such a manner as to allow accurate measurement of food intake. The high protein group consumed $8 \cdot 2$ g. of ration per animal per day in contrast to $9 \cdot 3$ g. per day for the low protein group. The mean weights for the two groups at the end of 23 days were 174 and 155 g. respectively. Calculation of the caloric values for the whole bodies of these animals shows that the high protein group contained 292 calories per average animal (1,710 calories per kg.) and the low protein group 263 calories per average animal (2,085 calories per kg.). Calculation of the calories utilized for physiological activity indicates that the low protein group expended 175 calories more per animal for the period of observation than did the high protein group. Increased spontaneous activity was clearly evident in the low protein groups during the period of

observation. Increased spontaneous activity with nutritional deficiency has been previously noted (Forbes *et al.*, 1935; Bevan *et al.*, 1950).
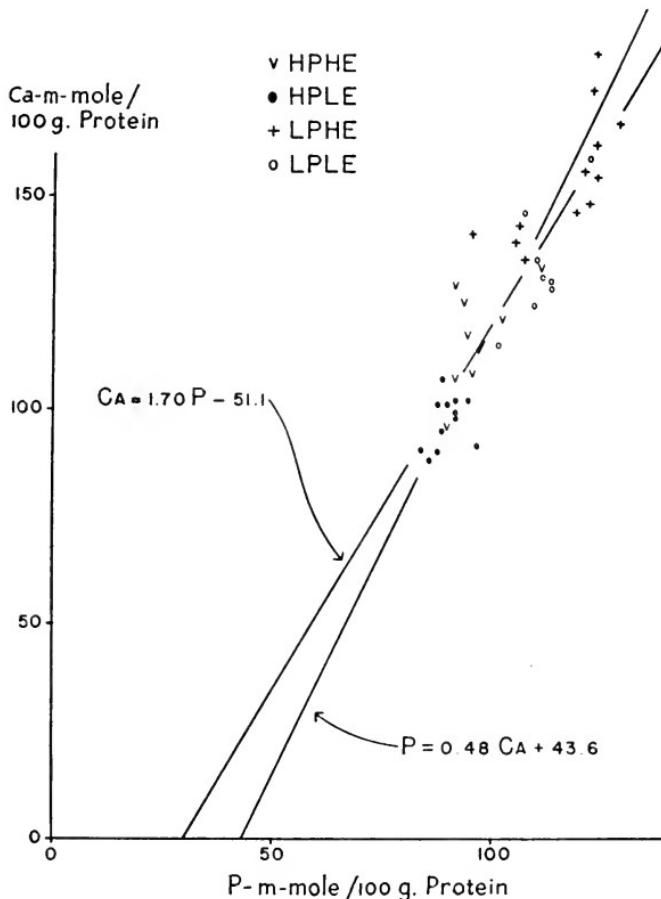
Fig. 4. Relationships of calcium and phosphorus to protein in the experimental groups. For description of method of construction, see text.

The data in Fig. 4 represent the calcium/phosphorus relationship in the four principal experimental groups. On the assumption that the protein content is a basic unit of structure, the values are compared in relation to protein. One advantage of this formulation is that the intercept of the

regression line on the X axis defines the amount of phosphorus present in 100 g. of calcium-free protein. This value should reflect primarily the phosphorus content of muscle tissue. From the statistical analysis of the calcium-phosphorus relationship, a correlation coefficient of $+0\cdot90$ was derived. Further, by the analysis of variance technique, it has been determined that the regression curve is a straight line, described by the equations calcium $=1\cdot70$ phosphorus $-51\cdot1$ and phosphorus $=0\cdot48$ calcium $+43\cdot6$ when both are expressed as m-mole/100 g. protein, and calcium $=2\cdot19$ phosphorus $-2\cdot04$ and phosphorus $=0\cdot37$ calcium $+1\cdot35$ when both calcium and phosphorus are expressed as g./100 g. protein. The X intercept is between $30\cdot3$ and $43\cdot6$ m-mole phosphorus/100 g. protein or between $0\cdot93$ and $1\cdot35$ g. phosphorus/100 g. protein. It is of interest that the calcium/ phosphorus ratios of the four groups of rats studied by Light and co-workers (1934) and the infants analysed by Widdowson and Spray (1951) also lie on this regression line when their values are calculated in this manner. This indicates that the changes in phosphorus content of the various groups are related to the changes in calcium and to the total amount of protein present. The phosphorus concentration is constant in the "soft tissue" (calcium-free protein), and the phosphorus has a constant ratio to the calcium in the "skeleton" (calcium-containing tissue).

It is also apparent from the figure that the calcium to protein ratio is highest in the low-protein, high-electrolyte-fed animals and lowest in the high protein, low electrolyte group.

Discussion

The present data, like the very similar data of Widdowson and McCance (1957) and Stanier (1957), indicate no real evidence for storage or depletion of protein with varying intake. The basis for such a judgment is made by examination of data calculated using either a kilogram of fat-free whole body or 100 g. of fat-free dry solids as a standard of reference.

The rationale for the use of the latter standard has been discussed in detail elsewhere (Cotlove *et al.*, 1951). While such a reference point is essential for evaluation of acute shifts of water and electrolytes in tissues, it may not be equally applicable where the growth of a complex of tissues is involved. In this latter situation it is essential that the relative gain or loss of a substance in question be examined in regard to a number of reference standards, as has been done here (see **Results**). When the change in any constituent is consistent in direction, regardless of the reference basis, it is probably a real one, as has been noted above. However, when the change is in one direction on one basis and in the opposite on another, the question of gain or loss is difficult to assess. An example of this from the current data is found in the change in potassium content with change in protein intake in the animals on the low electrolyte diets. The high-protein-fed animals were larger and contained more potassium on an absolute basis. When calculated per kilogram of fat-free body the potassium concentrations were equal, but on a litre of water basis the potassium was greater in the low protein group. Again, referring this ion to fat-free dry solids, the high-protein-fed animals would seem to have the highest content. For the purposes of nutritional evaluation, it is valid to calculate constituents as per unit of whole body inclusive of fat. When this is done, an even greater number of permutations and combinations can be found with regard to relative contents of all substances. Until more is known concerning the distribution, function and relationships of protein and electrolytes in tissues, it would seem advisable to emphasize only those changes which are relatively consistent.

When the present data are considered on this basis, the composition of the body with regard to water, protein and ash is the same despite variation of the components of the intake. The whole body may be smaller or larger as limited by the availability of certain crucial nutriments but its relative composition remains unchanged. Only the relative size of the skeletal mass in relation to soft tissue seems to be significantly

susceptible to some variation by variation of dietary intake. Even in relation to skeletal tissue the possibility of variable composition is limited by another parameter, i.e. protein. Thus, the composition of the body achieves an independence from the environment, an independence that would seem essential in a living system where metabolic function is carried on by protein with its critical requirement for constancy of water and ionic concentration.

The concept that the whole body or the cells of the body may be enriched or depleted of their various chemical constituents by variation of the dietary intake is widely supported in the nutritional literature. By examination of retentions during balance observations on growing infants, it may be concluded that the higher the intake of a substance, the greater will be its final concentration in the body per unit of weight (Rominger and Meyer, 1927; Swanson and Iob, 1933; Stearns, 1939).

Correlation of weight gains of premature infants with the protein and ash content of the milk fed has shown high positive correlation with the increasing ash content (Kagan *et al.*, 1955). Conversely, possible support for the concept of variable body composition stems from nitrogen losses after trauma. Both animals and men maintained on low protein intakes lose less nitrogen after trauma than do those with prior optimal intakes (Munro and Cuthbertson, 1943; Cuthbertson, 1948). Holmes, Jones and Stanier (1954) found evidence indicating that men shifted from very low protein intakes to optimal intakes retained nitrogen far in excess of that calculated from weight gain and external losses. The use of the terms "depletion" and "deficiency" bears tacit evidence for the belief in the concept of cellular impoverishment during nutritional deprivation. The majority of the evidence for the concept of variable storage of protein and minerals and loss during deprivation stems from the technically hazardous techniques involving measurement of external balances. The possibility of low correlation between apparent retentions or losses and changes in body weight has not been

commonly realized. The shortcomings of the balance method are functions of such items as the effects of variable caloric intake, quality and quantity of protein intake and mineral ratios on the fat content of the body, the distribution of body water and the relative size of body components such as skeleton and muscle. These problems have been most completely explored in relation to evaluation of the problem of protein adequacy (Mitchell, 1944; Allison, 1954; Calloway and Spector, 1953; Spector and Calloway, 1953). It is also little appreciated that systematic errors occur in the calculation of apparent retentions that are cumulative in a positive direction, the magnitude of the cumulative error being in direct proportion to the magnitude of the intake. This makes difficult the comparison of retentions at variable intakes. The relevance of this criticism with regard to calcium retentions has been discussed by Mitchell and Curzon (1939) and by Mitchell and co-workers (1945).

Examination of the composition of growth increments by direct body analysis has shown that, once chemical maturity is reached, the composition of the fat-free body with regard to protein and ash is nearly constant, regardless of any procedures taken to modify weight gain (Moulton, 1923; Moulton, Trowbridge and Haigh, 1922; Pickens, Anderson and Smith, 1940). As determined by direct body analysis the body composition of rats growing on mineral-poor diets shows little change except for a deficit of calcium (Light *et al.*, 1934).

The concept of variable cellular composition of the body is difficult to reconcile with the knowledge of the composition of tissues. All of the individual tissues of the albino rat have been analysed for their water, protein, fat and mineral content by many investigators. All of these data show a monotonous constancy when calculated on a fat-free basis. This occurs despite almost infinite variation in the rations fed to the animals. Unless special experimental conditions are imposed, individual tissues seem to hold fast to their chemical composition. The principle variation occurs with age (Lowry *et al.*, 1942). At any given age composition is constant. Even

with age the maximum change of water content is no more than 1 per cent and of potassium 5 per cent.

Examination of whole body data, with certain salient exceptions, also shows rather remarkable constancy. Fat is probably the only component of the total body that can vary within rather wide limits and still allow reasonable well-being to exist. Variation from 10 to 50 per cent can occur without apparent evidence of malfunction. The water content of the fat-free body is more closely guarded. Variation of much more than $\pm$ 5 per cent from a rather rigid norm results in rapid increments of physiological disability. Moreover, allowable variation of body water is primarily extracellular; cellular water content, within the limits of viability, must be confined to much smaller variations. Since protein is the critical parameter against which water content must be judged, it follows that protein concentration must also be highly critical and susceptible to only minute variation. The consideration applying to water must also hold for the chief extracellular electrolytes, sodium and chloride. Deficit of potassium in the whole body to the extent of approximately 25 per cent does occur, and is replaced by variable gains of total body sodium (Schwartz, Cohen and Wallace, 1953; Cheek and West, 1956). The studies of Sherman and Booher (1931) show that the calcium content of the whole body is widely variable in response to variation in the dietary intake. Definition of the optimal body content of this ion is elusive.

In the discussion so far the point of view has been taken that in order to justify the terms *stored protein* or *mineral*, these must exist as physically demonstrable entities comparable to glycogen and fat in the body. It would appear that the essential organic structure of the body cannot be affected in quality by adjustment of the diet. The careful chemical analyses by Luck (1936) of rat liver proteins from animals maintained on varying levels of protein intake indicate that all fractions of the liver proteins have participated equally in any "storage" process. Madden and Whipple (1940) have defined the reserve store of protein as " . . . all of the protein

which may be given up by an organ or tissue under uniform conditions without interfering with organ or body functioning." This definition indicates primary physiological significance, not anatomical. In this view the primary requirement for furthering understanding would be methods for characterizing and distinguishing physiological depletion. The response to repletion has been used to assess the degree of depletion in such a physiological sense. The work of Madden and Whipple (1940) and Cannon (1954) illustrates the fruitfulness of the method for studying the metabolism of protein under conditions of deficit. Cooke and co-workers (1952) and Schwartz, Cohen and Wallace (1955) have applied the technique to experimental potassium deficiency and Hansen (1956) to the potassium deficit in kwashiorkor. The ability to survive in stressful situations provides a further avenue of approach. Baur and Filer (1957), employing the weanling pig growing on diets similar to those used in the present experiments, have shown differing abilities of animals growing on different diets to resist water and caloric deprivation. Their data indicate that animals maintained on low protein intakes survive caloric deprivation to a greater degree than do those maintained on high protein intakes. Conversely, the high-protein-fed animals withstand water deprivation to a greater degree than do their low-protein-fed companions. Sherman (1946) has correlated calcium intake with life span and reproductive life. A newly opened approach to the problem of characterizing and assessing deficits in a physiological sense is that of distinguishing structural versus enzyme protein in tissues. Potter and Klug (1947) have shown that liver octonoate and succinate oxidases are depressed in animals fed varying levels of protein. Miller (1948) Lightbody and Kleinman (1939) and Williams and Elvehjem (1949) have extended these observations to a number of other tissue enzymes.

Summary and Conclusions

The composition of growth of the albino rat on high protein-high electrolyte, on high protein-low electrolyte, on low

protein-high electrolyte and on low protein-low electrolyte diets has been examined. Analysis of the whole body for protein, water, fat, ash, sodium, potassium, chloride, calcium, phosphorus and magnesium was performed on animals allowed to double their weaning weights on the enumerated diets.

The animals on the low protein intakes grew significantly less and their bodies contained more fat. The composition of the fat-free bodies on a unit basis were all essentially similar despite the variation of the food intake. The principle difference resulting from variation in intake was in the quantity of the skeletal constituents in the various groups. The animals consuming the low protein rations contained more calcium and phosphorus on a unit basis than did the high-protein-fed animals.

On the high protein intakes accretion of skeletal minerals was dependent upon the level of electrolyte intake, being higher in the high-electrolyte-fed animals. In the low-protein-fed animals accretion of skeletal minerals was less affected by the level of electrolyte intake.

Only in the animals on the high electrolyte diets did increased protein intake result in increased protein content of the body.

The significance of the data for nutritional evaluation is discussed.

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DISCUSSION

Widdowson: May I suggest, Prof. Wallace, that you started your experiments far too late. If you had started at 21 "Adolph days" instead of 21 "Wallace days", you might possibly have got different results. We have the feeling that a great deal happens during these first three weeks of suckling and the whole subsequent growth and development of the rat depends upon the amount of milk it receives during that time. Rats suckled in litters of three weigh two to three times as much at weaning as others suckled in litters of 16–20. This difference in weight persists even though all the animals receive unlimited food from weaning onwards. The chemical maturation of the tissues of the body, particularly the skeletal muscle, is more rapid in the fast-growing rats.

Wallace: How are they different? Are they dilute?

Widdowson: The proportion of extracellular fluid in the bodies and muscles of all the rats decreases with development, and the proportion of intracellular constituents, nitrogen and potassium, rises, but the changes take place more quickly in the fast-growing animals, so that they reach chemical maturity at an earlier age.

Kennedy: We can say, too, that the general developmental history is altogether different. For example, puberty in the female rat, as measured by vaginal opening, is at 30–35 days in the big rat and it may be 60 days in the small rat. All subsequent growth is also quicker.

Wallace: What happens if the smaller young rats are specially fed?

Kennedy: This experiment was first done by Parkes (1926 and 1929. *Ann. appl. Biol.*, **13**, 374, and **14**, 171). He did fantastic things like suckling mice with rat foster-mothers and getting them up within 21 days to something like 75 per cent of an adult mouse's weight. I went over this again, breaking the changes down week by week (1957. *J. Endocrin.*, **16**, 9). I found the acceleration in growth rate due to an unlimited milk supply was achieved almost entirely in the first week of life. The difference between birth weight and the weight at the end of one week might be fourfold; after that there was roughly a 50–60 per cent increase per week and this went on after weaning, when food was unlimited. Something happened within the early part of the suckling period which determined the shape of the subsequent exponential growth curve, and I think that one of the things was probably the development of appetite regulation. The amount the animal ate became fixed in relation to body weight, so naturally the bigger rat ate more and continued to grow faster.

Wallace: Can you change them by feeding them different diets?

Kennedy: After weaning this has no effect. I have increased the concentration of protein in our stock diet, which is usually 13 per cent, to as high as 30 per cent, which is about what rat milk contains, without significantly changing the growth rates of the large or the small weanlings. We have not tried to change the diet of sucklings.

Widdowson: It would be most interesting to give some rats in a litter electrolyte and protein supplements by stomach tube from the day of birth onwards, and allow the mother to suckle the whole litter so that some would get a higher protein and electrolyte intake than others. Analysis of the bodies at three weeks of age might show much bigger differences than those reported by Prof. Wallace for his older rats.

Talbot: When you give a high as contrasted to a low protein intake, how much protein do you give the rats per day relative to their absolute growth increment?

Wallace: I suppose that you are referring to the question of "feed efficiency"—the relation of grams of food consumed to grams of weight gained. This was $1 \cdot 81$ g. food per gram gain of weight for the high-protein-fed animals and $2 \cdot 51$ g. consumed per g. of gain for the low protein group. Thus the low protein group were less efficient in this regard. If gain of weight per gram of protein consumed is calculated the values are $0 \cdot 41$ g. per g. gain and $0 \cdot 30$ g. per g. gain for the high and low protein groups respectively. The high protein animals, however, have a greater gain of protein per unit of weight gain.

Kennedy: In the two curves you showed us with 100 per cent difference in concentration of protein, there was nothing like 100 per cent difference in growth. Therefore it seems to me that the feed efficiency must have been in favour of the low protein diet.

Wallace: One of our reasons for doing this type of experiment was to find out whether or not we could rely on balance measurements to measure the composition of growth. I think that the answer is a negative one. Except for change in body fat content, the composition of the body of the growing individual remains relatively constant over the periods in which it is feasible to carry out such measurements. There are probably extreme experimental conditions which do change body composition but I do not believe that one can change lean body composition significantly by changing the plane of protein intake. One can probably determine more accurately the composition of growth by dilution techniques than by the balance method.

McCance: What would be the effect of change in diet on electrolytes in the body? Our conclusion at the moment is that it has little effect on the composition of the cell.

Wallace: We cannot change the electrolytes in the cell; we can only change the amount in bone. Muscle can be made to grow faster or bigger, but its composition in terms of electrolytes cannot be altered.

Heller: Our experience is that you have to decrease the protein content of the diet very considerably to produce changes in body composition. We have recently been feeding weanling rats on cassava flour and African plantains, that is to say on diets that produce kwashiorkor in infants.

After about four weeks there was an increase of 5–7 per cent in total body water, but the interesting thing is that the plasma potassium and plasma sodium concentrations remained unchanged.

Milne: Prof. Wallace, the main change in calcium with these diets was in the skeletal calcium. Have you any information on changes in soft tissue calcium, particularly kidney calcium? In my experience it varies tremendously in rats on different calcium diets.

Wallace: The calcium in the body is almost entirely skeletal and with this kind of data it is impossible to say just where this calcium is. You have to study the individual tissues.

Fourman: Do you think that the increase in bone which you suggested took place is an increase in trabecular bone—so-called freely available, mobilizable, bone tissue?

Wallace: We are not certain but think it is probably both cortical and trabecular. We would like to know if the large animals on the high electrolyte intakes have more easily mobilizable bone tissue under conditions of stress.

McCance: You began by putting up charts of balances showing that if the diet contained more sodium and potassium, the child absorbed and retained more. Yet you find by experiment that you do not alter the composition of the body. Can you reconcile those observations?

Wallace: This is a purely technical matter on which I have strong feelings. In a balance experiment the quantity of food entering the body and the excreta recovered are always slightly less than the measurements indicate. The more refined the technique the smaller this error is. Also, the greater the concentration of a nutriment in the intake the greater will be the error when compared with intakes of lower concentration but of equivalent caloric value. When subtraction is used to calculate the balance these errors accumulate. The errors in doing a balance are not randomly plus or minus as is generally believed, but systematically positive. Much of the arithmetical difficulty arises because one must subtract two quite large numbers to obtain the usually very small balance value. At zero intake the balance method becomes more accurate. Body composition estimates such as can be made from Benedict's and Gamble's fasting data agree with direct analysis data quite well. However, body composition estimates made from balance data with infants fed with cow's milk and human milk are always widely divergent, even when weight gains are equivalent. The higher the intake of a constituent the greater the apparent retention. Eventually the retention becomes patently absurd.

THE EFFECT OF AGE ON THE BODY'S TOLERANCE FOR FASTING, THIRSTING AND FOR OVERLOADING WITH WATER AND CERTAIN ELECTROLYTES *

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As is well known, the body is equipped with homeostatic systems designed to maintain water and electrolyte content and concentration values at physiologically optimal levels. The systems accomplish this task largely by equating output with input. While rates of input can be varied widely without overreaching the capacities of the homeostatic systems concerned, nonetheless there are limits beyond which one cannot go without getting into difficulty (Talbot, Crawford and Butler, 1953; Talbot *et al.*, 1955). Thus for each substance there is a *physiological minimum requirement* or *floor*, which is the least intake of the substance in question needed to balance output and hence to prevent deficits where conservation forces are acting maximally. There is also for each substance a *physiological maximum tolerance* or *ceiling* which is defined as the largest amount of the substance that can be taken and eliminated without seriously disturbing body composition. Rates falling between these two parameters may be said to fall within the *physiological* or *safe working range*. When the rate of administration of a substance falls outside this range for an appreciable length of time, body composition deviates from normal and manifestations of disordered homeostasis develop as outlined in Table I.

The manner in which a limit to homeostatic capacity can be recognized and defined is illustrated in Fig. 1 (Talbot *et al.*, 1956). Here it can be seen that this patient maintained a normal potassium status, as judged from electrocardiographic T waves and from serum potassium concentration, and remained in potassium balance at rates of intake up to approximately 70 m-equiv. per m.² per day. These rates of

Table I

INDICATIONS THAT INTAKE IS PHYSIOLOGICALLY EXCESSIVE OR INSUFFICIENT
(ADULT VALUES)

Sub-stance	Too Much	Too Little
H_2O	Water intoxication Serum water $>3\cdot8$ ml./m-osm.*	Hypohydration Serum water $<3\cdot4$ ml./m-osm.*
Na	Extracellular oedema $Na_E \uparrow > 20\%$	Extracellular dehydration $Na_E \downarrow > 12\%$
K	Weakness; ECG T waves $\uparrow$; Serum K $>6\cdot5$ m-equiv./l.	Weakness; ECG T waves $\downarrow$; $K_I \downarrow >20\%$
P	Serum P >6 mg.%	Osteomalacia

Na_E = extracellular sodium. K_I = intracellular potassium.
* Corrected for urea.

intake could therefore be considered to be within his safe working range. By contrast, higher rates of intake led to a sustained positive balance and to the appearance of elevated T waves and hyperkalaemia, which are taken to be signs of potassium intoxication. Accordingly, it may be said that this individual's ceiling of tolerance for potassium was about 70 m-equiv. per m.² per 24 hours, a subnormally low value in comparison with a normal ceiling of at least 250 m-equiv. per m.² and in keeping with the fact that he was suffering from marked impairment of renal function.

The same principles have been used in estimating the upper and lower limits of body tolerance for water and certain electrolytes for normal individuals of various ages, depicted in Fig. 2. The upper limits shown in this figure are of necessity approximate, being based on the relatively few data available in the literature and the files of our metabolic unit (Talbot *et al.*, 1952; Talbot, Crawford and Butler, 1953; Talbot *et al.*,
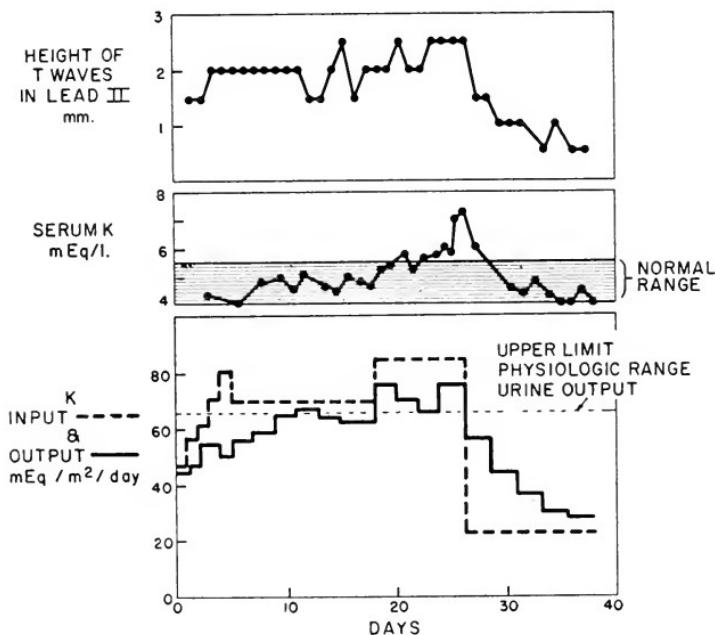
FIG. 1. Demonstration of physiological maximum tolerance for potassium in a patient with impaired kidneys. (From Talbot *et al.*, 1956).

1955, 1956; Talbot, Richie and Crawford, 1958). In all instances, they are intended to represent levels which can be attained by healthy individuals within a day or so and not the uttermost levels which can be attained after extensive prior conditioning. The lower limits include normal growth requirements for infants and children, a factor of relatively small size after the first few months of life. It can be seen that with the exception of young infants, individuals normally

utilize but a small segment of their homeostatic capacities. In early infancy, the margins of safety are relatively quite narrow, a fact long recognized by those interested in paediatrics.

The clinical significance of these homeostatic parameters
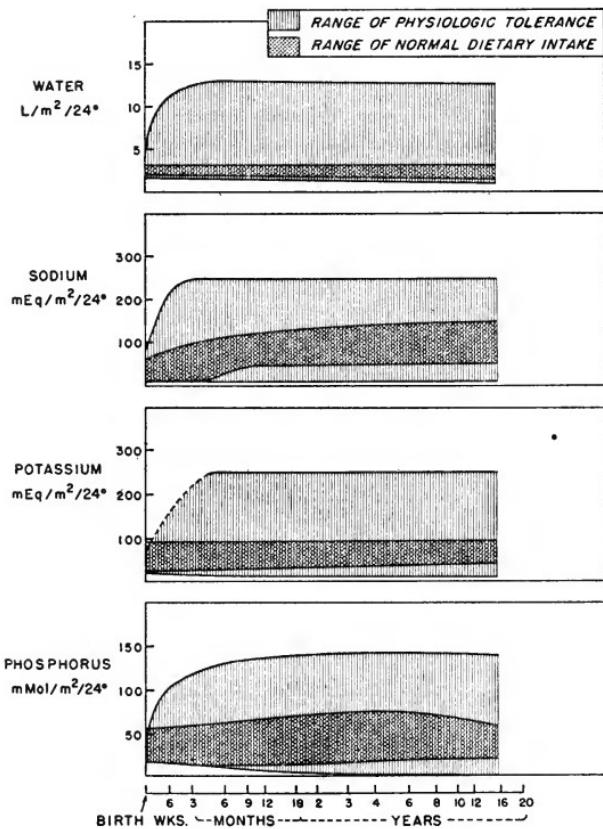
Fig. 2. Estimates of the safe working ranges of intake for individuals of various ages and of the portions of these ranges used by persons taking ordinary diets for age.

may be visualized by considering the length of time needed for individuals of various ages to lose a significant portion of their body stores when totally deprived of water or certain other substances (Fig. 3). In calculating these time values, attention has been given to the changes in body composition

which occur during the growth period; in each case average normal values for body composition and content were used (Shohl, 1939; Forbes and Perley, 1951; Corsa *et al.*, 1956; Friis-Hansen, 1957). Each substance has been considered separately. In dealing with water, sodium and potassium,
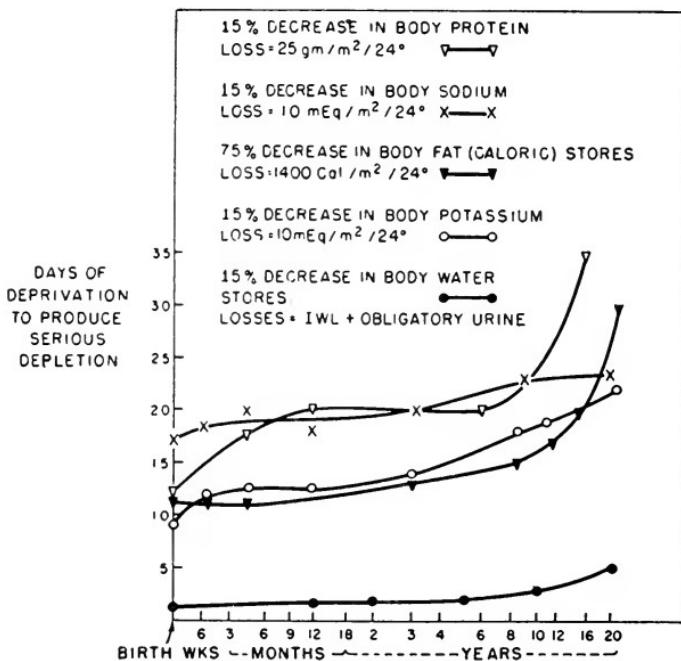
FIG. 3. Days of deprivation (ordinate) needed to produce the percentage decrease in body content indicated for each substance in individuals of various ages (abscissa). The rates of loss indicated for each substance approximate to physiological minimum output rates, of which some are indicated by the lower boundaries of the physiological tolerance ranges shown in Fig. 2.

rate of loss was taken as the physiological minimum requirement value indicated in Fig. 2. In considering body fat (calorie) stores, energy expenditures were assumed to be at the rate of 1,800 calories per m.² per day (Macy, 1942) and to be derived entirely from body fat. Body protein losses were calculated assuming a basal rate of loss amounting to 25 g. per m.² per day, the minimum value attained by individuals

receiving at least 75 grams of carbohydrate per m.^{2} per day (Gamble, 1946–7). It was arbitrarily decided that a 15 per cent decrease in body water, sodium, potassium or protein or a 75 per cent depletion of body fat (calorie) stores constituted a significant and potentially serious loss.

As indicated by the upward trend from left to right of the curves of Fig. 3, infants and children up to three years of age, when deprived of any one of the substances represented, are apt to become depleted two to four times faster than adults. For example, infants will develop as serious a degree of water depletion within one and a half days as adults do in the course of about five days of total thirsting. Likewise, infants deprived of electrolytes or protein or calories may lose an appreciable portion of their body stores of these items after nine to 17 days of deprivation.* By contrast, it takes 20 to 35 days for adults to become similarly depleted under conditions where homeostatic conservation forces are operating efficiently. These observations indicate that in infants who must be maintained by parenteral fluid therapy for more than a few days, special attention should be given to the provision not only of water, carbohydrate and the main extracellular and intracellular electrolytes, but also of maintenance allotments of calories and either preformed protein or amino acids. The same would apply to older children and adults who are depleted or have to be sustained by parenteral fluid therapy for more than a week or ten days.

Fig. 4 deals with the opposite phenomenon of overloading. Here again it has been necessary to make arbitrary decisions concerning the size of the overload and the degree of retention to be considered significant. It was decided to postulate rates of input that were ten per cent in excess of *adult* physiological maximum tolerance or ceiling values. The end-point values for the retentions of toxic degree resulting from these physiologically excessive rates are related to the respective average normal body content values at each age as follows: total body

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* The rate of loss would be considerably greater under conditions of zero carbohydrate intake (Gamble, 1946–7).

water, $+7$ per cent (Wynn, 1956); potassium, $+5$ per cent (Drescher *et al.*, 1958); total body sodium (euproteinaemic subjects), $+30$ per cent (Leaf, personal communication). In the case of phosphorus the end-point chosen was elevation of extracellular inorganic phosphorus concentration to 12 mg.
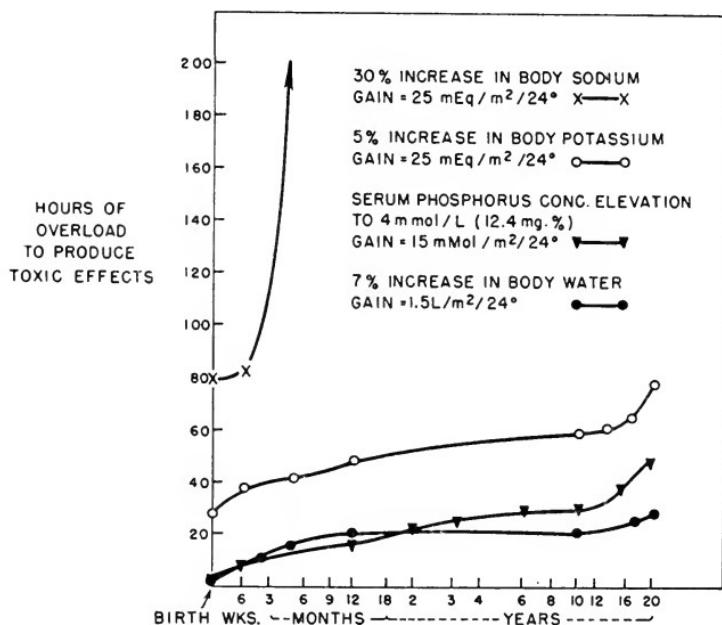
FIG. 4. Hours of overload (ordinate) needed to produce the percentage increase in body content indicated for each substance in individuals of various ages (abscissa). The rate of gain is that which obtains when rate of input exceeds the physiological maximum tolerance levels for adults shown in Fig. 2 by approximately ten per cent.

per cent.* Individuals who have surpluses of these degrees are apt to show the signs of intoxication listed in Table I.

As might be expected, Fig. 4 indicates that infants are relatively much more vulnerable to overloading than older children and adults. This is true not only in the relative terms depicted here, but also in absolute terms because the quantity needed to produce intoxication in a small individual

* This assumes no bodily capacity for cellular or skeletal storage of surplus inorganic phosphorus, a point on which we have no objective information.

is not very great. The curves indicate that one is apt to become water and phosphorus intoxicated before one becomes potassium or sodium intoxicated. It is interesting that these relations are in keeping with clinical observations on patients with marked limitation of renal function (Talbot *et al.*, 1956).

One of the areas where the foregoing considerations appear to have practical implications is with respect to parenteral fluid maintenance therapy. Review of hospital practices
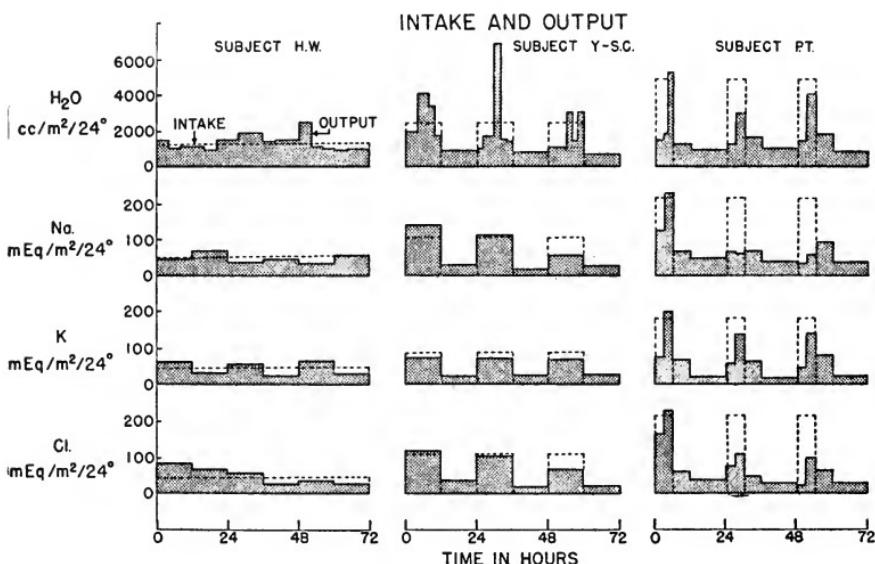
Fig. 5. Intake and output of water and electrolytes by normal adult subjects receiving a standard maintenance allotment of multiple electrolyte plus dextrose solution in 24, 12 or 6 hours each day. (From Neyzi, Bailey and Talbot, 1958).

reveals that some physicians give the total daily fluid, carbohydrate and electrolyte allotment in a slow continuous manner while others administer the total daily dose in a few hours, allowing the patient to fast and thirst for the remainder of the 24-hour period. The data shown in the right-hand sections of Fig. 5 (Neyzi, Bailey and Talbot, 1958) indicate the ranges of output rate observed on two sets of three normal adults maintained for three days on an ordinary dose (1,200 ml. per m.^{2} per day) of a solution containing, per litre, 50 g. of

dextrose, 40 m-equiv. of sodium, 35 m-equiv. of potassium, 40 m-equiv. of chloride, 20 m-equiv. of lactate and 15 m-equiv. of phosphate (Talbot, Crawford and Butler, 1953; Talbot *et al.*, 1955). The first set of subjects received their allotment by mouth in an essentially continuous (hourly dose) manner, the
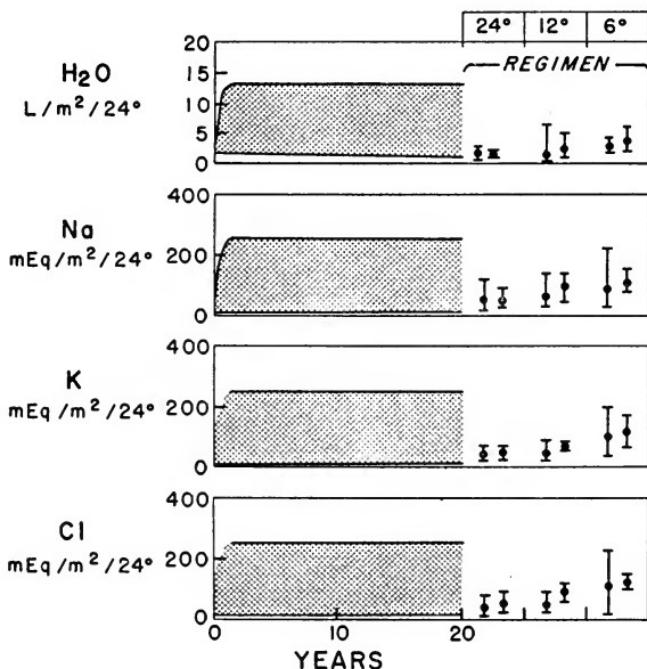
Fig. 6. Relations between rates of output observed for subjects on various regimens shown in Fig. 5 (right-hand section) and physiological ranges of excretory capacity shown in Fig. 2 (left-hand section). The solid black circles indicate the average and the vertical bars traversing them the ranges in output rate noted for the individual subjects per the scales along the left-hand ordinate. (From Neyzi, Bailey and Talbot, 1958).

second set at twice the rate for 12 hours each day and the third set at quadruple the rate for six hours out of every 24. As indicated by the length of the vertical lines at the right of Fig. 6, those on the 24-hour regimen utilized but a small fraction of their physiological ranges of excretory capacity in accomplishing metabolic homeostasis. By contrast, those on

the 12-hour and especially those on the six-hour regimens used almost fully their normal adult ranges of renal excretory adjustment in the course of each 24-hour period. When the homeostatic adjustments in water and electrolyte excretion exhibited by these adult subjects are viewed with relation to the infant ranges of homeostatic adjustment indicated by the shaded zones of the left-hand sections of Fig. 5, it can be seen
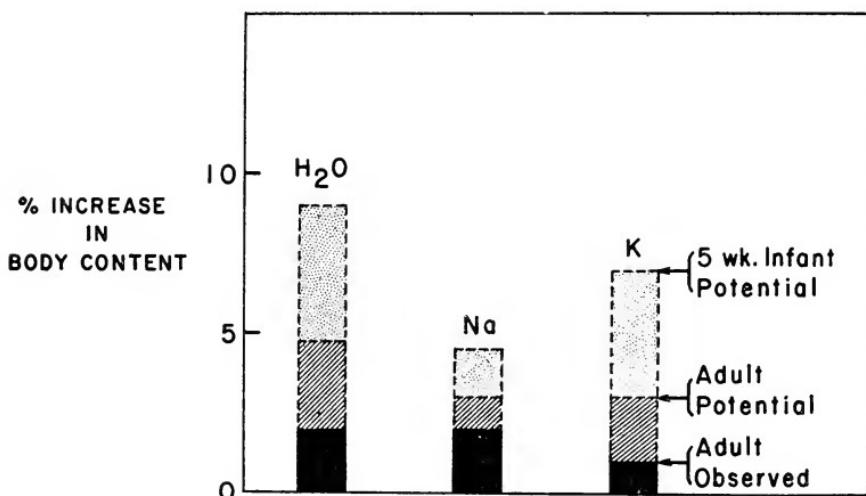
Fig. 7. Percentage increases in body water, sodium and potassium content (a) which actually occurred (black sections) during the 6-hour infusion period in the 6-hour regimen subjects of Figs. 5 and 6; (b) which would have occurred in these adults (adult potential levels), and (c) which would have occurred in a small infant (5-week infant potential), had no homeostatic increase in output rates above basal levels occurred.

that they are considerably greater than those of which such young individuals are capable.

Fig. 7 depicts the percentage increases in body water, sodium and potassium content which would occur during the course of the infusion period if a day's total maintenance allotment of 1,500 ml. per m.^{2} per 24 hours * were administered in six hours to a patient who was unable to increase rates of urinary output above the physiologically low levels characteristic of fasting and thirsting, a situation which one

* This is an ordinary allotment for infants and children on our Service.

may encounter in young infants and in patients undergoing the stress of anaesthesia and surgery. As the columns show, the percentage gains to be expected for infants are approximately twice as great as those to be expected for adults. While the gains indicated for adults are borderline as regards toxicity, those shown for infants are large enough to produce distressing manifestations.

In summary, an attempt has been made to indicate in approximate terms the limits of capacity of the body to adjust output of water and certain other substances in accordance with homeostatic needs, and to illustrate the clinical implications of such knowledge.

These thoughts are presented in the hope that they may elicit constructive suggestions concerning these highly significant, yet rather elusive phenomena.

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DISCUSSION

Black: There seems to be some conflict between Dr. Talbot, who says that large intakes should produce retention, and Prof. Wallace, who tells us that large intakes produce large arithmetical errors. In this matter I am on Dr. Talbot's side, and that is not entirely the emotional reaction of someone who has done a certain amount of balance experiments. I think we have some supporting evidence in that if balance experiments are done on an adult person who has just had an operation and is on a milk intake (in which the errors of measurement should be much the same as those of excreta), there is quite a definite correlation between intake and retention (Davies, H. E. F., Jepson, R. P., and Black, D. A. K. (1956). *Clin. Sci.*, **15**, 61).

Bull: What was the nature of the load imposed in the experiments on the tolerance of loading?

Talbot: The rate of intake of the substances in question is increased in a stepwise manner which allows time for compensatory homeostatic adjustment in rate of output to take place. At each step, measurements are made to find out whether the body content and/or concentration of the substance is being kept within physiological limits by appropriate adjustments of the rate of output. As rate of input is increased, it eventually reaches a point where the body is unable to keep its content and concentration values within normal limits by suitable adjustment of rate of output. This point is considered to be the upper limit of physiological tolerance or physiological ceiling for the substance in question. Rates of input in excess of this ceiling level produce a tendency to abnormal retention. For example, in the case of potassium, when the rate of input exceeds the physiological ceiling value, body potassium content increases above normals levels and hyperkalaemia develops, together with signs of potassium intoxication.

McCance: I would like a firm definition of what you mean by tolerance and capacity to eliminate. De Wardener did some experiments in which he took large amounts of water every day for 7 or 14 days and although he did not succumb and appeared to tolerate them perfectly well, there were finite changes in his responses, sensitivities, etc. (de Wardener, H. E., and Herscheimer, A. (1957). *J. Physiol.*, **139**, 42 and 53).

Talbot: In the case of water, the body normally can tolerate up to approximately 15 litres per square metre or about 25 litres per adult per day. These large quantities are eliminated simply by increasing the ratio of water to solutes in urine to levels of 20 to 30 ml. per m-osm. It is difficult to exceed this ceiling value in the normal individual. On the other hand, it is easy to exceed the water tolerance ceiling value in pan-nephritics and postoperative patients who are unable to increase the water/solute ratio of their urine above a few ml. per m-osm. and whose rate of solute output may be low. Such individuals may be unable to take more than 2 or 3 litres of water per square metre per 24 hours without retaining water and developing water intoxication.

Kennedy: Some of these substances were orally administered, and some

parenterally. It is said that one of the safeguards in oral ingestion of water is the fact that elimination goes on about as fast as absorption.

Talbot: As far as water, sodium and potassium are concerned, it is six one way and half-a-dozen the other whether they are taken by vein or by mouth. With phosphorus, where calcium and other substances may carry it out in the gut, there may be some large differences.

Bull: I believe there is a speed of infusion beyond which this theory is not correct. If, for instance, very frequent samples of blood are taken during transfusion, when a solution which is not isotonic is being given, very high values may be found. I agree that if the balance studies are taken for 24 hours, the result will be the same. But you can reach values acutely which are well outside what you consider to be the normal range, though fortunately without apparent ill effects. The picture of homeostasis varies very markedly with the period over which you are considering it. My colleague Dr. Graber finds that if you go back to the finer detail you may pick up oscillations in values which reveal the mechanism more clearly than do the long-term studies.

Talbot: Rates of input which are expressed per square metre per day mean are intended to represent the average rate of input throughout the 24-hour period. In other words, the fact that one may take as much as 15 litres of water per m.[2] per day does not mean that one could tolerate this volume if it were given in a fraction of the day. Indeed, were one to give the 15 litres in 12 rather than 24 hours, one would be giving it at the rate of 2×15 or 30 litres per m.[2] per 24 hours. Such a very high rate of input would produce signs of intoxication only if it were sustained for a sufficient length of time. Thus, 30 litres per m.[2] per 24 hours would be 30 divided by 24, or $1 \cdot 3$ litres per m.[2] per hour. One would have to infuse water at this rate for at least 70 minutes to produce the 5 per cent gain in body water necessary to induce overt signs of water intoxication.

Another factor which enters into such consideration is adaptation time. Some of the body's homeostatic mechanisms, such as those concerned with water, potassium and sugar, can adapt quite fully within two or three hours, while others, such as those responsible for phosphorus and sodium homeostasis, may require two or more days. In considering the ceiling and floor levels reported here, an effort was made to take this variable into account and to set forth ceiling and floor levels which the normal individual should be able to attain without becoming seriously disturbed metabolically either during the period of adaptation or later.

While it may be possible to set up experimental circumstances in which there are differences in the body's tolerance for water and the various electrolytes when given intravenously as compared to orally, for all ordinary practical purposes the body's tolerance for these substances is about the same whether they be given by mouth or by vein.

Fourman: Is it not true to say that with an excessive intake of water the individual will vomit, and with excessive intake of potassium the individual will, extraordinarily promptly, get diarrhoea?

Talbot: It is true that loss of thirst and nausea constitute accessory mechanisms which serve to protect the organism against the development of water intoxication by the oral route. On the other hand, we have

observed that rats offered gradually increasing quantities of potassium in their diet ate and absorbed the relatively very large quantities needed to produce a lethal degree of potassium intoxication. They did not develop diarrhoea, nor did they vomit; they just became weak and died. Likewise, we have seen a patient with marked limitation in tolerance for potassium due to advanced pan-nephritis become fatally intoxicated with potassium as a result of drinking fruit juices.

Adolph: The study of tolerances is a very important aspect of the general physiology of regulatory processes. Dr. Talbot, you estimated tolerances in terms of single constituents, but in some of the situations you described, such as the intravenous administrations, you were concerned with several constituents at a time. Now when there is depletion or excess of more than one constituent at a time the picture is very different with respect to tolerance. For instance, there is a great difference between taking pure salt and taking an isotonic solution of salt. I recognize that this work is exploratory and that you are making your estimates in the simplest way possible when you consider one component at a time, but eventually I hope we shall have some estimates of tolerance to multiple components.

This consideration of components seems to me to extend also to your studies of composition, Prof. Wallace. If you went to your statisticians still more often, would you not get into the study of multiple correlations which would get us further than comparisons made two at a time?

Wallace: We have made a number of statistical multiple correlations. It is often difficult to know just what they mean, once certain correlations become evident. Our biggest problem has been to have any assurance as to the proper parameter to which to refer growth. Should the reference basis be body weight, fat-free weight, protein, ash, or water?

Adolph: What I want to bring out is that an organism probably has some way of measuring the bodily composition which is very much more complicated than saying, for instance, that magnesium is the fixed constituent around which all others revolve. I think that without a study of multiple correlations we will never be able to find whether there is a key fixity by which homeostasis is guided to a definite volume and concentration to which the organism always returns. I do not know whether any of our methods of representing homeostasis will be so similar to that of the organism that we can predict what it does to get back to its fixity.

I should also like to remark on Dr. Talbot's choice of a key variable. No doubt he has great reservations about the use of this term. What he is trying to do, I gather, is to out-guess the organism as to what it is using as a measuring stick by which it will return to its original composition, or by which it will estimate what has to be done in order to defend itself against disturbances. When we think that an organism is restoring its potassium concentration, have we any assurance that that one restoration is a prime objective in the adjustments which are going on?

Talbot: We agree with you that most if not all of the variables under consideration are related to each other. For instance it is known that body tolerance for potassium is impaired under conditions of zero sodium intake and that tolerance for sodium is abnormally limited under condi-

tions of zero potassium intake. On the other hand, it was thought that a thorough exposition of available information on these relations at this time would serve only to confuse the picture without adding greatly to its significance. Certainly one cannot take and eliminate large loads of electrolyte without an ample supply of water etc. Accordingly, it was decided to define physiological maximum tolerance and minimum requirement levels for each substance under circumstances where the influence of these types of factors should be minimal, i.e. under conditions where the rates of intake of substances other than the one under consideration were well within normal limits. Should these preliminary definitions prove to be of value, it may become worthwhile to undertake to extend and refine them more by detailed definitions of certain of the most important interrelations.

You are correct in your deductions concerning our aims in defining physiological key variables. The present definitions are of necessity approximate and potentially subject to modification and refinement. At the same time they are proving to be of value as indices of patient status and as a point of departure for investigation.

CLINICAL CONSEQUENCES OF THE WATER AND ELECTROLYTE METABOLISM PECULIAR TO INFANCY

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Disturbances in the volume and composition of the body fluids occur more frequently in infancy than at other ages. Among the reasons for this are:

(1) The high incidence of diarrhoea, malnutrition, and certain congenital defects.

Diarrhoea is still one of the paediatrician's major concerns, one of its main causes being colon bacilli, pathogenic only for this age group.

Owing to their high caloric and protein requirements infants easily succumb to malnutrition, which progresses rapidly. The resulting expansion of the volume of their extracellular body fluids, sometimes accompanied by asymptomatic hyponatraemia, is a common disturbance of homeostasis in some countries.

Congenital defects of the oesophagus, the pylorus, the renal tubules, the adrenals, and the central nervous system may also cause serious disturbances in the body fluids; their discussion is beyond the scope of this paper.

(2) Circulation, metabolism and renal excretion are all maintained at high levels relative to the volume of the body fluids.

(3) When growth is arrested by disturbances which diminish the utilization of food, a fraction of the intake normally retained is rejected, thus raising the solute load on the kidneys.

* In the absence of Prof. Kerpel-Fronius, his paper was read for him by Dr. Winifred Young.

(4) Partly due to the interrelationships (2) and (3) kidney function is readily impaired by stress.

Thus the high incidence of body fluid disturbances is partly due to the occurrence of disease and partly to relatively inefficient homeostatic defence mechanisms. The latter is well
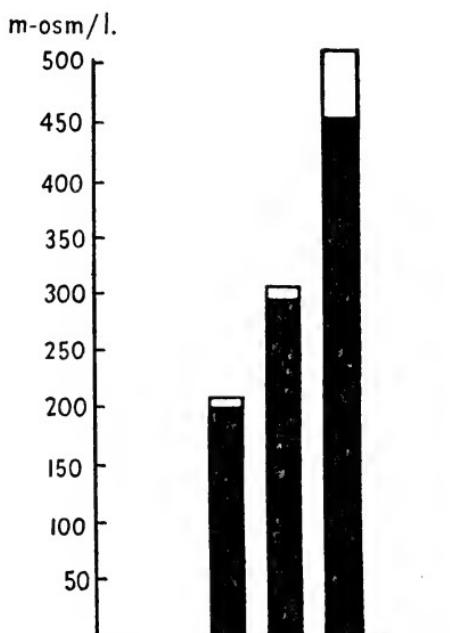
Fig. 1. Lability of osmotic regulation in 10-day-old puppies.

Left column	:	salt- and protein-free diet
Central column	:	normal
Right column	:	concentrated milk

illustrated by the observation that diets such as milk evaporated to one-quarter of its original volume, or salt- and protein-free food, bring about great changes in the tonicity of the body fluids (Csapó and Kerpel-Fronius, 1933; Kerpel-Fronius, 1933). After the first, the osmolarity of the blood plasma in puppies rose to 526 m-osm./l., 457 m-osm. being accounted for by "hyperelectrolytaemia"; after the second, the electrolytes decreased to 232 m-osm./l. (Fig. 1). There

was a water loss of over 20 per cent of body weight in the
first case, while in the second an increase in the water content
of all organs was observed. Such gross disturbances of
homeostasis may partly be due to the fact that although the
extracellular body fluids occupy a relatively high percentage
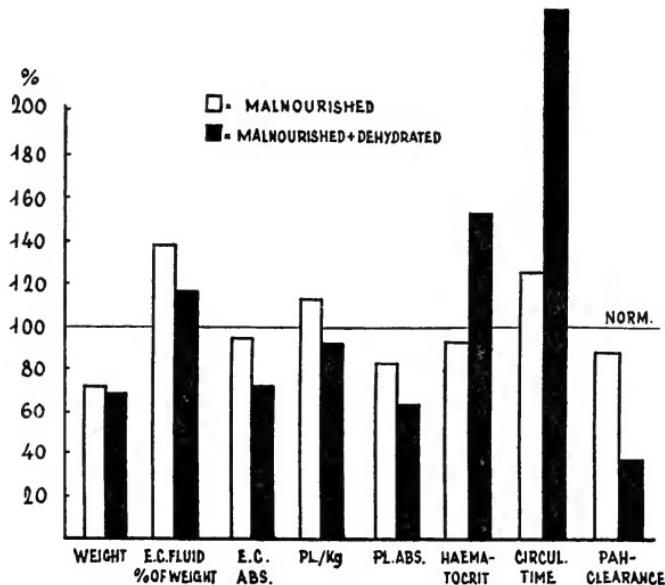
Fig. 2. Extracellular fluids, circulation and PAH clearance
in the dehydration of a malnourished infant.

Values are represented as percentages of those found in
normal infants of the same age. The horizontal line indi-
cates the normal values (100 per cent); the distance of the
top of each column from the normal line shows percentage
deviations.

White column   :   before diarrhoea
Black column   :   after diarrhoea
E.C. — extracellular; PL. — Plasma.

of the body weight, the water reserves in infants are low in
relation to the functions they may be called upon to perform.

In order to reconcile this apparent contradiction, it is
helpful to consider the relationship of body fluid reserves to
circulation and kidney function in malnourished infants.
Malnutrition does not affect all systems of the body equally,
fat and muscle sustaining greater losses than the extracellular

fluid compartment. Hence the size of the latter appears to increase with the progress of malnutrition (Kerpel-Fronius and Kovách, 1948; McCance, 1951; Keys *et al.*, 1950). Haemodynamically, however, it is not the amount relative to body weight but the absolute amount of extracellular fluid which is of importance. Fig. 2 illustrates a striking example of a case studied in comparison with well nourished infants of the same length, first in a state of malnutrition and later after dehydration due to diarrhoea had supervened. In the malnourished infant the volume of the extracellular fluid showed a percentage increase before and even after diarrhoea. However, the "absolute amounts", i.e. the fluid volumes calculated as percentages of those in normally nourished infants of the same length, were decreased. Since the haematocrit readings were high, the circulation time prolonged, and the renal clearances low, high water reserves calculated as a percentage of the body weight were clearly insufficient to maintain circulation and kidney function. The absolute volume of the water reserves, and not just the amounts proportional to the body weight, must be maintained in order to conserve a normal circulation and good renal function.

Let us now consider the normal infant. When compared with the adult, his extracellular water reserves—although high in terms of percentage of body weight—are strikingly low in relation to other physiological needs, namely oxygen consumption, insensible perspiration and cardiac output (Fig. 3).

Thus when compared on the basis of *body surface*, the infant appears to have the same oxygen consumption and cardiac output as the adult, but his systolic output (stroke volume) and plasma volumes are only half those of the adult; in order to achieve the requisite cardiac output with a relatively low plasma volume, the pulse rate is double that of the adult. His inulin and p-aminohippuric acid (PAH) clearance values are low in comparison with those of the adult and also in relation to his own cardiac output and metabolism. All his fluid compartments are strikingly low in proportion to metabolism, insensible perspiration and cardiac output.

Alternatively, on the basis of *body weight*, the infant's metabolism, dermal loss of water and cardiac output appear to be very high in relation to his total body water and plasma volume, which occupy approximately the same space as in the
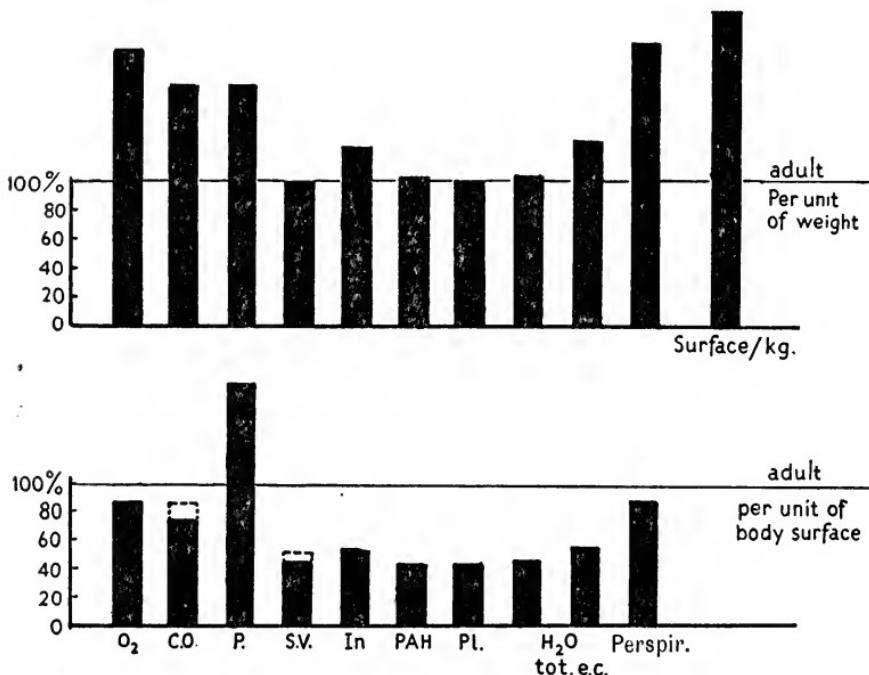
FIG. 3. Haemodynamics, fluid spaces and renal function of the infant as percentages of values for the adult.

The data represent mean values for five infants aged 4 months, with body weights of 5·5 kg., lengths of 61 cm. and surface areas of 0·30 m.². The basis of comparison in the upper part of the figure is the unit of body weight, in the lower one that of body surface. The horizontal line, 100 per cent, shows the normal values for adults, the height of each column giving the percentage differences between adults and infants.

C.O. — cardiac output; P. — pulse rate; S.V. — systolic volume;
In. — inulin; Pl. — plasma; e.c. — extracellular.

adult. This relationship holds true also for the extracellular fluid volume, although this is higher than in the adult. Renal clearances are proportional to fluid volumes and therefore low in relation to circulatory and metabolic rates.

Despite the marked differences between adults and infants in some of the physiological constants which have been mentioned, these functions are certainly nicely adjusted to each other even in the infant, and his defence mechanisms are fully capable of meeting the normal demands upon them. When put under stress, however, the fragility of the whole system which maintains body fluid homeostasis is exposed.
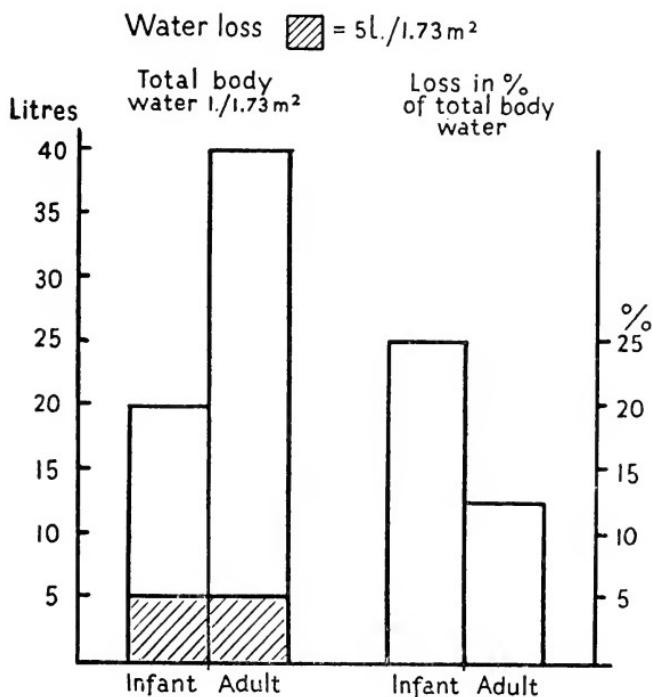
Fig. 4. Significance of "equal" losses when expressed per unit of body surface.

Under pathological conditions the consequences of the peculiar interrelationship of these functions are as follows:

(a) Water or salt loads calculated according to surface area will, in relation to total body water content, be double the values of the adult. The same holds true for loss of water, equal losses per unit of surface area being twice as high in the infant in proportion to the body water (Fig. 4).

(b) Water deprivation quickly exhausts the water reserves which are low in relation to metabolism and, consequently, to obligatory urine volume and dermal loss of water.

(c) Because of the high cardiac output required for metabolic processes, and the low reserves of water to guarantee its maintenance, circulation is endangered by even smaller water deficits, the more so since water losses occur rapidly. It will be remembered that the small plasma volume of the infant relative to the cardiac output is compensated for by a high pulse rate to ensure adequate circulation.

(d) The vulnerability of the circulation facilitates a rapid decrease in renal clearances, which even in the healthy infant are low in relation to his high metabolic rate. Obviously, the infant's rather poor renal blood flow is adjusted to, and only maintained by a relatively high cardiac output. The renal fraction has been calculated to be 10 per cent of the total output of the heart in infants whereas it is 20 per cent in adults.

As pointed out by McCance and Widdowson (1957) stagnation of growth plays a rôle in the easily disturbed equilibrium. In a growing animal a certain amount of the food goes to the building of its tissues. If growth is arrested, an additional solute load formed by this fraction of the intake presents itself for excretion by the kidneys. This will result either in a higher urine volume, or, if the kidneys are incompetent, in hyperelectrolytaemia and azotaemia. McCance and Widdowson (1957) have shown that these effects are striking in fast-growing animals and may under certain circumstances be of importance to the human infant. On the basis of some of the data compiled by the American Academy of Pediatrics (1957) an estimate has been made of the effect of arrested growth on solute load and renal water expenditure. Solute load may be expected to rise 13 per cent in the infant who is fed on cow's milk, and 57 per cent in the breastfed child, causing a considerable increase in urine volume. When at the same time extrarenal water expenditure is increased by high environmental temperature, or diarrhoeal losses, the water balance

may be threatened either by high urine volumes or, in the case of renal inadequacy, by uraemia.

In summary, the mechanisms defending body fluid equilibrium in the infant are more easily broken down owing to the water reserves being low in relation to the high metabolic rate and "strained" circulation. In circumstances of shortage this small water pool is quickly exhausted, and it is also easily flooded by loads which, in terms of body surface, are equal to those for adults. By decreasing the small plasma pool rapidly, water losses lead to slowing down of circulation. Owing to the rapidly decreasing renal clearances, as well as the high metabolic rate producing solutes at great speed, the relatively small water pool cannot then keep up its constancy. Deterioration is accelerated by arrested growth.

In conclusion a particular type of dehydration in which the infant seems to be in a somewhat less difficult position than the adult may be mentioned. In infantile pyloric stenosis, a condition in which starvation and dehydration develop together, a sharp decrease of about 50 per cent in oxygen consumption has been observed by Varga (1957). We have found that this diminution in oxygen requirements protects against stagnating anoxia brought about by the slowing down of circulation due to dehydration (Kerpel-Fronius *et al.*, 1951). A low metabolic rate will most probably also diminish obligatory water expenditures and thus delay the progress of dehydration. Since the metabolic rate decreases less in the semi-starved adult (Keys *et al.*, 1950), the infant may possibly be more resistant to dehydration when he is already suffering from starvation than an adult under similar circumstances.

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DISCUSSION

Davson: Has the subject of size *per se* been considered as opposed to immaturity? The pulse rate of the baby was mentioned as being faster than that of the adult and the reasons for it were based on the immaturity of the organism, whereas one finds that small adult animals have very fast pulse rates. The rabbit pulse, for instance, is well into the hundreds and the mouse pulse is even faster.

Young: I do not think it has been suggested that the pulse rate is high because of immaturity: it is high because of the high metabolic rate in relation to the other constants, and in order to keep up the cardiac output.

Adolph: The effect of body size on functions such as pulse rate and respiration rate varies considerably in any one species. Among various species of adults it is very definite because you can get a wide range of body sizes and can calculate what the average difference of function is. In one species, the rat, the breathing rate is almost constant with age, whereas the ventilation varies enormously with age, and even relative to body size it varies somewhat with age. The pulse rate varies in accordance with body size only after the age of weaning, and I should say that none of the body size rules apply uncomplicatedly during infancy. There are other factors, and perhaps the factor of metabolic peculiarities is one of them.

McCance: Would anyone with paediatric experience like to comment on the metabolic rate in pyloric stenosis?

Young: Prof. Kerpel-Fronius only quoted the example of the metabolic rate in pyloric stenosis because dehydration is so likely to occur in that condition, where the baby is also malnourished. Dr. Varga has studied a series of malnourished cases in which he showed that the metabolic rate and the oxygen uptake were low.

Talbot: Could the results shown in Fig. 2. (p. 156) be explained on the basis of starvation with hypoproteinaemia? As in the nephrotic patient, hypoproteinaemia tends to result in hypovolaemia. This in turn leads to sodium and water retention and to a tendency to the formation of extracellular oedema. It is thought that these reactions represent an attempt on the part of the body to restore vascular volume to a satisfactory level.

Young: When this infant became dehydrated he still had a relatively high volume of extracellular fluid as a percentage of body weight, but

the absolute volume was very low relative to that of normal infants. At this time he showed an increase in all these handicaps of failing function.

Talbot: Did he have a low absolute plasma volume?

Young: Yes, but it was not very low per kg./body weight.

Talbot: That might be the answer to the problem.

Bull: I should like to support that because we often find changed plasma volumes in burns, where the situation is similar to that of nephrosis. The extracellular fluid volume is not a good index of circulatory competence; the plasma volume can alter independently of it.

Fejfar: The longer circulation time showed in this case would mean that the cardiac output was lower, and one can say that in all circumstances where the cardiac output is inadequate, there is a decrease in renal blood flow. It is not necessary for it to be connected with a decrease in blood volume.

Black: With a very high pulse rate and low cardiac output there must be a fantastic decrease in stroke volume. That may be just a part of the diminished blood volume, or the newborn infant may have a diminished stroke volume. Perhaps the heart size is small in relation to body size.

Young: The great value of this paper is in explaining why the baby is more susceptible to stress than the adult, although he appears to have plenty of water. This particular way of setting out these relationships is very valuable from that point of view. To some people it has always been rather a puzzle that although the extracellular fluid volume is relatively high, it still is not high compared with the physiological demands made on it.

Heller: We are always talking about the large body water content or the high extracellular fluid volume in babies and young animals. Are they accidental, as it were—due for instance to some prenatal endocrine influences—or have they any functional significance? I have always been struck by the similarity between the water metabolism of the newborn animal and baby and animals with experimental nutritional oedema.

Davson: It depends whether the large water content is necessitated by the geometry of the animal. If you had a sparse number of muscle fibres, then you would have a bigger extracellular space to fill out the gap. The animal's extracellular geometry changes gradually and the space really has no functional significance except in so far as a muscle with more muscle cells in it per unit of weight is a more efficient muscle.

Fourman: There is not a bag, to be filled either by muscle or by water. Again, it all depends on the size of the cells.

Is the extra water of the baby in the muscle, the connective tissue or the skin?

Davson: In the adult animal you can correlate the amount of collagen with the amount of extracellular fluid.

Widdowson: Most of the extracellular fluid is in the skeletal muscle. This is one of the biggest tissues of the body and it is the one which changes most in composition with development. Tissues like the heart and the liver change very much less in their extra- and intracellular relationships with development. The heart, for example, is very much

nearer its adult composition in foetal life than the skeletal muscle. I think a great deal of this change is in the skeletal muscle and not in connective tissue.

Fourman: Then is there a difference in the mode of growth of skeletal muscle on the one hand, and liver and heart on the other? Does skeletal growth occur simply by hypertrophy without cell multiplication, and do heart muscle and liver grow by cell multiplication? Are babies' muscle cells smaller than those of adults and their liver cells the same size?

Kennedy: By and large what you have said is right. There is considerable hyperplasia in liver during growth although there is an over-all expansion in size of the cells with age. There is a much bigger change in muscle cell size than in the liver cells.

Fourman: If the extracellular fluid is considered as a film over the cells, that would account for the fact that the percentage of extracellular fluid does not change with age so much in liver as it does in muscle.

Kennedy: Within any one tissue it should be quite easy to test that, because cell size data based on nucleic acid determinations are available for many different ages in a number of species, and equally, extracellular fluid determinations are available in the same tissues.

Wallace: Muscle composition does not change much with age per unit of muscle; you are talking about more muscle, not per kilogram of muscle.

Widdowson: I am talking about per unit of muscle. As I have just said, skeletal muscle changes very much in composition during development.

Fourman: Dr. Shock, is the water content in the muscle larger in old people than in the young ones, since muscles do atrophy in old age? We have had that answered indirectly in Dr. Olesen's paper, but are there any direct analyses?

Shock: I cannot answer for the human, but we have some data on the electrolyte and water composition of rat muscle tissue. We found that the total water content per kilogram of muscle tissue does not change significantly with age. There was a definite shift in the water distribution in that the extracellular phase increased as the intracellular phase decreased. The potassium, phosphorus, and nitrogen contents all went down, but the chloride and sodium contents went up. The ratio of potassium to nitrogen and of phosphorus to nitrogen remained constant. Our interpretation of this was in the light of our beliefs about the reduction in active protoplasm in old age. It is as if a certain mass of protoplasm had disappeared and been replaced by extracellular compounds with the appropriate amount of sodium and chloride to make up the total water composition.

Fourman: As I said, it is not a replacement, but—to borrow Dr. Davson's expression—a geometrical necessity to keep a film of water around the cells.

Kennedy: But you would need to know whether the atrophy was due to a loss of whole structural units or to a change in the size of each unit.

Shock: We do not really know this. We have not done the histology on these muscle tissues, but we have sent some to Dr. Warren Andrew for examination.

THE EFFECT OF HORMONES OF THE PITUITARY AND ADRENAL GLANDS ON THE ELIMINATION OF SODIUM, POTASSIUM AND A WATER LOAD IN INFANT RATS DURING THE WEANING PERIOD

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Homeostatic mechanisms in infant animals differ from those in adults of the same species. Mechanisms regulating the metabolism of water and electrolytes change immediately after birth, during the period the eyes open, at the time of weaning, in connexion with sexual maturation and perhaps also at other stages of postnatal development. In the present paper we should like to draw attention to the time of weaning, which seems to us to be one of the important stages in the development of the regulation of water and electrolyte metabolism.

The preweaning period in rats is relatively long. Up to the 14th day of life infant rats cannot survive without the mother rat. They are usually weaned at the end of the third week but according to breeders natural weaning occurs only at the end of the fourth week. This agrees with the development of thermoregulation, for infant rats can survive very low environmental temperatures without the mother only at the end of the fourth week (Čapek *et al.*, 1956).

Up to the 14th or 18th day infant rats live on breast milk only. This is the only source of water and electrolytes, if we disregard the urine of litter-mates that is sometimes sucked by the infant animals. From that time onward infant rats in addition to breast milk also actively feed on solid food and

drink water. Gradually the mechanisms for compensation of thirst and hunger separate. At the end of the fourth week infant animals cease to feed on breast milk and take in food that is normal for adult animals.

We studied the active intake of water, electrolyte solutions and milk in infant rats using the method of free choice as known especially from the work of Richter (1936), Young (1949), and Young and Chaplin (1949). We observed that in infant rats weaned at the beginning of the third week of postnatal life there is a significant change in the regulation of water, electrolyte and milk intake at the end of the fourth week. The regulation of sodium intake in relation to water intake, especially, changes. According to Richter (1936) appetite for individual components of the diet is an important homeostatic mechanism and is determined by the needs of the organism.

In order to be able to offer a physiological explanation for changes in the regulation of sodium intake it is necessary to throw light on the relation between mechanisms of self-selection and other components of water and electrolyte metabolism that can be studied better and more objectively.

The adrenals and the posterior lobe of the pituitary are of special significance for the regulation of water and electrolyte metabolism. For this reason we have studied the effects of hormones from these two glands. Up to the present nothing is known of a change in function of the adrenals or in the effect of their hormones at the end of the fourth week of life in the rat. Indirectly one might expect such a change from the fact that the regulation of sodium intake depends on the function of the adrenals (Richter, 1936). There is also no difference in the size of the glands in males or in females during the fourth week.

More is known about changes in the rôle played by the posterior lobe of the pituitary during this period. Heller (1952) showed that up to the end of the fourth week of life the rat kidney does not react to vasopressin during a water load in the same way as that of the adult. In addition the ability

of the kidneys to eliminate an administered water load changes and its ability to concentrate increases. According to Falk (1955), however, infant rats older than three days already react to vasopressin by cessation of diuresis and an increased excretion of chloride. As both authors use different methods it seemed useful to study this problem first, using several methods, and also to study the effect of vasopressin on the elimination of sodium and potassium. Opinions on the natriuretic effect of vasopressin also differ and we believe that this is due to different methodological approaches. Schaumann (1949) and Heller and Stephenson (1950) observed that vasopressin decreases the excretion of sodium in adult rats, while Sawyer (1952) observed an increased elimination of this electrolyte. The former authors administered the hormone at the same time as the water load. Sawyer first slightly prehydrated his animals and then gave them the hormone and the water load. According to Heller (1952) the ability of the rat kidney to eliminate a water load changes at the time of weaning. We therefore always used rats with a water load.

Infant rats were weaned on the 15th-16th day after birth and the whole litter left in one cage. They received a standard synthetic diet without sodium chloride. They were allowed to choose between water and a 3 per cent sodium chloride solution. As we expected changes in the mechanisms studied to occur at the end of the fourth week, infant animals aged 23 and 33 days were used. Loads of warm distilled water were administered via a stomach tube in amounts of $4 \cdot 5$ ml./100 g. body weight. Subcutaneously the animals received saline ($0 \cdot 5$ ml./100 g. body weight) in which the substances studied were dissolved. The elimination of a water load was studied for three hours after its administration or, in the case of vasopressin, for three hours from the first micturition. Urine was collected at hourly intervals. The amount of urine, together with the concentration of sodium and potassium, was determined by use of a flame photometer.

Adult rats rapidly excrete urine with a low content of

sodium and potassium after administration of a water load.
Males excrete a water load less well than females.

In our experiments the excretion of a water load was the
same in infant rats as in the experiments of Heller (1952).
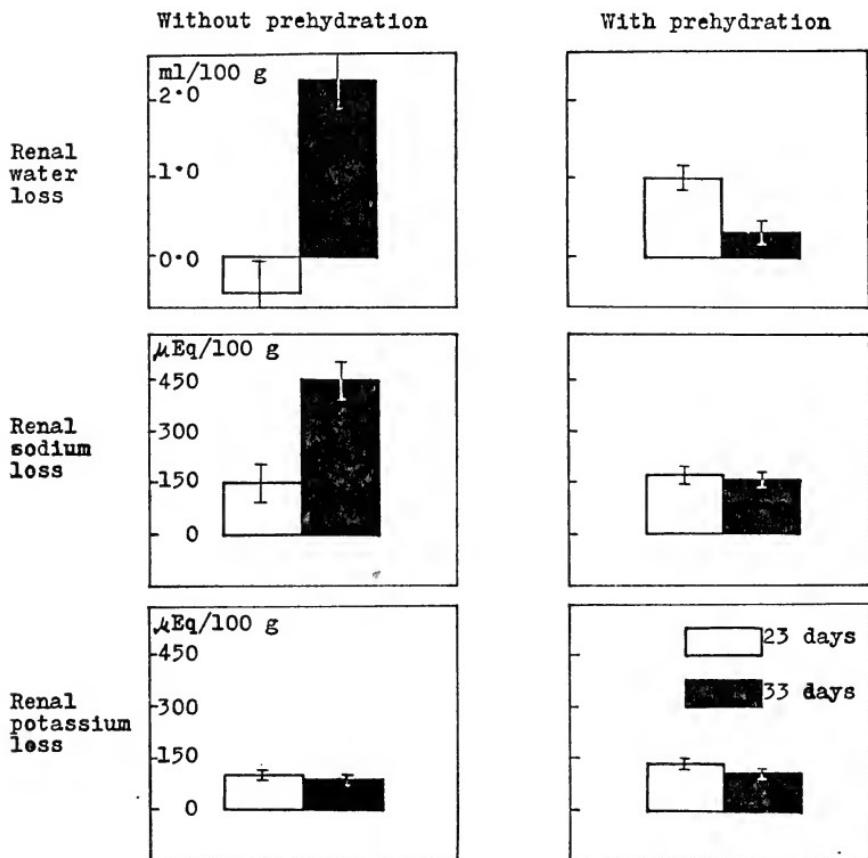
FIG. 1. The renal loss of water, sodium and potassium during the first three
hours after administration of a water load (4·5 ml./100 g. body wt.) to
young rats aged 23 and 33 days without prehydration or with prehydration
(2·5 ml./100 g. body weight).

There are no sex differences. There are, however, consider-
able differences between infant animals aged 23 and 33 days.
These can be seen in Fig. 1. Twenty-three-day-old animals
do not eliminate the total water load within three hours.

Older animals, however, excrete nearly half the water administered and thus excrete body water via the kidneys. Differences in sodium excretion are also apparent. Thirty-three-day-old animals excrete three times as much body sodium as younger rats. The difference between both age groups studied disappears completely, or becomes much smaller, if $2 \cdot 5$ ml. water/100 g. body weight is put into their stomachs two and a half hours before the actual water load. In that case more urine is excreted by the younger animals and losses are reduced in the older age group. Sodium losses are also decreased in the older age group to the same level as in 23-day-old animals. No significant changes in potassium excretion were observed.

Differences between the two age groups are thus not constant. For this reason we assume that the difference is not due only to changes in renal function but that regulatory mechanisms are also concerned.

The effect of vasopressin was studied in animals receiving one water load and in prehydrated rats. The elimination of the water load was studied according to the method of Falk (1955). In addition the effect on total water loss three hours after the first micturition was studied. This procedure was similar to that of Heller (1952) who determined total renal excretion of a water load 145 minutes after administration of the hormone and the water load.

After 10 or 25 m-u. vasopressin/100 g. body weight, no significant differences between the two age groups could be observed during water diuresis. This is in agreement with Falk (1955). Yet 23-day-old animals react differently to vasopressin than 33-day-old rats. This difference can be seen in Table I. After a single water load vasopressin (the table shows the results with 25 m-u./100 g. body weight) increases renal water losses in the younger animals, while in the older group total renal water losses are reduced. The sodium loss in older animals treated with vasopressin becomes greater after prehydration only. In younger animals the elimination of potassium is significantly greater than in the

Table I

THE EFFECT OF VASOPRESSIN ON SODIUM AND POTASSIUM EXCRETION AND WATER LOSS AFTER WATER LOAD IN PREHYDRATED AND UNPREHYDRATED RATS OF DIFFERENT AGES

	Age of animals in days	Sodium excretion		Potassium excretion		Renal water loss	
		No. of animals	μ-equiv./ 100 g. body wt./3 hr.	No. of animals	μ-equiv./ 100 g. body wt./3 hr.	No. of animals	ml./100 g. body wt./3 hr.
Water load 4·5 ml./100 g. body wt. H_2O via stomach tube + 0·5 ml./100 g. body wt. saline s.c. without prehydration	23	7	147±50·4	8	99± 9·7	8	−0·5±0·4
	33	8	450±45·5	7	85±13·7	8	2·3±0·38
Water load 4·5 ml./100 g. body wt. H_2O via stomach tube + 25 m-u./100 g. body wt. vasopressin in 0·5 ml./100 g. body wt. saline s.c. without prehydration	23	7	187±28·3	5	123±62·5	7	0·9±0·36
	33	8	350±36·0	7	113±18·1	7	1·4±0·18
Water load 4·5 ml./100 g. body wt. H_2O via stomach tube + 0·5 ml./100 g. body wt. saline s.c. with prehydration	23	15	168±24·3	16	135±13·9	15	1·0±0·24
	33	14	154±21·3	14	109±11·4	16	0·3±0·215
Water load 4·5 ml./100 g. body wt. H_2O via stomach tube + 25 m-u./100 g. body wt. vasopressin in 0·5 ml./100 g. body wt. saline s.c. with prehydration	23	16	245±36·2	16	173±10·3	16	1·4±0·32
	33	13	255±29·0	13	123±13·0	16	−0·1±0·41

33-day-old rats. Thus vasopressin has a different effect in 23-day-old than in 33-day-old animals. Evidently there is a
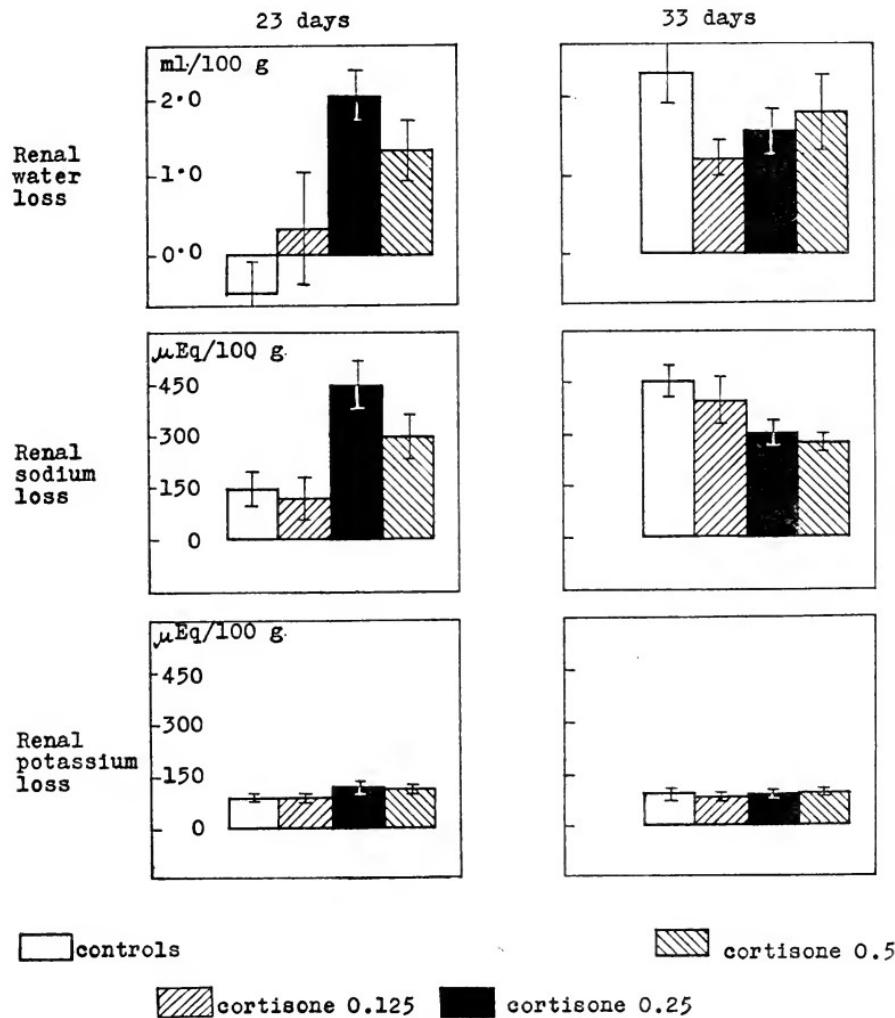
FIG. 2. The effect of cortisone administered for six days in different doses on renal loss of water, sodium and potassium during the first three hours after administration of a water load (4·5 ml./100 g. body wt.) to young rats aged 23 and 33 days.

change in the reactivity of the kidneys to this hormone at that period. This might be due to functional differences in

kidney parenchyma or to the fact that from the end of the fourth week a regulatory factor is present which can be influenced by loading the organism with water. It therefore seemed all the more interesting to us to find out whether the function of the adrenals changes at the time of weaning.

After adrenalectomy the ability to eliminate a water load is strongly reduced in infant rats. It is difficult therefore to use this method for solving the problem. A less direct way was chosen — a study of the effect of substances that act in a similar way to the main corticoids. Cortisone or cortexone was administered for six days in various doses to 18–23 and 28–33-day-old animals. Then a water load was given. It appeared that the effect of these substances also depends on the age of the rats.

The effect of cortisone is shown in Fig. 2. The elimination of a water load, sodium and potassium was determined in rats that received $0 \cdot 125$, $0 \cdot 25$ or $0 \cdot 5$ mg. cortisone/100 g. body weight. The hormone has opposite effects in the younger and in the older age groups. In 23-day-old animals it increases the excretion of water (as it does in the 3-day-old rats of Falk, 1955) and sodium, while in the 33-day-old rats it decreases both. After a dose of $0 \cdot 25$ mg./100 g. body weight, renal water and sodium losses in the younger animals reach approximately the levels of the older control animals. It appears as if the administration of cortisone compensates for a factor missing in the younger animals but present in the older rats. This, however, is not borne out by the way in which a water load is eliminated by the younger rats after cortisone. Fig. 3 shows changes in the concentration of sodium in the urine during the course of water diuresis in normal animals and after cortisone ($0 \cdot 25$ mg./100 g.). In the control 33-day-old animals the concentration rises as the intensity of water diuresis falls. In the younger group there is no such relationship and the concentration is not lowest during the highest diuresis. If cortisone were only a substituting substance the course of the curves of sodium concentration ought to be the same in 23-day-old rats receiving cortisone

and 33-day-old controls. As this is not the case and as in the younger animals increased natriuresis is mainly due to increased concentration at the time of maximum water
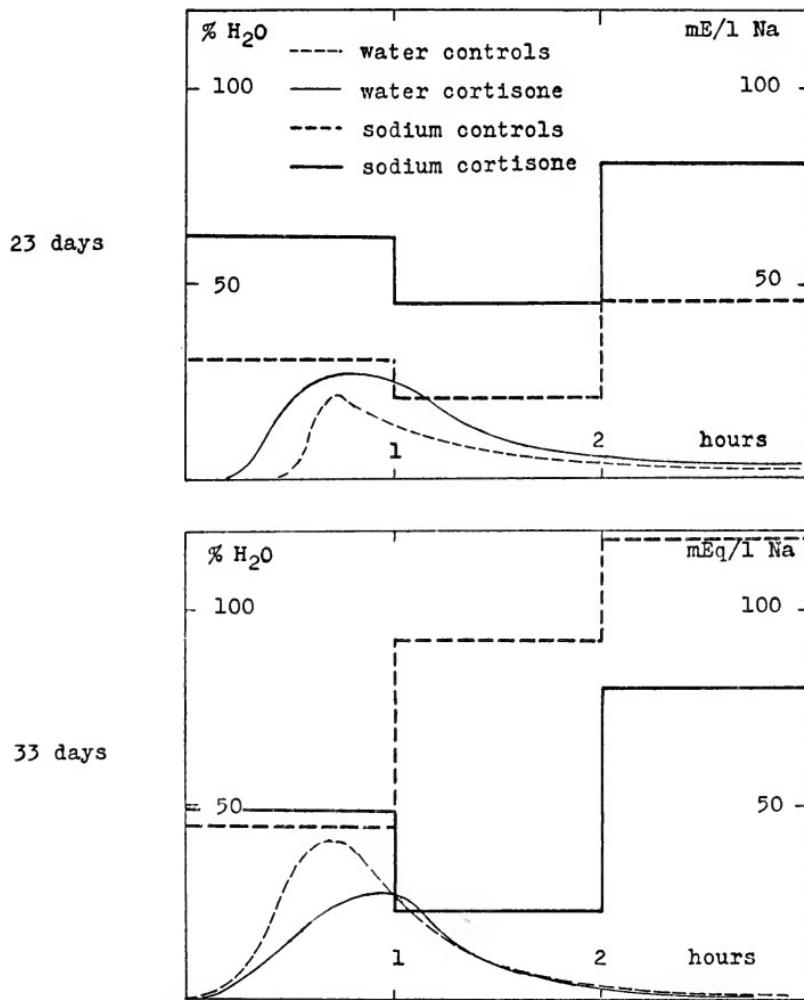
Fig. 3. The effect of cortisone (0·25 mg./100 g. body wt./day) on the course of the excretion of a water load and the concentration of sodium in the excreted urine in infant rats aged 23 and 33 days.

diuresis, relations are evidently more complex. This is also borne out by the fact that the effect of cortisone in the younger group is variably dependent on the dose used.

This is even more evident in the case of cortexone. This was administered by the same route as the former substance
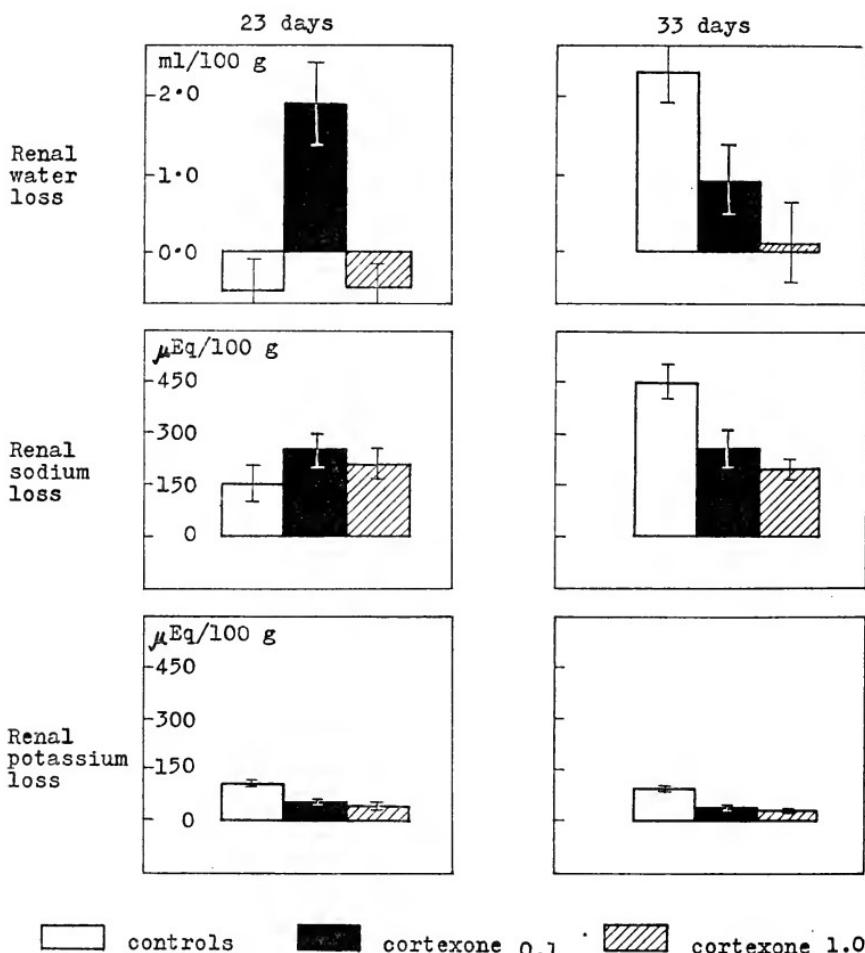
FIG. 4. The effect of different doses of cortexone, administered for six days, on renal loss of water, sodium and potassium during the first three hours after administration of a water load (4·5 ml./100 g. body wt.) to young rats aged 23 and 33 days.

but in doses of 0·1 and 1 mg./100 g. body weight. Results are shown in Fig. 4. Lower doses of cortexone had an effect similar to cortisone, quantitatively different in younger and

Controls

Cortisone

ACTH
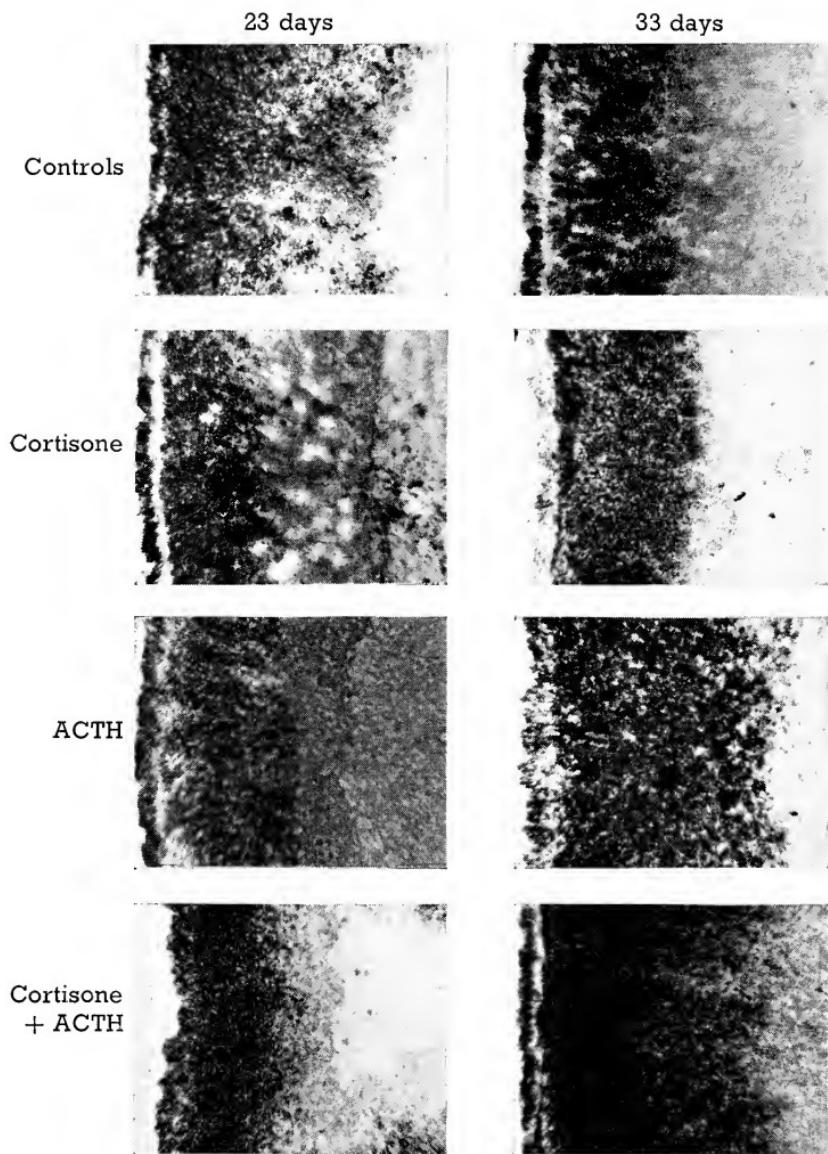
Cortisone
+ ACTH

FIG. 5. The effect of cortisone (0·25 mg./1C0 g. body wt./day) and ACTH (0·2 i.u./animal/day) administered for six days on the size of the adrenal cortex in young rats aged 23 and 33 days. Stained with Sudan Black.

older animals. In younger animals it increased renal losses, which thus nearly reached the levels of the older controls. In 33-day-old animals water losses decreased after cortexone. The higher dose, however, had no effect on renal losses of water in 23-day-old animals, whereas in 33-day-old rats it further decreased renal losses. These doses, however, are probably toxic. Sodium losses were never significantly altered by either dose of cortexone in the younger group. In 33-day-old animals they changed in direct proportion to the dose used. In both age groups cortexone decreases renal potassium losses significantly.

Thus corticoids have a different effect on the elimination of water and electrolytes after a water load in infant rats that have not yet reached the age at which they are normally weaned, than they have in older animals. The opposite effects in 23-day-old animals, depending on the dose used, indicate that these hormones cause changes that mutually interfere with each other.

We attempted to determine whether in addition to the pharmacodynamic effect of these hormones there is also an effect on the regulation of adrenal activity.

The weight of the adrenals of animals receiving cortisone or cortexone, as indicated above, dropped to about the same extent in both 23- and 33-day-old animals. Simultaneous administration of ACTH in amounts usually sufficient to maintain adrenal weights of hypophysectomized animals (0·2 i.u. per animal) prevents adrenal atrophy in both groups. This reaction is less obvious on histological studies. Fig. 5 shows microphotographs of the adrenal cortices of 23- and 33-day-old animals (controls; after cortisone [0·25 mg./ 100 g./day]; after ACTH [0·2 i.u. animal/day]; and after simultaneous administration of cortisone and ACTH). Preparations were stained with Sudan Black so that both the width of the cortex and the sudanophil layers can be seen. After ACTH there are no obvious changes in the width of the cortex and the sudanophil layer. After cortisone and cortisone plus ACTH differences are evident. This is even more apparent

in Fig. 6, which shows the results of micrometric measurements of the width of the cortex and the sudanophil layer as obtained from serial sections of the adrenals. Four adrenals from each group were measured. One hundred sections from each gland were used and measurements were taken from
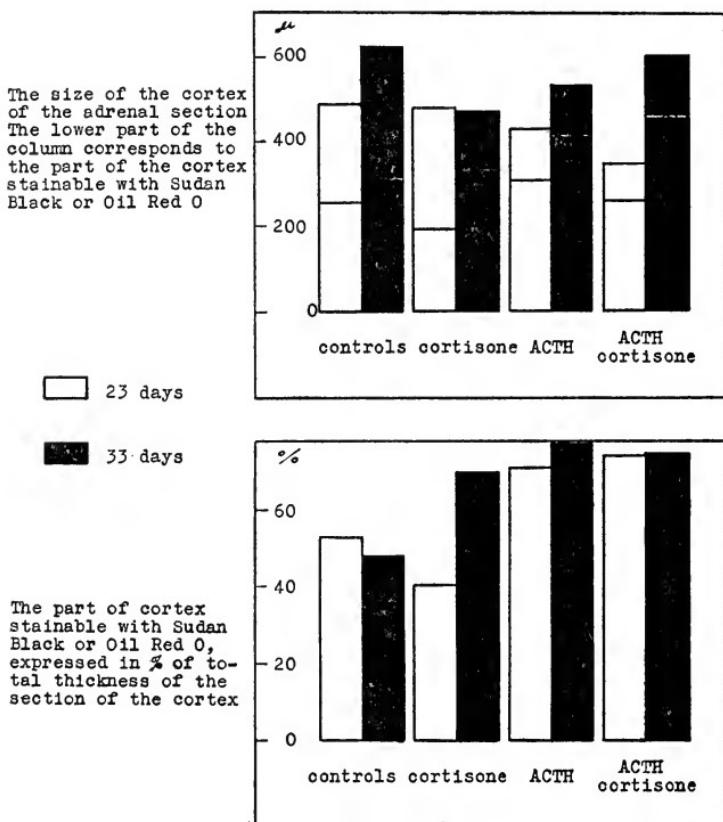
FIG. 6. See Fig. 5.

several sites of those sections. Differences are largest after cortisone. In 23-day-old animals the sudanophil layer decreases in size while the total width of the cortex remains unchanged. In 33-day-old animals the width of the cortex decreases and thus the relative width of the sudanophil layer is increased. After ACTH and cortisone the proportion

of the sudanophil layer increases in both age groups but in the younger group the size of the whole cortex is smaller. It is difficult to interpret these changes. It is certain, however, that according to morphological criteria the adrenals of the 23-day-old animal react differently from those of the animal aged 33 days. This would indicate that changes in the reactivity of infant rats to a water load at the end of the natural period of weaning, and to corticoids, are also conditioned by a different reactivity of the adrenals and the adrenopituitary system.

This hypothesis is further supported by results from experiments in which the effect of ACTH and a combination of ACTH and cortisone (0·25 mg./100 g.) was studied on the elimination of water, sodium and potassium after a water load. Results are shown in Fig. 7. As has already been shown, cortisone prevents retention of a water load in 23-day-old animals and considerably increases renal water losses. ACTH is without effect. After simultaneous administration of ACTH and cortisone, water losses decrease in comparison to losses after cortisone only. In 33-day-old rats results are less evident because of the large scatter. ACTH itself causes an increase in sodium excretion in 23-day-old animals but in combination with cortisone it is without effect on sodium elimination and thus removes the latter's natriuretic effect. This effect is probably due to the lower renal water losses. In 33-day-old animals ACTH decreases sodium losses just as do cortisone and cortisone combined with ACTH. The same holds good for ACTH when combined with cortexone. ACTH prevents atrophy of the adrenals after cortisone in infant rats aged 23 days and also prevents the effect of cortisone on sodium and water elimination. This is not the case in older animals. This is in agreement with the histological picture and with the differences between 23 and 33-day-old animals.

We have thus been able to show that there is a time correlation between changes in homeostatic mechanisms regulating the intake of water and electrolytes appearing in infant rats at the time of natural weaning, and adrenal

pituitary mechanisms regulating the metabolism of water and electrolytes. At the end of the fourth week of life the effect
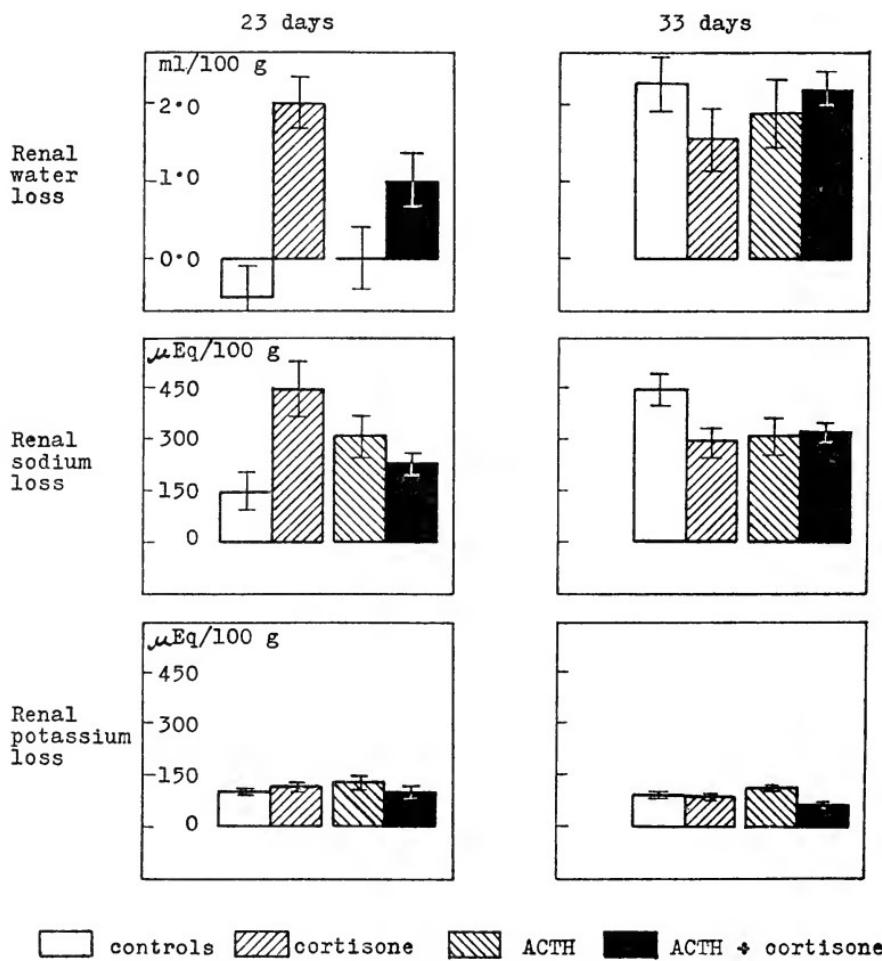
FIG. 7. The effect of cortisone and ACTH (for doses and duration of administration see Fig. 5) on renal losses of water, sodium and potassium during the first three hours after administration of a water load (4·5 ml./100 g. body wt.) to young rats aged 23 and 33 days.

of vasopressin on elimination of a water load changes. This is in agreement with Heller (1952). In addition, at this time vasopressin begins to have an effect on sodium elimination.

This is probably conditioned by the presence of a regulating mechanism which after previous loading with water increases the reabsorption of sodium. At the end of the preweaning period there is a considerable change in the effect of cortisone and cortexone on elimination of water and sodium after a water load. Even 33-day-old animals, however, do not react quantitatively in the same way as adult animals. This is evidently due to the fact that only after the 33rd day does the male adrenal begin to differ from that of the female. It may be assumed from the results presented here that the reactivity of the adrenals changes at the time of weaning. That change can be in relation to the change in homeostatic mechanisms regulating the intake of water and sodium which occurs at the time of weaning.

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[Discussion of this paper was postponed until after the paper by Dr. Desaulles.—EDS.]

DIFFERENCES IN THE PATTERN OF ELECTROLYTE AND WATER EXCRETION IN YOUNG AND OLD RATS OF BOTH SEXES IN RESPONSE TO ADRENAL STEROIDS

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IT is a known fact that, with advancing age, the cell mass and, correspondingly, the cell water content of the animal decrease. This, together with a constant or increasing extracellular water content, appears to be one of the true signs of ageing (McCance and Widdowson, 1951; Olbrich and Woodford-Williams, 1956).

Although the adrenals, and more especially the adrenal steroids, play an important part in the maintenance of the water and electrolyte balance, only comparatively little is known about the influence of age on the activity of the adrenals or on the sensitivity of the organism to adrenal steroids in animals. We were therefore prompted to study in rats of different ages the pattern of urine and urinary electrolyte excretion after treatment with two genuine adrenal steroids, aldosterone and cortisol, following a load of physiological saline solution amounting to 20 ml. per kg.

In view of the very complex interrelationship existing between pituitary, gonads, and adrenals during the development of the animal from birth to maturity and old age, we have also studied rats of both sexes. These animals were chosen in three different groups, ranging in age from (a) five weeks to (b) fifteen weeks to (c) one year and more.

Methods

All experiments were performed on adrenalectomized rats of the same breed, in order to avoid interference between the steroids injected and the steroid output of the animal's

own adrenals, as well as to avoid strain-bound differences in sensitivity.

To test the action of steroids on urinary and electrolyte excretion, we have used the method described in detail by Desaulles and Meier (1956), the only difference being that, instead of collecting urine from the fifth to seventh hour after treatment and loading, we collected it in different groups from the 30th minute to the second hour and a half, from the first to the third hour, from the second to the fourth hour, and from the seventh to the ninth hour following treatment, this procedure enabling us to follow closely the excretion of urine and of electrolytes. Both male and female animals were used, the age groups being:

(a) animals about five weeks old and about 50 g. in weight,
(b) animals about 15 weeks old and about 150–180 g. in weight,
(c) animals about one year old and exceeding 300 g. in weight.

All animals used in these experiments were kept isolated in metal cages at constant temperature (26°) and relative humidity (75 per cent), the number of animals per group varying from six to 12. The animals were given full standard rat cake (Nafag A.-G., St. Gallen) and water *ad libitum* until the beginning of the experiment.

The steroids chosen, aldosterone and cortisol, are known to be secreted by the rat adrenals (Bush, 1953; Singer, 1957). Cortisol was used as free alcohol, aldosterone was used as DL-aldosterone acetate, the activity of which is just one half of D-aldosterone (Schmidlin *et al.*, 1955, 1957). All substances were dissolved in sesame oil and injected intramuscularly.

The doses used in these experiments were chosen from previous experiments (Desaulles and Meier, 1954; Desaulles, 1958) and lay within a dose range corresponding to sub-maximal effects. For aldosterone acetate $0 \cdot 01$ mg./kg. was given, and for cortisol 5 mg./kg.

As the excretion of urine and urinary electrolytes differs

in amount in animals of differing age and weight, the results are expressed as percentages of the values of control animals for urinary excretion in ml., and for sodium and potassium excretion in m-mole. The differences between the sodium/potassium ratios of treated and control animals are, on the other hand, expressed in absolute values.

Results

Effect of aldosterone

In the *male rat*, aldosterone produces a marked inhibition of urinary output that is most pronounced in young animals
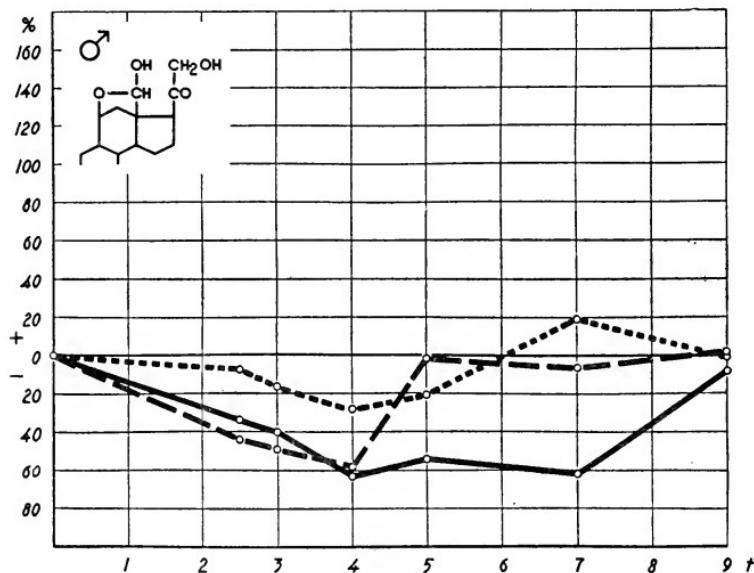
FIG. 1. Urinary excretion of adrenalectomized male rats of different age groups treated with aldosterone (0·010 mg./kg.).

Abscissa: Duration of experiment (hours); collecting period 2 hours.

Ordinate: Urinary excretion as a percentage of the values of control animals.

Continuous line: 5-week-old rats.
Interrupted line: 15-week-old rats.
Dotted line: one-year- and more-old rats.

and tends to diminish—at first in duration and then in intensity—with increasing age (Fig. 1).

Aldosterone prevents sodium excretion in a very marked manner in about the same intensity and for about the same duration (five to seven hours) in all age groups (Fig. 2).

The only difference to be noted is that in old animals the onset of the sodium-retaining effect of the steroid is retarded,
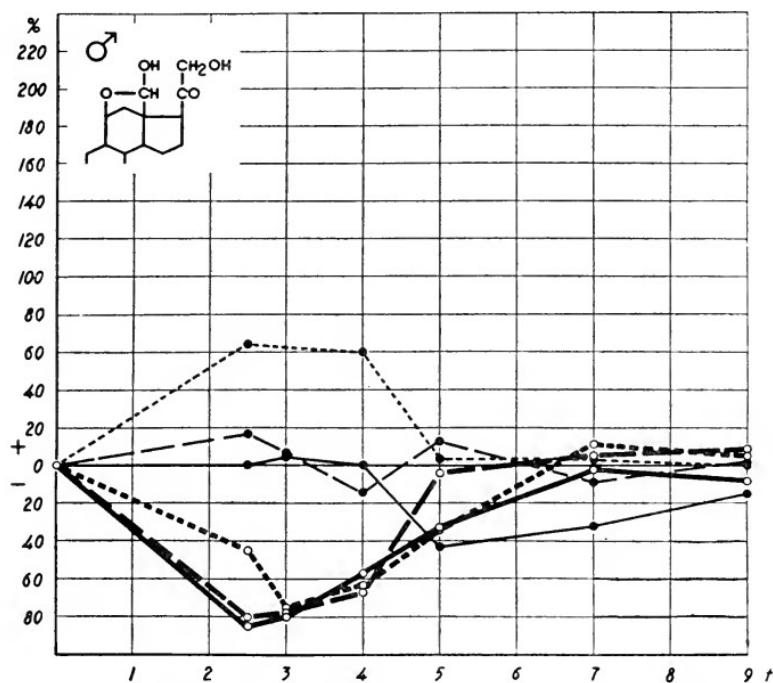
FIG. 2.   Urinary sodium and potassium excretion of adrenal-ectomized male rats of different age groups treated with aldosterone (0·010 mg./kg.).

Thick line: sodium excretion.
Thin line: potassium excretion.
Other figures as for Fig. 1.

the maximal effect falling in the collecting period of the third hour, instead of in the preceding period.

The effects of aldosterone on potassium excretion depend upon the age groups in question.

In young animals, aldosterone does not affect potassium excretion until the fourth collecting hour. From the fifth

hour onward it induces a clear-cut reduction in potassium excretion, which reverts to normal in the ninth hour. On animals of the adult group aldosterone has practically no effect at all. In old rats, however, aldosterone markedly enhances potassium excretion.
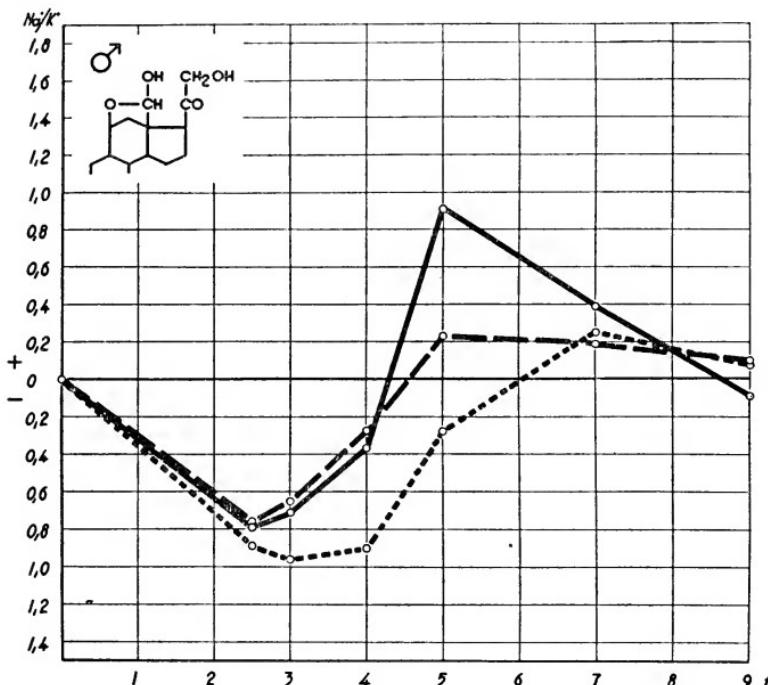
FIG. 3. Urinary sodium/potassium ratio of adrenalectomized male rats of different age groups treated with aldosterone.

Ordinate: Difference between sodium/potassium ratio of experimental animals and controls.

Other figures as for Fig. 1.

If we consider the sodium/potassium ratio, we observe that aldosterone reduces it markedly during the first hours of the experiment in all groups (Fig. 3), its maximum occurring in the first collecting period for young and adult groups, and showing a certain delay (three hours) and greater intensity (-0.95 against -0.75 to -0.80) in the old age group. From the fourth hour onward there is an increase in the ratio for

young animals (due to potassium retention), whereas adult and old animals return to a range within control values, the adult group reacting more readily than the old animals.

In the *female rat*, aldosterone also reduces the urinary output, but to a somewhat smaller extent than in males (—40 per cent on the average, against about —60 per cent in males) (Fig. 4). As in males, young animals tend to respond more
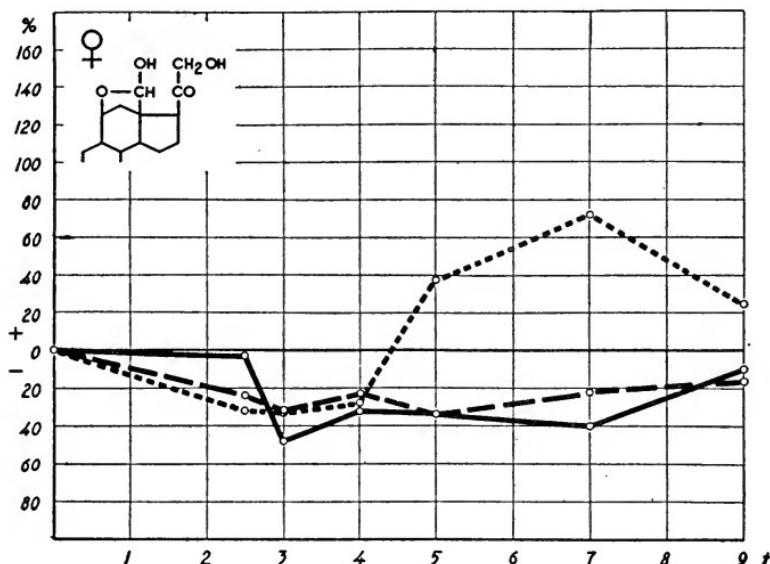
FIG. 4. Urinary excretion of adrenalectomized female rats of different age groups treated with aldosterone (0·010 mg./kg.).
Figures as for Fig. 1.

markedly although there is a certain delay in the onset of the effect. In contrast to males, with increasing age a short period of urinary retention is followed by a strong diuretic response.

On sodium excretion aldosterone exerts a very pronounced inhibiting effect of about the same relative intensity as in males in all age groups (Fig. 5). In contrast to that in males, this effect is followed by a period of sodium excretion, most marked in old animals (+80 per cent), the values returning towards the norm in the ninth hour.

On potassium excretion the enhancing effects of aldosterone are more marked and begin at an earlier age than in males, old animals showing the most pronounced effect.

On the sodium/potassium ratio the effects are much more marked than in the case of males (Fig. 6). Young animals respond with a reduction that is marked (−0·90), but of slow
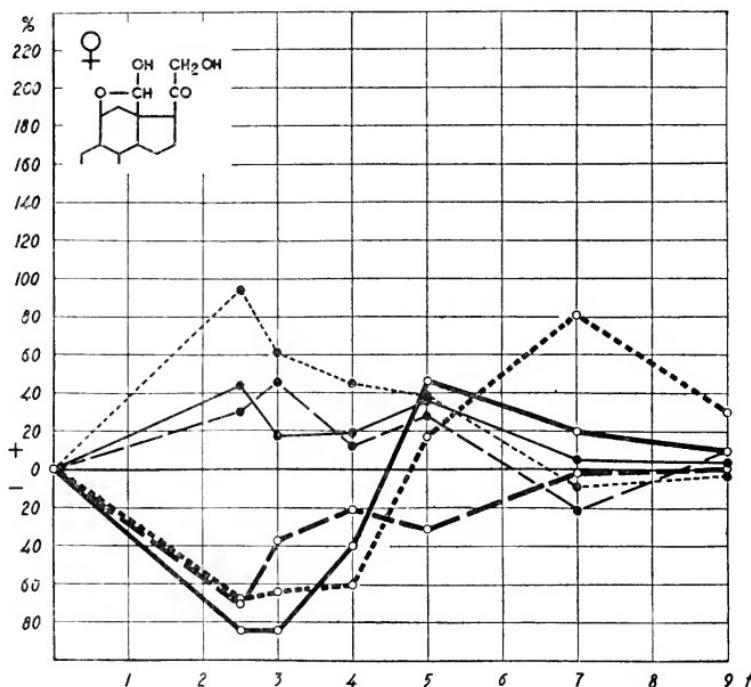
Fig. 5. Urinary sodium and potassium excretion of adrenalectomized female rats of different age groups treated with aldosterone. Figures as for Fig. 2.

onset (maximum in the fifth hour), the values returning to within control limits at the end of the experiment.

In adult and old females, the reduction in the sodium/potassium ratio is more intense (−1·29 and −1·40 respectively) and rapid in onset (maximum in the first collecting period). This effect lasts longest in old animals.

The rapid lowering of the sodium/potassium ratio is

followed, in contrast to the situation for male animals, by a very pronounced and rapid rise (more in adult than in old animals) to high positive values ($+0\cdot95$ and $+1\cdot28$, respectively), this effect tending to return within control values in the ninth hour.
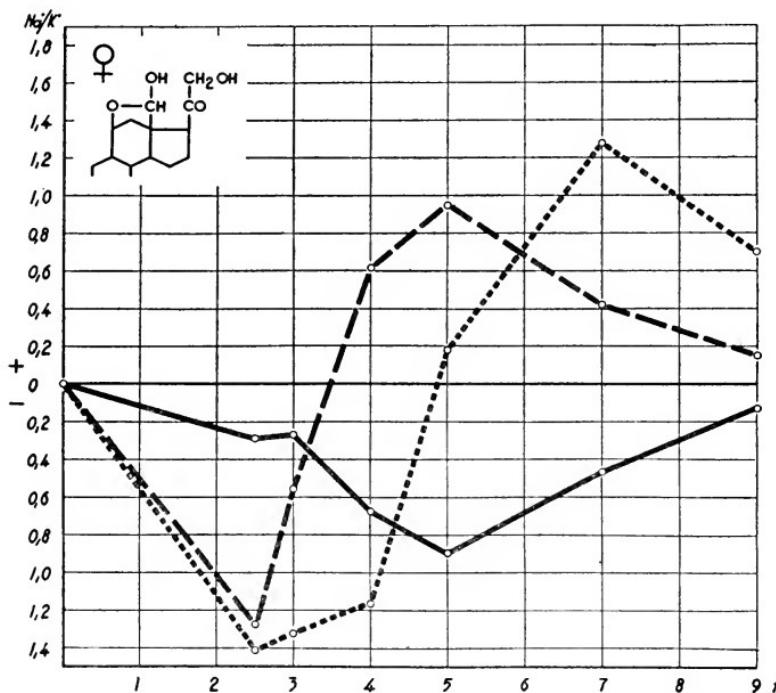
Fig. 6. Urinary sodium/potassium ratio of adrenalectomized female rats of different age groups treated with aldosterone.
Figures as for Fig. 3.

Effect of cortisol

On urinary output, cortisol has, as is well known, a marked enhancing effect (Marcus, Romanoff and Pincus, 1950; Desaulles, Schuler and Meier, 1955). In *male rats* this effect is well developed, and ageing does not seem to modify it markedly (Fig. 7.)

On sodium, cortisol exerts initially a slight retaining effect that has already been reported (Dorfman, 1949; Johnson,

1954; Desaulles, 1958) and which is followed by enhanced sodium excretion (Fig. 8). In males these effects tend to disappear with advancing age.

On potassium, one observes the characteristic excretory response whose intensity is particularly high in young animals, its onset being somewhat more rapid in adult and old animals.
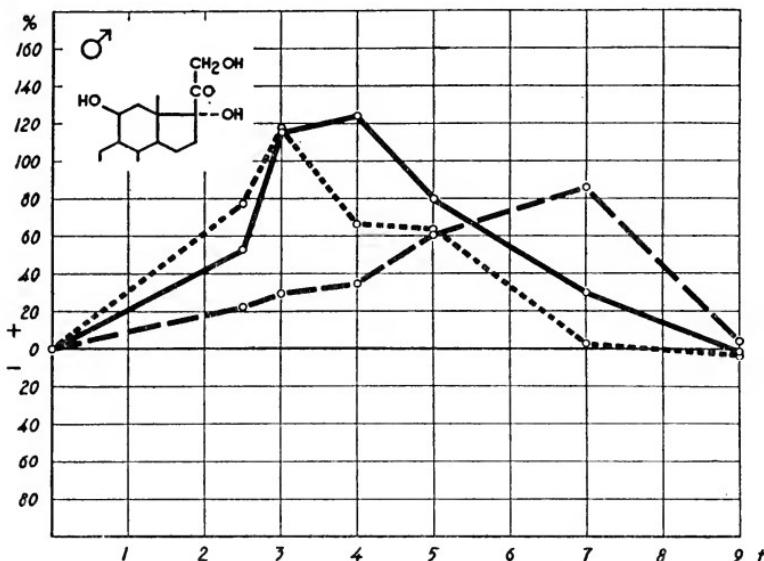
Fig. 7. Urinary excretion of adrenalectomized male rats of different age groups treated with cortisol (5 mg./kg.).

Abscissa: Duration of experiment (hours); collecting period 2 hours.
Ordinate: Urinary excretion as a percentage of the values of control animals.

Continuous line: 5-week-old rats.
Interrupted line: 15-week-old rats.
Dotted line: one-year- and more-old rats.

The effect of cortisol on the sodium/potassium ratio is first to lower it moderately in males, and to raise it afterwards to high positive values (Fig. 9). This effect, most marked in young animals, declines with increasing age.

In *female rats*, cortisol has a stronger enhancing effect on urinary output than in males (Fig. 10). With age, this effect increases and a certain latency of onset seems apparent.

On sodium, cortisol has similar retaining effects in females as in males and these disappear in old animals (Fig. 11).

As regards the enhanced sodium excretion which appears later, females react differently from males. Instead of dis-
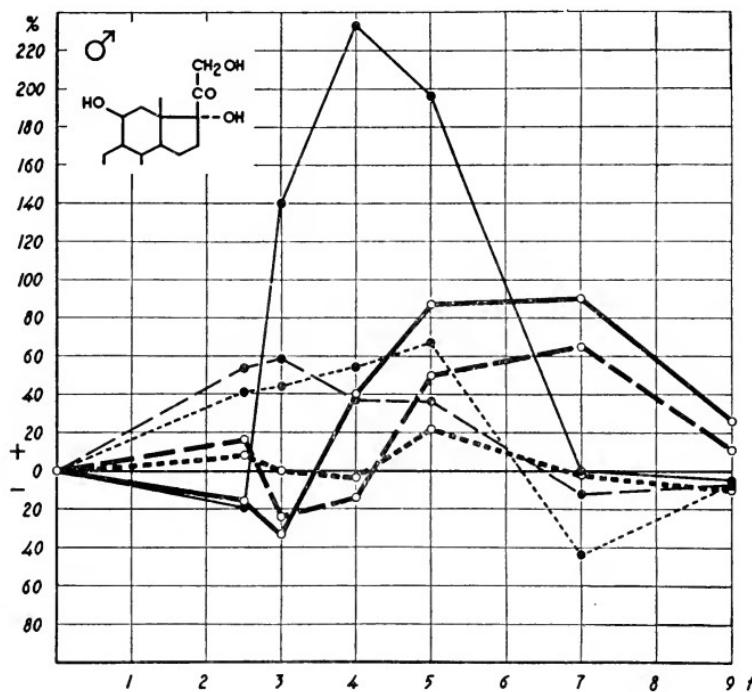
FIG. 8. Urinary sodium and potassium excretion of adrenalecto-mized male rats of different age groups treated with cortisol
(5 mg./kg.).
Thick line: sodium excretion.
Thin line: potassium excretion.
Other figures as for Fig. 1.

appearing with increasing age, the response remains high and its onset is more rapid in ageing females, although in this experiment animals of the adult group do not respond clearly.

The effect of cortisol on potassium excretion in females is similar to that observed in males, i.e. it is enhanced, the effects tending to decrease in intensity with age.
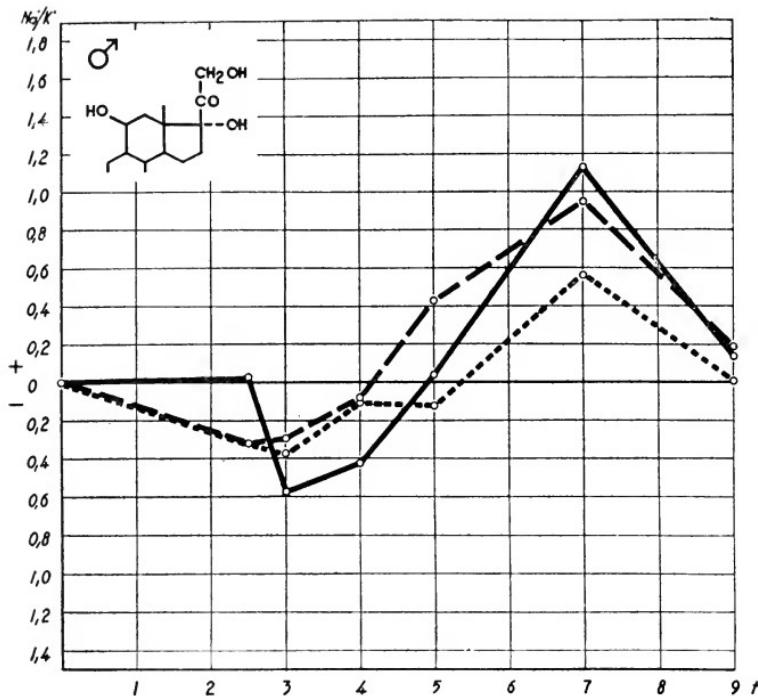
FIG. 9. Urinary sodium/potassium ratio of adrenalectomized male rats of different age groups treated with cortisol (5 mg./kg.)

Ordinate: Difference between sodium/potassium ratio of experimental animals and controls.

Other figures as for Fig. 1.
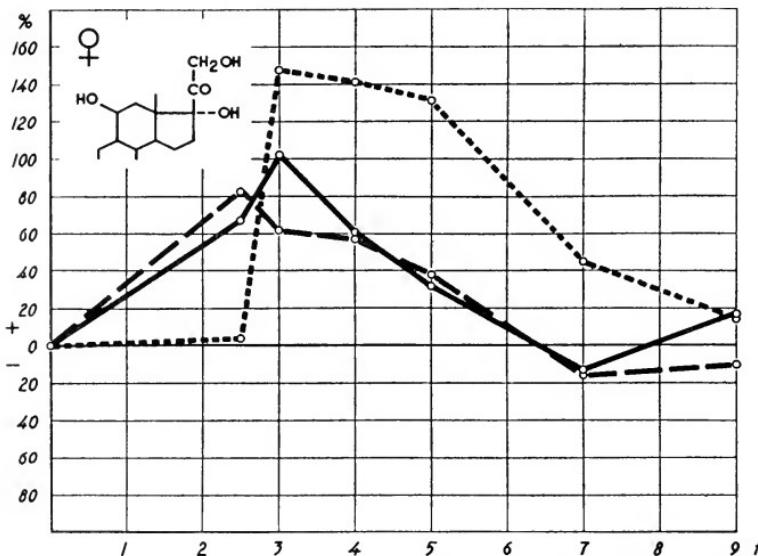
FIG. 10. Urinary excretion of adrenalectomized female rats of different age groups treated with cortisol (5 mg./kg.).
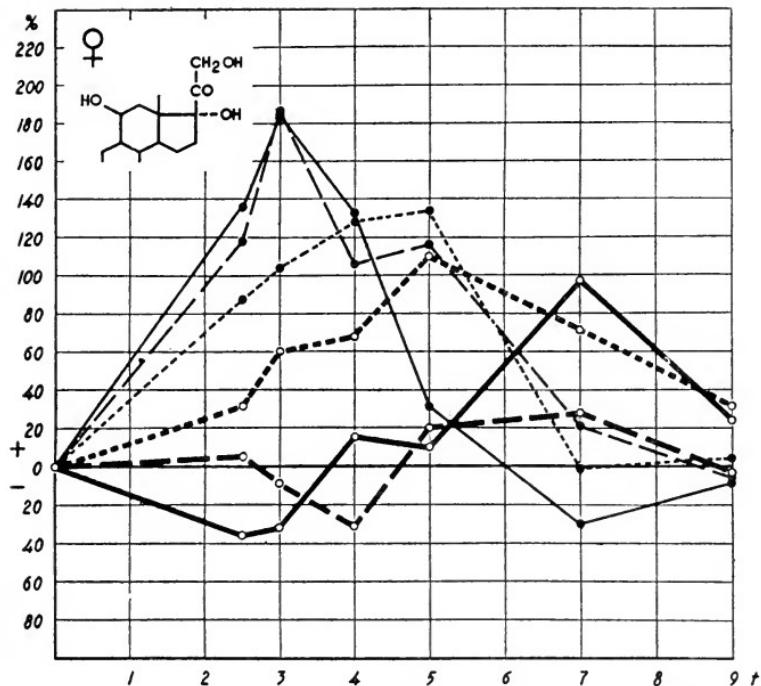
Figures as for Fig. 1.

FIG. 11. Urinary sodium and potassium excretion of adrenalecto-
mized female rats of different age groups treated with cortisol
(5 mg./kg.). Figures as for Fig. 2.
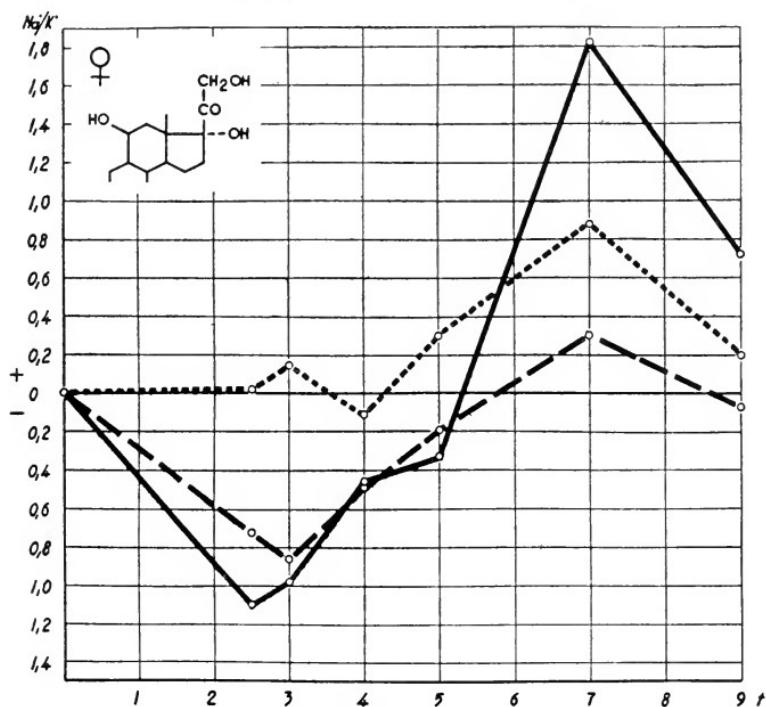
FIG. 12. Urinary sodium/potassium ratio of adrenalectomized
female rats of different age groups treated with cortisol (5 mg./kg.)
Figures as for Fig. 3.

On the sodium/potassium ratio, the effects are comparable to those obtained in males but are of greater intensity (more than twice the values observed in males) and of more rapid onset, and also tend to diminish rapidly with increasing age (Fig. 12).

Discussion

From the experimental results presented, it follows that age modifies the sensitivity of adrenalectomized rats to the influence of the adrenal steroids investigated. These modifications are qualitative as well as quantitative, the sex of the animals also playing an important rôle.

Whereas in male rats increasing age tends to reduce to control values the inhibiting effects of aldosterone on urinary output, it tends in females to induce a marked secondary diuretic response. The primary retention of sodium produced by aldosterone is of about the same order of magnitude in all animals, whether male or female, but ageing greatly increases the concomitant loss of potassium, this effect being particularly clear in male and female rats of the old age group.

In contrast, cortisol has an enhancing effect on diuresis which, especially in females, tends to increase with advancing age, whereas in males it is more intense from the onset and remains of about the same order. The effects of cortisol on sodium excretion are profoundly different with advancing age in rats of different sexes. In males these effects tend to disappear completely. In females, on the other hand, they appear earlier and remain of the same order of magnitude.

After cortisol treatment we can observe comparable differences in potassium excretion. Whereas young males respond with an intense potassium excretion which drops rapidly as the animals grow older, these changes are only moderate in females, potassium excretion remaining high until old age and its onset merely retarded.

These age and sex-bound differences become particularly clear if we study the variations in the sodium/potassium ratio. The sensitivity of the animals to the effects of aldosterone

increases with advancing age, females showing much greater differences than males. By way of contrast, sensitivity of the animals to cortisol diminishes with advancing age, females showing here too a greater sensitivity than males.

The similarity of the curves of the sodium/potassium ratio for aldosterone and cortisol is also striking and leads us to the problem of (a) the primary and (b) the secondary effects of these substances, and furthermore to the problem of the classification of adrenal steroids on the basis of what has been considered their most important physiological effects.

From previous experiments with aldosterone one is inclined to consider as primary effects both sodium retention as a consequence of increased tubular resorption of sodium ion, and potassium excretion as a consequence of the exchange between sodium ions in the tubule cells (Cole, 1957; Stanbury, Gowenlock and Mahler, 1958). Sodium retention remains of about the same order of intensity and duration from youth to old age in both males and females. It is concomitant potassium excretion that rises strikingly with advancing age both in males and females.

On the other hand, the diuresis induced by aldosterone, which is most apparent in old female animals in the later phases of the experiment, is most probably of secondary origin, its causes lying in the effect of aldosterone on the sensitivity of adrenalectomized animals to endogenous antidiuretic hormone (Gaunt, Lloyd and Chart, 1956).

As for cortisol, its essential effect seems to lie in the very marked potassium excretion which is regarded as running in parallel with its catabolic effects.

Its effect on potassium excretion tends to diminish with advancing age, male animals being here more susceptible than females. Conversely, the diuretic and sodium-excreting properties of cortisol seem to be caused essentially by the potent antagonistic effect of this steroid on the sensitivity of the animal to antidiuretic hormone; these properties tend to disappear with increasing age in males but not in females. Aldosterone and cortisol tend to induce a greater diuretic

response and concomitantly higher sodium excretion with advancing age, especially in females.

This, together with the similarity of the changes in the sodium/potassium ratio induced by aldosterone and cortisol during these experiments, even if the factors of ageing and sex act differently on them, underlines certain similarities of effect in a number of known adrenal steroids which have already been stressed (Meier and Desaulles, 1956; Gaunt and Chart, 1958). Relative dosage, time, age, experimental conditions and different stages of homeostasis are among the factors modifying these similar patterns of effect. The relation of homeostasis to the development of the animal organism is too complex to permit of any definite statement. We have simply tried to show that the properties of certain hormones may be profoundly affected by such factors as sex difference and increasing age, and that these differences may act in the same or in quite different ways and thus contribute towards a better understanding of pathophysiological changes due to age.

Summary

It has been shown that in rats of differing age and sex the sensitivity to the influence of aldosterone and cortisol on urinary electrolyte excretion varies greatly.

Whereas age tends to increase sensitivity of the animals to the effects of aldosterone, their sensitivity to cortisol by way of contrast tends to diminish.

On the other hand, female animals show a greater responsiveness to these changes than male animals.

These results are discussed.

Acknowledgement

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DISCUSSION

Adolph: Dr. Křeček, how do you account for what I take to be an absence of water diuresis in rats at 23 days of age? Is it because they are weaned early? Unweaned rats have a large water diuresis at this age.

Křeček: Water diuresis always occurs in rats of 23 days of age, but during the first three hours after a water load there is a retention of one-twentieth of the load. This figure was arrived at from balance tests, being the difference between water load and water excretion.

Heller: I am very pleased about the agreement between your findings and ours, Dr. Křeček. You use much the same technique as we did to estimate the response of your animals to vasopressin, and you say that you collect the urine for three hours after the injection. Did you have a special reason for choosing this time interval?

Křeček: Yes, it was because the pattern of diuresis changes after the administration of vasopressin, so that after three hours the excretion of the water load is complete.

Heller: For how long did your dose of vasopressin inhibit the water diuresis of the adult animals which you used for comparison?

Křeček: When we give enough vasopressin for maximum diuresis we

find that in young animals there is very little difference in water diuresis as compared to that in animals 33 days old. Between adult animals and 33-day-old ones there is no difference, but between 23 and 33 days there are variable, but statistically significant differences in the excretion of the water load.

Heller: That is almost exactly what we found; our age groups were 20–22 and 29–31 days after birth.

Borst: Has diurnal rhythm been taken into account by Dr. Desaulles and Dr. Křeček? Big differences can arise if the controls and experiments are not done at the same time each day.

Křeček: Our experiments and controls were always done at the same time in the morning. They were done in summer and in winter, with the same results.

Desaulles: Ours were done very early in the morning.

Adolph: Did you run controls without the hormones?

Desaulles: Every group was run with controls.

Borst: Light is not important. In blind people the diurnal rhythm remains normal if they are in light during the night and in the dark during the day.

Desaulles: We cannot cope with every activity, but we did think that light might be one of the problems.

Fourman: Dr. Desaulles, you drew an analogy between the effects of aldosterone and of cortisol on the excretion of sodium and potassium. If one considers the excretion ratio of these two ions in the urine, the effects do appear to be analogous. Fred Bartter and I first became interested in this question in 1949, when we began some studies which we completed about a year ago (1957. *J. clin. Invest.*, **37**, 872). In the human we were impressed with the fact that cortisol produces a large increase in the excretion of potassium which is transient even if the administration of cortisol is continued. It is not necessarily accompanied by a retention of sodium, but it is associated with an increase in the pH of the urine. With aldosterone, on the other hand, the loss of potassium is not transient; it is accompanied by retention of sodium and the pH of the urine does not change. On the basis of these experiments we felt that the effects of these two steroids on the electrolytes were quite different. We even suggested that the early effect of cortisol on potassium was secondary to a release of potassium from the tissues.

Desaulles: That is my opinion too.

Fourman: What strikes me is that pharmacologists are mistaken as long as they equate these end-effects of excretion of sodium and potassium, and as long as they speak about the alteration in Na/K ratio and use this as a measure of aldosterone effect. I think the early effect of cortisol on potassium may be a tissue effect; on the other hand the effect of aldosterone on the secretion of sodium may well be a renal effect, and I think you think so too.

Desaulles: Partly, yes. The cortisol effect on potassium is surely cellular.

Fourman: The early rise in potassium excretion with cortisol is probably a cellular effect. The results will not be very reliable if you assay a

hormone by a change in Na/K ratios in the urine, when the change is produced by two different mechanisms.

Desaulles: I just wanted to show in this experiment that ages bring changes, and sex too.

McCance: We are dealing here with the reactions and responses of an end organ, and it is a little difficult, apparently, to disentangle them.

Fourman: The effects that I am speaking about concern the immediate loss of potassium within eight hours of giving cortisol. This immediate large loss is completely out of proportion to any nitrogen loss, and in fact precedes a measurable nitrogen loss from the body. I was not concerned with the later catabolic response, only with the early potassium loss which is quite transient, and which is what people are concerned with when they assay so-called aldosterone activity in urine by Na/K ratios.

Milne: I am confused by your statement, Dr. Fourman. You make a clear distinction between potassium excretion following (a) cortisol, and (b) aldosterone. You tell us that the potassium excretion following cortisol is out of proportion to the nitrogen loss, and therefore is a true potassium excretion. You say that the difference is that potassium comes from the cells, but where do you think the potassium comes from after aldosterone excretion?

Fourman: It does appear that potassium excretion after aldosterone may be attributed to a change in the sodium-potassium exchange in the renal tubule, whereas the large and early transient potassium excretion with cortisol is not necessarily accompanied by any retention of sodium, and is associated with a rise in pH of the urine. Ultimately the potassium has got to come from the cells in both cases. But in the first case we are concerned with a primary renal effect, and in the second case I suggest—and it is only a suggestion—that there may be a liberation of potassium—presumably organically bound (in view of the alkaline urine)—from the cells, and that may be called a primary cellular effect.

Kennedy: Dr. Desaulles, when you spoke about the influence of sex were you thinking in terms of the actions of androgens or oestrogens? Your animals were not spayed, but is there a true sex difference?

Desaulles: The effect could be changed by ablation of one of the so-called specific sex organs. If you castrate males, you modify the results of the experiment quite considerably; if you spay the female, the changes are much less impressive, but there still remains a great difference between the two sexes. I want to stress here a point that is always a little puzzling to me: if you spay a female you produce a marked adrenal enlargement, but if you castrate a male the enlargement of the adrenals is not so obvious. We do find quite a lot of sex-bound differences in different functions of the animal, so I think that a very important part is played by the gonads.

Kennedy: If I understand you rightly, there is still a difference in the absence of both the adrenals and the sex organs.

Swyer: Is this difference after castration in the two sexes one which is independent of the time after castration, i.e. after a long time do the differences between the sexes become less?

Desaulles: That is a very important point, because it is very well known that if you castrate an animal and the time-lag is too great, the responsiveness of certain sexual adnexal organs disappears. We used the following method in our work. We castrated the animals, in these and other similar experiments, and at different periods after castration we tested the sensitivity of their sexual adnexa. We observed that what was found by Parkes and Deanesley about 20 years ago is still absolutely valid. You must begin the experiments between two and three weeks after castration; after that the sensitivity diminishes very rapidly. If you wait from one to three months some responses disappear completely, and you need very high dosages of the substance to obtain resensitization of certain organs.

Swyer: I was thinking not so much of that, but of whether the response to the adrenal steroids shows a sex difference which is diminished but not entirely removed by castration.

Desaulles: I have not enough experience of all the effects that may be considered to say anything definite about this point, but it still seems to me that castration in itself does not suffice to abolish certain existing differences between the sexes in their response to adrenal steroids.

THE EFFECT OF AGE ON THE ELECTROLYTES IN THE RED BLOOD CELLS OF DIFFERENT SPECIES

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Two kinds of age changes may occur in the red blood cells. The erythrocytes themselves have a definite length of life which may be determined in various ways, whereas the longevity of "fixed" tissue cells generally cannot be as exactly indicated. Thus, as cells erythrocytes may be "young" or "old". On the other hand, like any other cells of the body, the red cells may be a part of a young or of an old organism.

Cellular age

In order to study age changes in the erythrocytes as cells, two principal ways are open. One of them is to produce anaemia, e.g. by bleeding, and thus to stimulate erythropoiesis, so that a large proportion of the circulating cells will have been produced within a relatively short period. The writer is not aware of any systematic study of the red cell electrolytes throughout the regeneration after acute bleeding. In microcytic anaemias of man—which is the type seen also in bleeding anaemia—the concentration of potassium in erythrocytes is lower than normal (Maizels, 1936). In other types of anaemia a change in the opposite direction may occur (Maizels, 1936; Selwyn and Dacie, 1954; McCance and Widdowson, 1956). However, changes in the electrolytes observed in any type of anaemia are not necessarily dependent on the age of the erythrocytes, but may be caused by many other factors associated with anaemia.

Recently it has been claimed that other methods for studying young or old erythrocytes might be feasible. According to Borun, Figueroa and Perry (1957), after centrifugation of blood the bottom layer contains the oldest cells, and the surface the youngest ones. An analysis of the different layers has shown that—at least in human adult blood—the packing is closest and the amount of intercellular plasma lowest in the bottom layer, but when the effect of different packing is corrected there is no difference between the sodium and potassium concentrations of the bottom and the surface erythrocytes (Leppänen, personal communication). Serial osmotic haemolysis has also been suggested as a means for differentiating young and old erythrocytes (Simon and Topper, 1957). The value of these methods is not yet clear. However, the nature of the methods used suggests that changes in the electrolyte metabolism of the erythrocytes may be involved in their ageing, though such changes may not necessarily result in differences in the concentration of sodium and potassium.

Age of the animal

As a mixed population of different cellular ages, erythrocytes are easily available. The availability and development of flame photometric analysis have been a stimulus for several investigations of the electrolyte content of the red cells. It has been found that in general the sodium and potassium content of the erythrocytes *in vivo* is fairly stable, typical of the species, and resistant to many physiological and pharmacological agents. However, in disease, particularly in febrile states, erythrocytes tend to lose potassium and gain sodium: in other words, the electrolyte composition of the erythrocytes moves closer to that of plasma.

Sheep and other ruminants. It may be inferred from results published by Green and Macaskill (1928), and by Wise and co-workers (1947) that the intracellular potassium concentration is higher in the blood of young calves than in that of adult cattle. These two papers were the first to indicate that

the red cell electrolytes may change with age. The subject was taken up by Hallman and Karvonen (1949) in another species, sheep. A distinct difference between foetal and adult Finnish sheep was observed, in the sense that the concentration of potassium in erythrocytes was higher in foetal than in adult sheep. Fig. 1 shows the differences in both
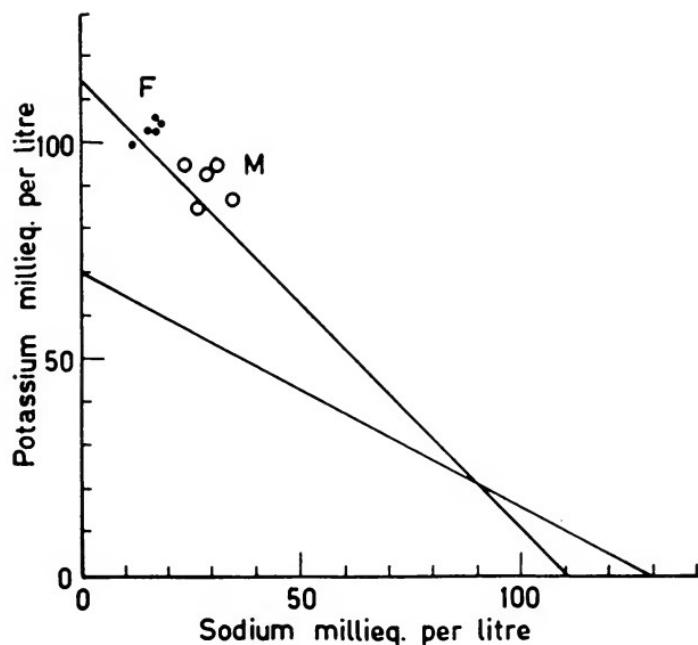
FIG. 1. The concentration of potassium and sodium in the erythrocytes of sheep foetuses (F) and their mothers (M) belonging to the Finnish breed (Hallman and Karvonen, 1949). The corresponding figures for the red blood cells of adult sheep of other breeds fall along the two straight lines (Evans, 1957).

sodium and potassium concentrations. The sum of the two electrolytes tends to remain constant with age.

Widdas (1954) confirmed this observation and found a gradual decrease of the potassium and an increase of the sodium with advancing foetal age.

The study by Hallman and Karvonen (1949) brought out another interesting finding. In 1898 Abderhalden published

the first values for the sodium and potassium concentration of adult sheep erythrocytes, and found that they belong to the "low potassium—high sodium" type. In 1937 Kerr observed higher potassium concentrations, with a large variation between individual sheep. In the determinations of Hallman and Karvonen, the erythrocytes of the Finnish sheep turned out to be—contrary to those of Abderhalden—of the "high potassium—low sodium" type, containing still more potassium than the red cells of Kerr's sheep. Sheep erythrocytes thus show a large range of individual variations in the electrolyte composition.

The electrolytes are not the only constituents of the red cells in which individual sheep differ. The solubility characteristics of sheep haemoglobin obtained from different countries, from different breeds, or from different sheep may also differ (Karvonen, 1949; Karvonen and Leppänen, 1952). It was natural, as a working hypothesis, to connect with each other these differences in the red cell electrolytes and in the type of haemoglobin. In the first five samples representing different breeds of sheep, haemoglobin prepared from the low potassium erythrocytes actually showed a crystal habit different from that of the high potassium cells (Karvonen and Leppänen, 1952).

Since these early attempts the red cell electrolytes of sheep have become the subject of intense study. The individual differences in the electrolyte composition have been shown to be permanent characteristics (Evans, 1957). The occurrence of different types of red cells in a number of breeds has been studied, and the genetics of the inheritance have been worked out (Evans, 1954, 1957; Evans and King, 1955; Evans et al., 1956; Evans and Mounib, 1957).

The application of paper electrophoresis to sheep haemoglobins has shown that though there is a definite association between the electrolytes in the red cells and the haemoglobin, this association is not absolute (Harris and Warren, 1955; Evans et al., 1956; Evans, Harris and Warren). On the other hand, the haemoglobin present in the red blood cells has an

influence on the concentration of potassium in the whole blood of both high potassium and low potassium sheep, and thus presumably also on the concentration of potassium in the cells themselves (Evans *et al.*, 1956).

The study of individual differences between adult sheep thus shows that the type of haemoglobin is associated with the red cell electrolytes, but that other factors also play a rôle.

Haemoglobin changes with age: the haemoglobin of a foetus differs from that of an adult, but after the production of the adult type is once established, no further changes with age are known to occur.   For instance, the haemoglobin of a sheep of the age of 14 years showed solubility characteristics identical with that of younger animals (Karvonen, unpublished.)

The transition from foetal to adult life involves a change of haemoglobin and of the red cell electrolytes. In sheep, these two changes appear to start before delivery and to be completed some time after birth (Karvonen, 1949; Hallman and Karvonen, 1949; Widdas, 1954). Whether the changes are exactly parallel would be a subject of considerable theoretical interest.

Other species. The effect of age on the electrolyte concentration of red cells has been studied in few other species. Remarkably enough, a relationship just opposite to that in ruminants has been found: the sodium concentration is higher and the potassium concentration the same or lower in foetal than in adult erythrocytes, at least in man (Hallman, Österlund and Vara, 1954; Österlund, 1955; McCance and Widdowson, 1956), pig (McCance and Widdowson, 1956), and in guinea pig (Widdas, 1954, 1955; Karvonen and Leppänen, unpublished). The concentration of chloride changes in the same direction as that of sodium.

Underlying mechanisms

It has been pointed out by Conway (1957) that the smaller a cell, the more work per unit cell volume a "sodium pump" must do in the same environment of plasma or extracellular fluid, in order to keep the intracellular sodium at constant

level. A similar conclusion applies to an eventual "potassium pump". The erythrocytes of a foetus are larger than those of an adult. With constant activity of the electrolyte pumps an increase in the cell sodium and a decrease in potassium would be expected from foetal to adult life. This is the direction of development in the ruminants, but not in the other species examined. It is rather questionable whether the decrease in cell size even in the ruminants is an important cause of the changes of the red cell electrolytes.

With the aid of *in vitro* studies much progress has been made in elucidating the mechanism of cation transfer across the red cell membrane. The application of these methods to the erythrocytes of the foetus suffers from a serious limitation: the cells of foetuses (at least human and sheep) haemolyse spontaneously and rather fast *in vitro*. To some extent the rate of haemolysis is dependent on oxygen tension, high oxygen tension increasing the rate of haemolysis, but haemolysis also occurs at an appreciable rate in blood exposed to nitrogen. Haemolysis in human cord blood may also be retarded by administering ascorbic acid to the mothers before delivery, but even so the rate of spontaneous haemolysis remains considerably higher than in adult blood. An addition of ascorbic acid *in vitro* is without effect (Räihä, 1956, and personal communication).

Summary

Information on changes in the electrolyte metabolism of individual erythrocytes during their life cycle is meagre. However, the claims that young and old cells may be separated with the aid of centrifugation or serial haemolysis suggest that changes in the electrolyte metabolism may be involved in the ageing of the red cells. Differences in the actual sodium and potassium concentrations have not, however, been demonstrated.

In sheep and cattle the erythrocytes of a foetus contain more potassium and less sodium than those of an adult. In man, pig and guinea pig, a difference in the opposite direction

has been observed. The eventual association of the difference in red cell electrolytes with a difference in haemoglobins and with a difference in cell size is discussed.

In vitro studies of foetal erythrocytes and, particularly, their interpretation, are handicapped by a fast rate of spontaneous haemolysis in foetal blood. In man this may be retarded by exposing the blood to nitrogen and/or by administering ascorbic acid to the mother before delivery, but even so the rate of spontaneous haemolysis remains far above that observed in adult blood.

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DISCUSSION

Davson: The most interesting thing here is the finding that these red cells have a very high sodium concentration and a low potassium one. One thinks of it at first as a primitive feature. On the other hand, when one looks through the animal species in which it happens, it is most prominent in the cat and dog, whilst the guinea pig, which we think of as a rather primitive animal, has a very high potassium just like man. So it has nothing to do with that. Then you also think of it as a failure to develop a potassium-accumulation mechanism. There again, it is probably not to be considered as a failure at all. The red cell is derived from a very highly developed nucleated cell and most likely the erythroblast has the ability to concentrate potassium. Then when the cell becomes a reticulocyte or an erythrocyte it loses the power of accumulation of potassium. This 'loss' could be a development in the interests of economy, because much less energy is required to maintain a cell with a low concentration of potassium than with a high one, and the erythrocyte has only an anaerobic source of metabolism.

Your results with the foetal cells are interesting, Dr. Karvonen. With sheep, you find that the foetal erythrocytes have the high potassium and it looks as if as they develop they lose the power of accumulating potassium. But then, with the other species, we get the reverse. I think a lot more work on the spontaneous haemolysis is necessary. Haemolysis usually has a very definite cause and is usually due to the fact that the permeability of the membrane becomes abnormally high and you get this Donnan difference of osmotic pressure being exerted between the plasma and the contents of the cell. Therefore, the most profitable line of research would be to try and get conditions in which you could prevent this haemolysis from occurring.

Milne: Is there any data available on the foetal levels in the cat and the dog, which have this very high sodium content in the erythrocytes?

Karvonen: No, we have none.

Davson: The sheep can have as high a sodium content as the cat, yet there is very little difference between foetal sheep. Would it be possible to get a nucleated stage in the erythrocyte of the mammal and study its potassium content? It could almost be done histochemically, just to get a qualitative idea of the content.

Karvonen: That would be a very interesting thing to do.

Fourman: Tosteson reported a low erythrocyte potassium in sickle-cell anaemia (1953. *J. clin. Invest.*, **32**, 608). That confirms your view that the level of potassium in the blood may be related to abnormal haemoglobins; is there any information on that, outside man?

Karvonen: In sheep, the type of haemoglobin affects the absolute level of electrolytes within the same group. If you have sheep with low potassium-containing red cells, and one of the animals has a different type of haemoglobin, the electrolyte level in its red cells is also slightly different.

Desaulles: Has not the same effect been described for some kind of

deer? Deer may have sickle cells, and this is correlated with a certain type of different haemoglobin.

Davson: I know the camel has ellipsoidal cells.

Bull: Is anything known about the relative efficiency of the different kinds of red cells with respect to their function of carrying oxygen, in relation to pH changes, carbon dioxide changes, etc.

Karvonen: Quite a lot is known about species and foetal-adult differences, but nobody has studied these aspects within one species, and at the same time paid attention to the intra-species variations in intracellular electrolytes.

Hingerty: It seems from the last three papers that there may be some sort of late development of function as regards sodium and potassium control. It may be something, according to Dr. Desaulles's work, that develops in the rat at about 5–6 weeks, or something that increases the efficiency of sodium—potassium exchanges across the cell membranes, or the reabsorption rates in the renal tubules. During our potassium-depletion experiments we found that in young rats up to six weeks of age we could replace about 25 per cent of the muscle potassium by sodium on potassium-deficient diets (Conway, E. J., and Hingerty, D. J. (1948). *Biochem. J.*, **42**, 372). When we repeated the experiment we happened to use rats of about nine or ten weeks old, and we found that the exchange rates were much lower. Probably a greater efficiency develops in the interval; either the cell holds on to the potassium more efficiently or the sodium pump works more efficiently. Possibly these changes are gradually developing in the growing animals and their responses to hormones may also develop gradually.

Shock: One of the problems that occurred to us was whether the erythrocytes that are formed in the normal course of turnover in the very old individual can act as effectively as those in the young individuals. We have not yet done the obvious experiment of producing a stress which causes haematopoiesis, but we have examined the osmotic fragility of red cells from individuals between the ages of 20 and 90, with about ten individuals in each decade. With careful control of the pH, which influences the fragility rather markedly, we found no striking evidence of differences in the osmotic resistance of red cells taken from individuals as old as 90, as compared with the young individual.

I also wonder whether there are subtle differences between the chemical structure of haemoglobin formed in an old individual as compared with that in the young or middle-aged person. If the haemoglobin from 80–90-year-old individuals had been subjected to as detailed and careful an analysis as that which resulted in the identification of the different types of haemoglobin in the foetus, perhaps we would have found that differences appear after a lifetime of utilizing the mechanism for making haemoglobin.

McCance: Dr. Davson, can you comment upon the genetic side of this? You spoke about the sodium pump; what about the difference in haemoglobin?

Davson: I cannot relate this at all. I do not see why a given type of haemoglobin should be associated with a given electrolyte content.

Black : On the genetic side it is very odd that one gets this scatter right along the line. One would think that, according to Mendel, one would get segregation at the two ends of the line.

I was not clear whether there was an excess of fluid in the red cells. In other words, in the foetal sheep or man was there an excess of potassium per litre of red cells? Was there any difference in phosphate content? Differences in phosphate content have been described, I think, by Prankerd (1955, *Clin. Sci.*, **14,** 381) and others in connexion with the sickle cell problem, and I wondered whether that side had been gone into with foetal versus grown-up sheep.

Karvonen : I am afraid I gave a wrong impression when I said the scatter was all along that line. There is a very clear concentration at each end of the line but there is also a group in between. Within each group, however, there is quite a considerable scatter which is due to a permanent, individual characteristic of each sheep. The statisticians say that there is quite a high intra-individual correlation.

The foetal cells contain more water than the adult cells. In sheep I do not think that any determinations of the phosphate have been done, but in man and in pig it has been found (McCance and Widdowson, 1956) that the phosphate of the foetal cells is higher.

Davson : It must be realized that when red blood cells are analysed, very large numbers are used; there may well be differences in concentrations of potassium and sodium amongst the individual ones, and they could well fall into groups which would never be discovered. Variations in the Na/K ratio could be reflections of variations in the proportions of high potassium and low potassium cells, which would give a continuous scatter right along the line.

THE DEVELOPMENT OF ACID-BASE CONTROL

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General Principles (as they apply to adults)

When the body of a healthy person is provided with the diet normally eaten in Europe and the United States, it produces in its metabolism more non-volatile anions than cations. These "surplus anions" are excreted by the kidney partly in combination with titratable hydrogen ions (the titratable acidity) and partly as ammonium salts. The ammonium salts usually account for rather more than 50 per cent of the total. If the excess of non-volatile anions increases, the pH of the urine falls and the titratable acidity increases, but the excretion of ammonia also increases because a fall in the pH of the urine is *one* of the things which raises the output of ammonia; and consequently the percentage of the surplus anions excreted as ammonium salts remains about the same. The excretion of ammonium salts is also increased (a) if the pH of the urine is maintained at its lower limits for some time by the continuous administration of acid or acid-forming drugs. This is thought to be due to an increase in the activity of the enzymes in the kidney which catalyse the formation of ammonia and particularly of glutaminase (Davies and Yudkin, 1952). (b) By an increase in the acid "load" (Rector, Seldin and Copenhaver, 1955). Both (a) and (b) increase the percentage of the surplus anions excreted as ammonium salts, and good examples of the effects which may be observed after continuous high dosage are given by Ryberg (1948). As the pH of the urine rises progressively above 6·5 the percentage of the total output of surplus non-volatile anions excreted as ammonium salts may also rise and ultimately

reach 100, because above pH 6·5 the excretion of titratable acid falls more rapidly than the ammonia and is extinguished before the excretion of ammonia, which continues at a decreasing rate up to pH 8. This tendency of the percentage to rise as the pH of the urine goes above 6·5 is therefore exaggerated if the urines are titrated, as they mostly are nowadays, to pH 7·4 instead of, as at one time, pH 8.

Dihydrogen orthophosphates are the main buffer acids which can be titrated in a normal adult's urine, but this may not be so in disease if there is a great excess of abnormal organic acids of the right buffer strength in the urine, such as β-hydroxybutyric acid or amino acids. Apart from the phosphates and weak organic acids which contribute by their presence to the titratable acidity, the surplus of non-volatile anions in the urine is very largely due to sulphates, derived from the metabolism of protein (Hunt, 1956). Chlorides are generally balanced by the equivalent amount of fixed base unless calcium or ammonium chloride has been taken to produce a chloride acidosis.

The ability of the kidney to excrete hydrogen ions into the tubules, and so to excrete the surplus non-volatile anions in the way described, depends upon the activity of carbonic anhydrase. Since it has been shown experimentally that the degree to which the pH of the urine can be lowered depends upon the activity of the carbonic anhydrase at any given time, it may be that the lower and well-known limit of urinary pH attainable by a normal person is an expression of the activity of his carbonic anhydrase, but this is merely a suggestion at the moment.

The Newborn Period and Later Infancy

Complete collections of urine from three healthy baby boys have been made for the first 48 hours of their lives, and again over the whole of the 7th–8th day. These babies all passed urine at the moment of birth and this was also collected. Urine passed by two other babies at birth has also been included in the series, and a 24-hour collection has been made

on four additional babies on the 7th to 8th day. Of the seven babies investigated one week after birth, six were breastfed and the seventh was fed on Ostermilk. Samples of blood have been taken from the cord at birth, and from the femoral vein at 48 hours and seven days. Urine has also been collected for 24 hours from one child aged eight months and from one aged one year, while six normal men and women have provided 24-hour urine collections to serve as the adult comparisons. The urines were collected and stored under toluene. Determinations of pH, titratable acid, ammonia, creatinine, phosphate, citrate and sulphate have been made on the urine, and the sera have been analysed for creatinine, CO_2, chloride, sodium and potassium.

The excretion of surplus anions

Fig. 1 shows the millimoles of surplus anions not combined with fixed base (i.e. titratable acid plus ammonium salts) excreted by the infants on the first, second and seventh days of life and by the older infants. A figure for the adults is indicated also. All the values are expressed per kg. of body weight per day. The average pH of the adult urine was 6 or a little over, while that of the babies was between $5 \cdot 5$ and $5 \cdot 8$, and this has to be taken into account in considering some of the results. The urine passed in the first and second 24 hours of life contained less surplus anions per kg. of body weight than that of the adults although the pH of the urine was lower, which would have led one to expect a higher rather than a lower anion excretion. This low rate of excretion was quite sufficient to maintain the acid-base balance of the body, for the serum CO_2 and chloride did not change. It is to be attributed to the fact that the urine contains very little phosphate or sulphate at this period (McCance and von Finck, 1947, and see later), owing to the small breakdown of tissue protein (McCance and Strangeways, 1954). By the seventh day the babies were taking nearly 500 ml. of breast milk a day, which contained $9 \cdot 5$ g. protein or about 3 g./kg., and they were passing about three times as much urine per kg. of

body weight as the adults. Their excretion of surplus anions, sulphates among them, per kg., had reached the adult level although they were still excreting little or no phosphate. The pH of their urine was a little higher than it was on the first two days, and the increased volume may have been one reason for this (McCance and von Finck, 1947; Hungerland, 1957).

At eight months to one year of age the babies excreted more
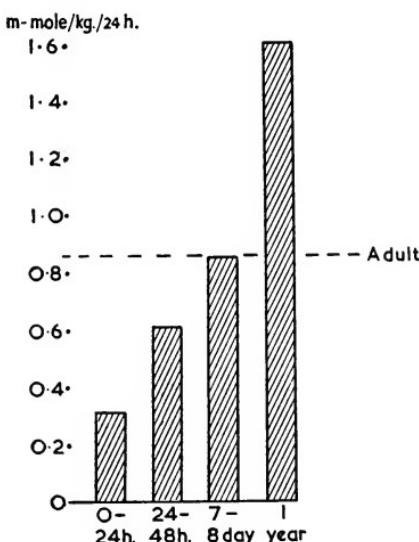
Fig. 1. Surplus anions (not combined with fixed base) excreted by babies during the first week and at 8 months to 1 year of life.

surplus anions per kg. of body weight than the adults. This is explainable by the high intake and metabolism of protein per kg. of body weight at this time of life. A child of one year consumes about $3 \cdot 5$ g. protein per kg., which is two to three times as much as an adult per kg., and only 8 or 10 per cent of it is used for growth in contrast to the 50 per cent or so retained in the neonatal period. The phosphates and the cystine and methionine in the milk and other protein foods were probably the main sources of the surplus anions.

Fig. 2 shows the percentage of the surplus anions excreted with ammonia. For this it is possible to give a figure for urine which was formed *in utero* and passed at the moment of birth and which had a pH of over 6. It will be observed that, although the pH of the urine passed at birth was higher than that of the urine passed afterwards, the percentage of the surplus anions excreted with ammonia was also very high before birth, and of the order to be expected in adults with
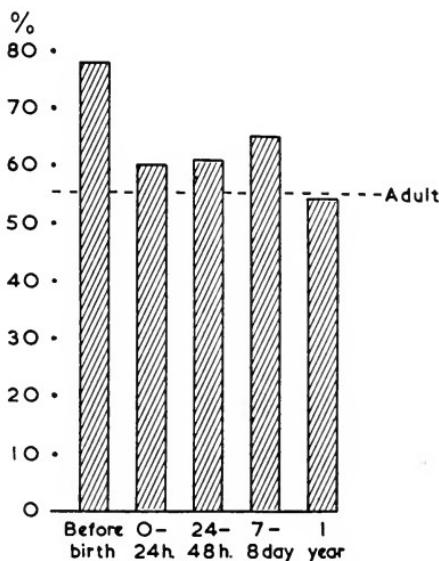
FIG. 2.   Percentage of the surplus anions excreted as ammonium salts.

very acid urines after taking large doses of ammonium chloride for some days. The percentage of the surplus anions excreted with ammonia in the first 48 hours and on the seventh day of life has also tended in our series to be higher than that in the urine passed by adults. This is probably because the babies' urine contained so little phosphate, and consequently the titratable acidity was low in relation to the total amount of surplus anions to be excreted. It was not because the ability of the newborn kidney to produce ammonia was greater than that of an adult, for all the evidence is against this. Work

which has been done on kidney slices *in vitro* (Robinson, 1954), and on renal glutaminase and ammonia production (Hines and McCance, 1954) goes to show that, weight for weight, the kidney of the newborn of other species contains less glutaminase and produces less ammonia than that of the adult. Fig. 3 shows that the total amount of ammonia excreted per kg. of body weight was in fact small in the first two days, but that by a week, when the baby was taking in three times as much protein as the adult per kg. of body weight, it had risen above the adult level. The ability to form ammonia in response to an acid load in the first day or two of life has not yet been
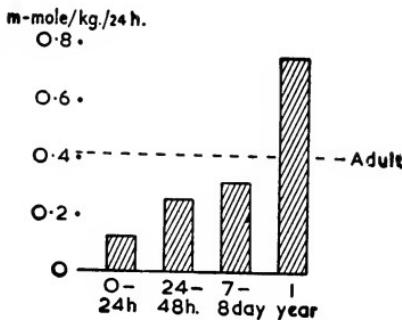
Fig. 3.   The amount of ammonia excreted.

studied in man, but Cort and McCance (1954) found it to be smaller in puppies two days old than in adult dogs. The matter requires further investigation.

Fig. 4 shows the excretion of ammonia in millimoles/24 hours divided by the glomerular filtration rate (as measured by the endogenous creatinine clearance) in ml./minute. It was possible to calculate this ratio for the urine passed at birth, even though the rate of urine flow before birth was not known, because the two functions being compared are both expressed in terms of rates of urine secretion. The excretion of ammonia was high *in utero* and in the newborn period in relation to glomerular filtration rate. The glomerular filtration rate at this time of life is very low by adult standards, and if the endogenous creatinine clearance is a true measure of it,

it is evidently lower even than the excretion of ammonia. By one year of age the glomerular filtration rate/kg. had risen above that of adults (McCance and Widdowson, 1952), and more ammonia and surplus anions per kg. were being excreted (see Figs. 1 and 3); the amount of ammonia excreted per ml. of glomerular filtrate was near the adult level.
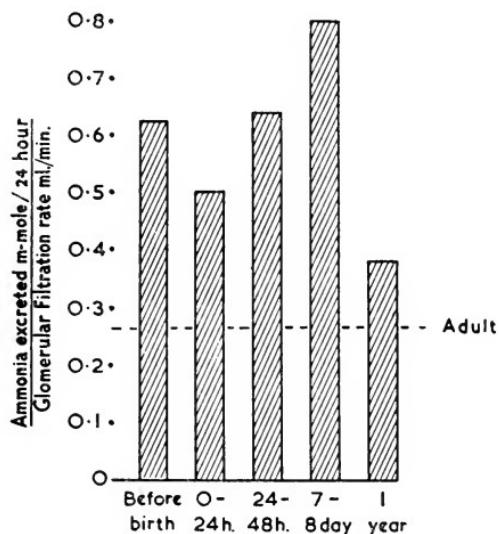
FIG. 4. The ratio of the ammonia excreted (m-mole/24 h.) to the glomerular filtration rate (ml./min.).

The nature of the titratable acidity

Fig. 5 shows the excretion of titratable acid per kg. of body weight by the babies and the adults. The amount excreted was low during the whole of the first week, but it was rising even though the urine still contained no phosphates. The high excretion at a year is again related to the high intake of protein at that age.

Fig. 6 shows the percentage of the titratable acidity due to phosphate in the urine of an adult and in the urine of a breast-fed baby in the first week of life. In the adult the percentage depends upon the pH and, since the pH of the urine passed

by the present series of adults was higher than that of the newborn infants, the value for adults shown in Fig. 6 (70–80 per cent) has been taken from Gamble (1942). Phosphates accounted for a very small fraction of the titratable acidity of
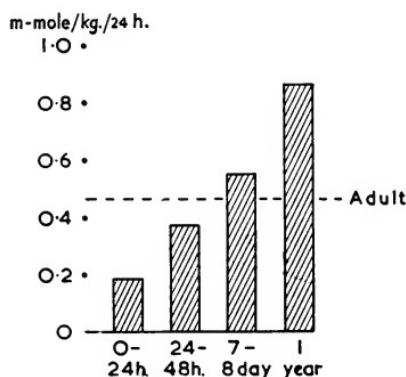
FIG. 5. The excretion of titratable acid.

the infant's urine, which is due to the fact, already mentioned, that the urine of breastfed infants contains so little phosphate at this time of life.

Investigations are being made on the organic acids in the
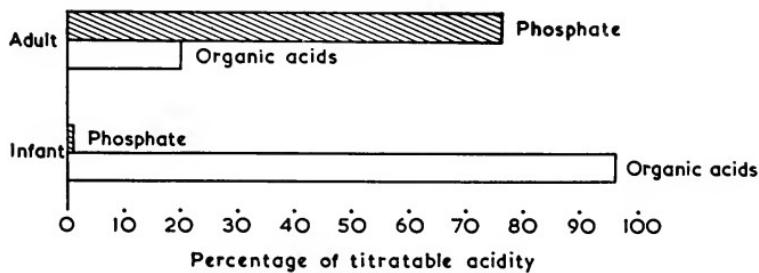
FIG. 6. The proportion of titratable acid due to phosphates and organic acids in the urine (pH 5·5–6·0) of adults and infants.

urine during the first week of life. Citric acid is one of the major constituents, and on the seventh day the breastfed babies were found to be excreting 33 mg. citrate/kg. body weight/24 hours (Stanier, personal communication). This

is more than the amount excreted by the adults in this series. In so far as citric acid may be regarded as a product of the metabolism of the kidney it cannot be classed as a surplus anion although it contributes to the titratable acidity.

It is well known that infants on cows' milk mixtures have a higher concentration of inorganic phosphorus in their serum than breastfed infants; they excrete phosphates by the seventh day of life, and the phosphate-organic acid relationship is of the adult pattern, as it is also in the urine of infants eight months to one year of age.

Foetal Life

In the uterus the acid-base balance of the whole conceptus is regulated ultimately by the mother's lungs and kidneys, but the foetal kidneys, membranes and placenta act as intermediaries.

Urine has been taken from the bladders of five human foetuses aged 10–20 weeks. It has always been found to be hypotonic, due mainly to very low concentrations of sodium and chloride. It appears to resemble the urine formed *in utero* and passed at term which has been better investigated and described elsewhere (McCance and Widdowson, 1953; Hanon, Coquoin-Carnot and Pignard, 1955, 1957).

The pig has a gestation period of about 120 days. Between the 20th and 60th day there is a rapid expansion in the volume of allantoic fluid. The sac containing the fluid has free connexion with the kidney through the urachus and bladder. Its membranes also participate in exchanges with the mother. Table I shows the composition of the fluid at 20 days, 45 days and 60 days. At 45 days both mesonephros and metanephros are functional, but the former is becoming less so. The volume of fluid in the sac is very variable (Wislocki, 1935), but it far exceeds the weight of the foetus. The osmolar concentration falls greatly so that from 45 days it is only one-half or one-third that of foetal serum (McCance and Dickerson, 1957). This fall in osmolar concentration is due largely to a fall in the concentration of sodium and chloride. The

concentration of potassium does not fall in the same way, and the concentration of calcium rises. This calcium appears to be held in solution by citric acid (Economou-Mavrou and McCance, 1958).

The fluid at 45 days has been found to have a pH between 5·5 and 6, and a titratable acidity of about 10 m-equiv./litre. The fluid contains ammonia, and ammonia appears to account for about 25 per cent of the titratable acid plus ammonia found in it. The concentration of phosphates is always small, and the acidity is almost entirely due to carbonic acid. The

Table I

THE WEIGHT OF THE FOETAL PIG AND THE VOLUME AND
COMPOSITION OF ITS ALLANTOIC FLUID

Foetal age	20 *days*	45 *days*	60 *days*
Weight of foetus	0·1 g.	20 g.	100 g.
Volume of allantoic fluid	5 ml.	110 ml.	350 ml.
Composition of allantoic fluid			
Osmolar concentration m-osm./l.	256	120	92
Urea m-mole/l.	3·1	8·4	10·3
Chloride m-equiv./l.	69	30	18
Sodium ,, ,,	114	13	14
Potassium ,, ,,	14	8	6
Calcium mg./100 ml.	6	30	—
Inorganic phosphorus mg./100 ml.	9	6	—

pH rises quickly if the fluid is shaken or even if it is left in a tube exposed to the air, and it was found necessary to collect and analyse the fluid out of contact with air. Lutwak-Mann and Laser (1954) found no "bicarbonate" in pig's allantoic fluid at 20 days' gestation, but there is no doubt about the presence of carbonic acid at 45 days.

Further investigation has confirmed the fact, first noted by Lutwak-Mann (1955), that the chorioallantoic membrane contains carbonic anhydrase. At 45 days the allantoic fluid itself also had some carbonic anhydrase activity. On the basis of material from three pregnant pigs the activities of carbonic anhydrase may be given as foetal kidney $+++$, chorioallantoic membrane $++$, allantoic fluid $+$. It is

hoped to extend the study to later stages of gestation, to other membranes and to glutaminase.

It is an open question at present whether the fluid found in the sac at 45 days is a hypotonic urine elaborated by the foetal kidney and similar to that formed by the human kidney before birth, or whether the fluid has been made hypotonic and acid by the action of the membranes themselves. Small amounts of fluid have been removed from the bladders of foetuses at 45 days and it is probably possible also to withdraw fluid from the large mesonephric duct, so that this problem may be soluble without recourse to large-scale experimental veterinary obstetrics. Should it turn out that the composition of the fluid is being altered by the membranes, their activities may contribute materially to our ideas about the function of the renal tubules.

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DISCUSSION

Zweymüller: The identification of organic acids in urine by paper chromatography is elegant and of general application. The R_F values of the different organic acids are distinctly different and therefore a clear separation on the paper is possible. We used the technique developed by Nordmann and co-workers (1954. *C.R. Acad. Sci., Paris,* **238,** 2459), and Fig. 1 demonstrates the position on a two-dimensional descending chromatogram of some non-volatile, water-soluble organic acids which
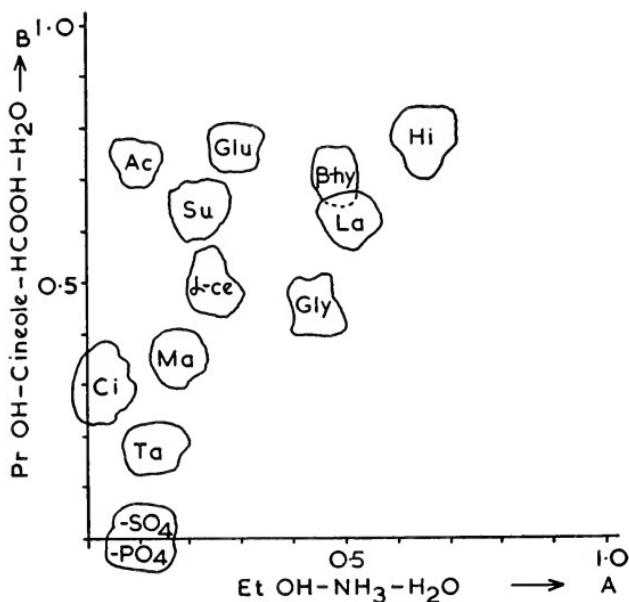
FIG. 1 (Zweymüller). The position of some organic acids in the urine of a normal adult on a two-dimensional descending chromatogram.

Ci = Citric acid, Ta = Tartaric, Ma = Malic, Gly = Glycolic, α-ce = α-ketoglutaric, Su = Succinic, Ac = Aconitic, Glu = Glutaric, β-hy = β-hydroxy-butyric, La = Lactic, Hi = Hippuric.

Nordmann has found in the urine of normal adults. There is clear separation of citric acid, tartaric, malic, α-ketoglutaric, succinic, aconitic, lactic, glycolic, hippuric, glutaric and β-hydroxybutyric acids. One spot applies to both sulphate and phosphate, if there is any phosphate in the urine. Using this method the organic acids give yellow spots on a blue-greenish background. These spots have the advantage that they do not fade but get more intense with time. We have so far examined urines passed by newborn babies on the first, second and seventh days of life, but we have not done enough to give a complete answer yet. Citric acid appears to be

the major organic acid constituent of the urine which is passed immediately after birth. In addition, urine passed during the first 24 hours of life contains malic acid, glycolic, lactic, β-hydroxybutyric, succinic, and α-ketoglutaric acids, but not aconitic acid. With this method one can detect a minimum of 20 μg. of each of these organic acids.

Adolph: Is there any appreciable accumulation of organic acids in the newborn during the first week of life? At this stage the individual is very insensitive to the hydrogen-ion concentration changes as far as the breathing is concerned, and I was wondering whether it is also insensitive as far as excretion is concerned.

Zweymüller: We are now working on the detection and identification of the organic acids found in the urine of normal newborn babies, and the next problem will be to identify those found in the urine of hypoxaemic newborn babies.

Karvonen: Did you find any pyruvate or does it come out with this method?

Zweymüller: We have not found a pyruvic acid spot, but we have not added pyruvic acid to the urine so we do not know exactly where the spot should appear on the paper.

Karvonen: I understand that increased excretion of pyruvate has been found during the first few days of life (Tallqvist, H. (1952). Thesis, Hámeenlinna).

Zweymüller: There is an interesting paper about some work on the output of organic acids in potassium depletion in which pyruvic acid, lactic acid, α-ketoglutaric acid, and citric acid were estimated, but this was done on normal adults (Evans *et al.* (1957). *Clin. Sci.*, **16**, 53).

Fourman: The hydrogen ion in the allantoic sac must come from somewhere, it cannot be manufactured. It must come in the end from the mother and since she cannot manufacture the hydrogen ion it must ultimately come from her diet. So what happens if you feed alkali to the mother pig?

Widdowson: We have not tried that.

Milne: It is well shown in your paper, Dr. Widdowson, how the newborn baby copes with its normal environment. I would agree that the organic acid level, especially that of citrate, is proportionally much higher than in the adult. Obviously in assessing the efficiency of the kidney, particularly in excreting an acid load, one must give it a maximum challenge and, though I see the difficulties of this in human experimentation, it would be extremely interesting to do this in the newborn animal. There seem to be two separate aspects of excretion of acid by the kidney. One is the ability of the kidney to excrete a maximum amount of hydrogen ion per day and clearly that can only be assessed by giving a prolonged acid load. The other is the ability of the kidney to maintain a hydrogen ion gradient between urine and plasma, in other words the production of a minimum urinary pH. I would be very interested in having data on whether the minimum pH of adult urine is similar to the minimum pH of newborn urine, whether the ammonia excretion can increase on prolonged acid ingestion proportionally to that of the adult,

and finally whether this very large citrate output in the newborn shows the same tremendous lability to acid-base effect as it does in the adult, in whom it can be reduced by quite small doses of acid or increased by alkalinization, say by sodium bicarbonate.

Widdowson: We have not yet given an acid load to newborn babies, although we should like to do so, but the experiment has been done on puppies. The question about citrate is one for the future. We have so far only studied three babies and this investigation is by no means complete.

McCance: The puppies have only been studied with respect to acute acidosis (Cort, J. H., and McCance, R. A. (1954). *J. Physiol.*, **124,** 358). The difficulty in an animal which is developing very rapidly is to separate the effects of several days' administration of an acid-forming drug and the natural development of the animal at that age. In the acute experiments the puppies were very defective in their ability to produce ammonia and they did not make a good response at all. They remained much more acid internally. We have unfortunately not yet tried the effect of altering the pH of the urine upon the excretion of citrates in the newborn baby.

Scribner: We carried out some experiments in rats which seem to indicate that the amount of citrate in the urine depends on the kidney tissue level of citrate rather than on the blood citrate level. After intraperitoneal injection of either sodium or potassium bicarbonate, urinary citrate increases 10- to 20-fold in one to two hours. Kidney tissue citrate increases two to threefold. Blood citrate rises 10 per cent at most. The response to intraperitoneal injection of citrate is quite different. We used ammonium citrate to get away from changes in acid-base balance, due to, say, injection of citric acid on the one hand or sodium citrate on the other. After the injection of $0 \cdot 0035$ m-mole/kg. ammonium citrate the blood level rises nearly 100 per cent, but there is little or no increase in either kidney tissue citrate or urinary citrate. We concluded from these experiments that the level of citrate in the urine under these conditions is determined by the citrate level in the renal tubular cell and is independent of the amount of citrate filtered through the glomerulus.

McCance: Dr. Milne, what determines the lower limits of pH which the human and other kidneys can achieve?

Milne: I think this can only be answered conditionally. First, one must state the stimulus, and secondly one must state the conditions of the kidney at that moment. Ammonium chloride has been used as the usual stimulus and I think no-one has ever produced a pH of human urine below $4 \cdot 4$ by that method, but other stimuli seem able to produce a considerably lower pH. The experiments of Schwartz, Jenson and Relman (1955. *J. clin. Invest.*, **34,** 673) showed this, where they infused sodium sulphate in a sodium-depleted individual. There, quite clearly, they got down to a urinary pH of $4 \cdot 0$, so that is a more effective stimulus, and indeed this agrees in the rat. It is very difficult to produce a highly acid urine in rats by most experiments. When it is given ammonium chloride the rat seems to be able to keep up with the ammonium intake and puts out ammonium chloride in its urine almost as quickly as it is either injected or taken in the drinking water. But an

acid urine in the rat can be produced by the same technique of sodium depletion and intraperitoneal sodium sulphate, which is clearly a more efficient stimulus to maximum acidity. Finally, one would agree that the condition of the kidney has been shown quite clearly to be dependent partly on body potassium stores. Potassium depletion, possibly by decreasing intracellular high-energy phosphate bonds—though that is purely speculation—will decrease the maximum osmolar gradient between urine and plasma, and similarly it will decrease the maximum possible hydrogen ion gradient. This effect is produced by potassium deficiency on the two stimuli of ammonium chloride or sodium sulphate injections.

McCance: The lower limits might be due to the activity of carbonic anhydrase having a ceiling in the human kidney. We know that if the carbonic anhydrase is defective the lowering of pH is correspondingly limited.

GENERAL DISCUSSION

Wallace: I should like to present a problem that arises when one attempts to interpret chemical analysis of tissues from deficient animals in terms of histological appearance. Skeletal muscle taken from potassium-deficient animals is low in potassium, high in sodium, high in its content of basic amino acids and probably low in bicarbonate content. When the muscle is examined histologically one sees apparently normal cells lying side by side with grossly abnormal cells. Which cells account for the chemical abnormalities? I have wondered if a cell can tolerate any deficit at all. Possibly, for the cell, it is an all-or-none phenomenon. Does a tissue as a whole become deficient in a sort of quantum fashion, cell by cell rather than by an over-all shared process by all of the cells? Is it not necessary to get down to a truly cellular level to further our understanding?

Fourman: May I add to Prof. Wallace's problem? The kidney and the heart show the morphological changes of potassium deficiency before the other tissues. These two tissues, when they are analysed in animals that have been made deficient in potassium, do not as a rule show chemical evidence of potassium deficiency. I suppose they do if you carry the deficiency far enough but as a rule they do not. It has always been a puzzle to me why two tissues that have a normal potassium content are the first tissues to show a potassium abnormality. These two tissues are also ones that never rest, in the way muscles do, and one wonders whether the fact that their function requires the maintenance of a normal potassium content, with the demand on the metabolic energy of the cell that this entails, carries the seeds of their own destruction.

Wallace: The analyses of Orent-Keiles and McCollum do show deficits of potassium in cardiac muscle taken from deficient rats (1941. *J. biol. Chem.*, **140**, 337). However, most workers have not shown the same thing.

Black: Jean Oliver and co-workers (1957. *J. exp. Med.*, **106**, 563) have done work on the localization of the morphological defect in the nephron of potassium-depleted animals, and this seems to be limited to the proximal and the collecting tubules. Dr. Fourman's difficulty may not be so real if the lesion is as sharply localized as that. With analysis of the whole kidney that may just be a failure to detect a limited local deficiency of potassium.

Milne: Part of the difficulty may be this: is not the necrosis or degeneration in the cell possibly due to the fall in intracellular pH, not primarily to potassium deficiency? I agree that kidney analyses

have not shown a potassium deficiency as in muscle, but they have shown a fall in intracellular bicarbonate, and therefore presumably a fall in intracellular pH. These experiments have not, as far as I know, been done with the heart muscle, but by analogy one would predict that the same situation may occur: the fall in intracellular potassium may be small, but the fall in intracellular bicarbonate and intracellular pH may be comparable to that in the kidney, and possibly greater.

Fourman: Yes, unless you think as I did, that the fall in intracellular pH is a result of the fall in intracellular potassium.

Milne: Direct analysis of tissue does not appear to support that.

Shock: The histological structure in Prof. Wallace's potassium-deficient animal, which I presume was a young one, is quite similar to the sections of muscle tissue from the old animals that Dr. Andrew has prepared from our material, which show a reduction in potassium content of the total muscle mass. There were fewer nice-looking muscle fibres in Prof. Wallace's animal than we see in the sections from the older animals, but there is a striking similarity in that there are good-looking areas, as described by the pathologist, with a lot of other material around them. I recall that a few years ago there was quite a flurry about the electron microscopic studies of mitochondria. In such pictures the mitochondria from cells of old animals were presumed to look frayed and woebegone. Subsequent experiments showed that dietary deficiencies and alterations could produce similar changes in the mitochondria taken from cells of young animals. If the few cellular changes we can observe in older animals can be produced by nutritional and dietary alterations in the young ones, it is possible that these 'age changes' are the result of chronic malnutrition of the cells. This brings us to the basic questions of what is adequate nutrition of a cell, and how can it be maintained.

Wallace: What is old and what is young? To me a 30-day-old rat is quite young, while to Dr. Widdowson it is as old as Methuselah.

Shock: To me a 10–12-month-old rat is a husky young adult, and when I talk of an old animal I mean one that is 24 months old or at least is at an age when 50 per cent of his contemporaries are dead.

Wallace: Young rats made potassium-deficient do show morphological changes in skeletal muscle. These changes can be almost completely reversed in as little as 36–48 hours after potassium administration. The lesions in cardiac muscle do not show this rapid type of healing. It would be interesting to see if your old rats have a slower repair time. Dr. Hingerty has already mentioned that older rats chemically repair potassium deficiency more slowly than do the young ones.

Kennedy: Morrison and Gordon (1957. *Fed. Proc.,* **16**, 366, and personal communication) have reported that a 24-month-old rat starved of food but not water for 24 hours loses far more urea, creatinine and potassium than a young one of comparable weight. So there is a state of incipient potassium deficiency. We have also found that the adrenals are usually pretty large in these old rats.

Shock: We have a done good many metabolic balance studies on the human (Duncan *et al.* (1951). *J. clin. Invest.,* **30**, 908; Duncan *et al.,* (1952). *J. Geront.,* **7**, 351; Bogdonoff *et al.,* (1953; 1954). *J. Geront.,* **8**, 272; **9**, 262; Watkin *et al.,* (1955). *J. Geront.,* **10**, 268). We consistently found that the older individuals, when given good protein intakes that resulted in positive nitrogen balances, retained potassium in excess of the theoretical amount required for the nitrogen retained. A good deal of this, I am sure, may be due to cumulative analytical errors, but it has always seemed to me that the older animal will work himself into a potassium deficiency if given the opportunity.

Black: Is not some of our difficulty here due to the limitations of morphology? If we take as our criteria of morphological change the fact that the tissue 'looks bad' or 'looks moth-eaten', then we are not going to get anywhere in deciding the cause of this change. You can hardly expect a cell to have a signpost saying 'I am too old', or 'I am potassium-deficient', and if we see the same change I do not see how we can expect morphology to decide its aetiology.

Talbot: When you use the term 'potassium-deficient', Prof. Wallace, do you wish us to think simultaneously about the correlated fact of the cellular sodium excess? Cellular sodium intoxication may actually be the provocative factor under some circumstances.

Wallace: Sodium excess is usually a corollary but not always. Some cation, it would seem, must replace the deficit. Basic amino acids have been shown to increase in potassium-deficient tissues as well as sodium.

Talbot: We have just done some experiments where the absolute losses of potassium due to starvation were greater per rat than some of the losses incurred when feeding a zero potassium-normal sodium intake. The animals which had lost this large amount of potassium by simple depletion were asymptomatic; it was only those that also had cellular sodium intoxication that showed all the symptoms commonly considered characteristic of marked potassium deficiency.

Hingerty: Prof. Wallace, when you restored the potassium, morphologically the tissue appeared perfectly all right in 36 hours. Did you do the chemical analysis?

Wallace: Yes, we did, stimulated by your work (Conway, E. J., and Hingerty, D. J. (1948). *Biochem. J.,* **42**, 372). Unlike you we found that sodium was lost simultaneously with a gain of muscle

potassium to normal (Schwartz, R., Cohen, J., and Wallace, W. M. (1955). *Amer. J. Physiol.*, **182**, 39).

Swyer: The sex difference in these responses to various hormones, and other matters which must either themselves have a hormonal basis or must be genetically determined, still puzzle me. What is the true sex basis? Is it a question of androgens and oestrogens, or the ratio of these two sex hormones, or is it in fact due to some characteristic which depends upon the presence of one X or two X chromosomes?

Kennedy: It is probably something to do with species, but the differences in size and growth between the castrate cockerel and the castrate hen, and the same sort of thing in male and female castrate rats, are very well known, and there is obviously a genetic difference in the subsequent behaviour of the neonatal castrate. Some of the early theories of ageing depended on body size, and one wonders how much actual size, or organ development and growth as such, rather than sex alone, affects the matter. The kidney of the male castrate rat, even though it is castrated very young, is a much bigger organ and in some senses, therefore, is a more developed or older organ than that of a female rat. Purely structural factors may determine some of the differences in what I think you call end-organ responsiveness.

Swyer: Is castration even shortly after birth early enough? After all, the foetal testis has a very important rôle to play and intra-uterine castration might avoid this difficulty.

Desaulles: That might possibly be helpful in determining the rôle of the X zone. It is hard to imagine how the interrelationship between pituitary, adrenals and gonads acts just at the beginning of life in the animal.

Milne: Is the control to the castrate male a spayed female?

Desaulles: They are quite different—that is the annoying point.

Kennedy: When he discussed renal function Dr. Shock pointed out that there was some similarity between the old and the young kidneys in their inability to sustain water diuresis and so on. It has been shown (Smith, H. (1951). The Kidney; Structure and Function in Health and Disease. New York: Oxford University Press) that if you take an animal of intermediate age and remove one of its kidneys and half the other, then the initial response, at least, is a great diminution in water diuresis, which may take four weeks to be restored to about two-thirds normal. This may suggest that the period during which the major changes in the newborn develop is during the unfolding of the anlage of the kidney; senescence in most animals that have been studied similarly involves a loss of structural units. So again, simply the amount of end organ which is there may be the important thing, apart altogether from what is called the endocrine climate.

Adolph: I wish the structural picture agreed so well with the physiological response to water loading. First of all, when you take out one kidney from, say, a middle-aged rat, you do not reduce the water diuresis much—it is more often a reduction of 20 per cent than of 50 per cent. Even if you take out a kidney and a half you still have 70 per cent of the response, and hypertrophy does not seem to be particularly important in restoring the response to near 100 per cent (Adolph, E. F., and Parmington, S. L. (1948) *Amer. J. Physiol.*, **155,** 317). Similarly in the kidney of the newborn the number of nephrons available, as far as anatomical studies show, is about 50 per cent of that in the adult, and yet the diuresis may only be 10 per cent of the adult's. As the diuresis develops in intensity with age, it gets far ahead of the development of the number of nephrons or of any other structure that has been counted in the kidneys. Enzyme studies have been made to try and find something that would be parallel either to the water diuresis or to the clearance increase with age. The clearances in the newborn kidney, as far as they have been measured, also develop rather slowly, but all of them are in parallel, at least in the rat. This is not necessarily true in all species, because there seems to be an exception in the rabbit (Levine and Levine. (1958). *Amer. J. Physiol.*, **193,** 123). However, phenol red and inulin clearances are proportional to one another at every age in the rat, while there is no clear parallelism between any two properties of excretion except the clearances.

Desaulles: When a heminephrectomized animal is submitted, eight or ten days after operation, to a physiological saline load of about 20 ml./kg., the output of urine during eight hours is much higher than in an animal with two kidneys. It is not at all clear to me why the output is so much higher; the dilution is greater, and less sodium is given out.

Richet: It may be dependent on the amount of solutes per nephron.

Bull: I think Dr. Richet's suggestion is a likely one. The kidney lesions in severe burns are probably due to a period of low circulatory volume which damages certain nephrons in several different ways. The resulting morphology may be very various but the functional lesion is usually rather similar in producing an oliguria, with a failure of concentration. This agrees best with the idea of fully functioning surviving nephrons; any nephrons that are damaged at all are right out of the picture. This explanation also agrees with our finding that in old patients there is a poorer response to water load and a slower excretion of sodium.

THE RÔLE OF THE KIDNEY IN ELECTROLYTE AND WATER REGULATION IN THE AGED

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THE kidney is the first line of defence in maintaining appropriate concentrations of water and electrolytes in the internal environment of all the cells in the body. Although there are other avenues through which salts and water may be lost from the body, and other factors which may enter into the regulation of concentrations in local areas, it is the kidney which carries the major burden of electrolyte and water regulation. The kidney responds to a multitude of stimuli and is blessed with large reserve capacities. It is the purpose of this report to describe briefly some of our findings with regard to age changes in renal function, to discuss the possible mechanisms of these changes, and to discuss their relation to the maintenance of certain physiological constants in the aged.

In order for the kidney to serve its functions of regulating water and electrolyte concentrations, as well as the volume of extracellular fluid, blood must be delivered to it in adequate amounts, glomerular filtrate must be formed, and the tubular cells must selectively reabsorb and excrete substances in accordance with a variety of stimuli to which the kidney must respond. The application of clearance techniques makes it possible to assess the nature of age changes in discrete renal functions. The studies to be reported are based on ambulatory male subjects between the ages of 20 and 90 years who were found to be free from clinical evidence of renal disease as judged by clinical laboratory tests and medical history. All

subjects were selected only after a thorough history and physical examination which excluded recent or remote renal diseases, cerebrovascular accidents, coronary artery disease, syphilitic or rheumatic heart disease, hypertension, or any recent alterations in body weight. All tests were carried out under basal conditions and subjects were hydrated with 600–800 ml. water, given orally 1–2 hours before the test, and 200 ml. water were given at half-hour intervals during the
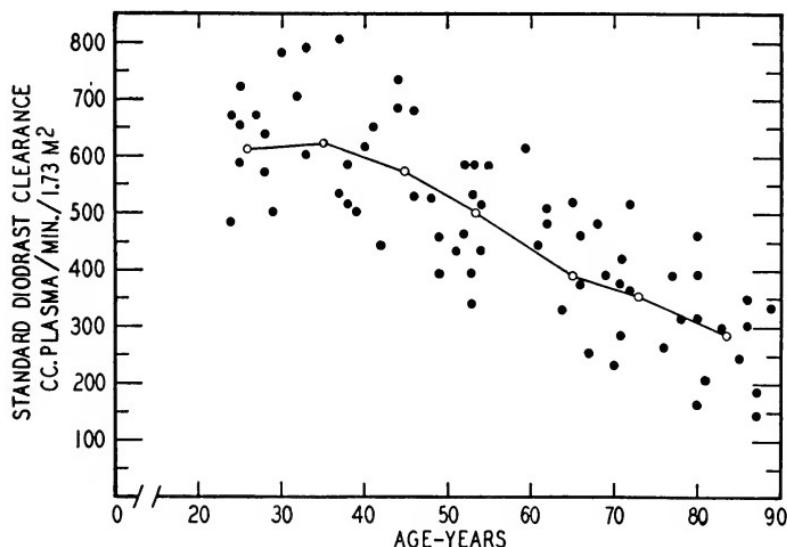
FIG. 1. Change in standard diodrast clearance or effective renal plasma flow with age. ○————○ average values ml. plasma/min./ 1·73 sq. m. body surface area.
(From: Shock, 1952).

test. The constant infusion method was followed, and four clearance and four Tm periods of 10–14 minutes each were taken according to the method of Smith, Goldring and Chasis (1938). Fig. 1 shows the age change in effective renal plasma flow as estimated from diodrast clearance (Shock, 1952). Between the ages of 20 and 90 years there was a decline in the effective renal plasma flow amounting to approximately 53 per cent. The regression equation relating the diodrast clearance to age is: $Cl_D = 840 - 6 \cdot 44 \times age$ (in years).

Although there is a substantial variation between subjects at any given age, the trend is highly significant.*

The age decrement in glomerular filtration rate, as measured by standard inulin clearance, is shown in Fig. 2. The regression of inulin clearance with age is expressed by the equation: $Cl_{In} = 153 \cdot 2 - 0 \cdot 96 \times$ age (in years). The average decline over the age span 20–90 years was 46 per cent in this instance.†
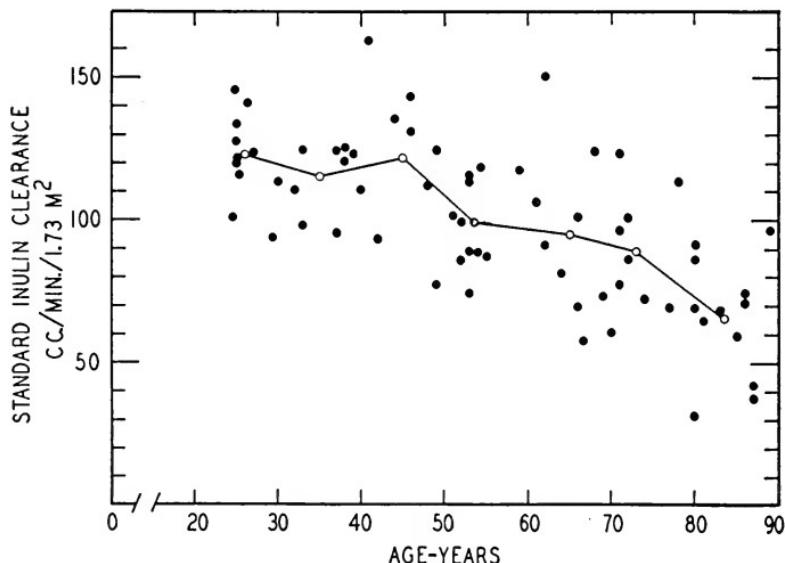
FIG. 2. Change in standard inulin clearance or glomerular filtration rate with age. ○————○ average values, ml. filtrate/min./1·73 sq. m. body surface area.

(From: Shock, 1952).

The fall in glomerular filtration rate is closely associated with the fall in plasma flow so that the filtration fraction, calculated as ratio of inulin clearance to the diodrast clearance, shows only a slight increase with age (Fig. 3).

* In a different sample of subjects in whom renal plasma flow was estimated from PAH (p-aminohippuric acid) clearance (Watkin and Shock, 1955), the regression equation was: $Cl_{PAH} = 820 - 6 \cdot 75 \times$ age (in years).

† In other groups of subjects the regression of inulin clearance on age was: $Cl_{In} = 157 \cdot 0 - 1 \cdot 16 \times$ age (in years) (Watkin and Shock, 1955), and $Cl_{In} = 150 \cdot 9 - 0 \cdot 904 \times$ age (Miller, McDonald and Shock, 1952).
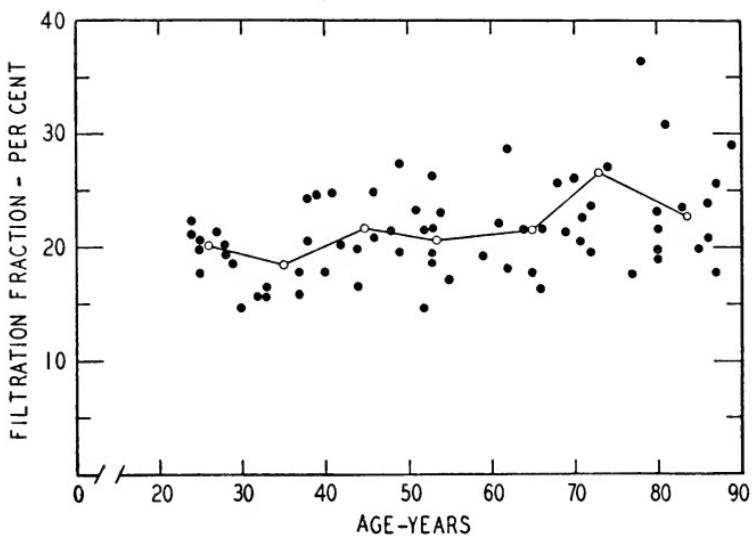
FIG. 3. Change in filtration fraction with age. ○────○ average
values, per cent of plasma filtered.
(From: Shock, 1952).
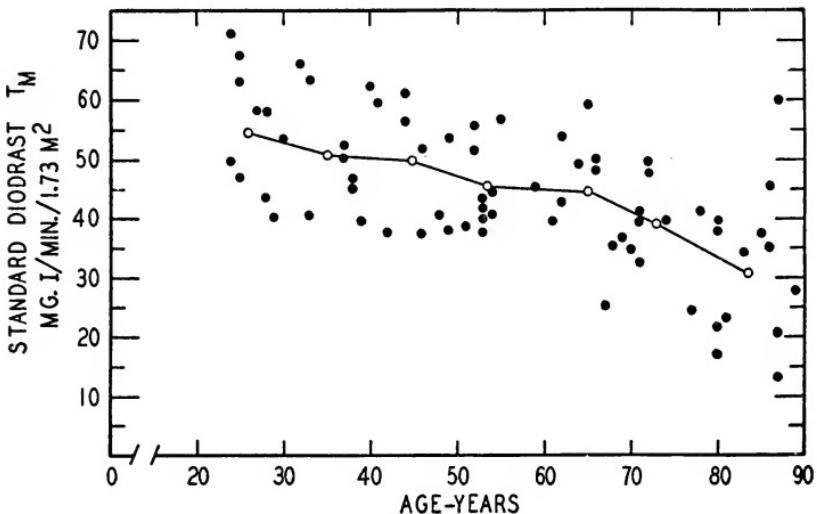
FIG. 4. Change in standard diodrast Tm with age. ○────○ average
values mg. diodrast iodine/min./1·73 sq. m. body surface area.
(From: Shock, 1952).

The maximum capacity of the renal tubule to excrete diodrast also diminishes with age. Fig. 4 illustrates the results of this test in the subjects studied. The average diodrast Tm fell from $54 \cdot 6$ to $30 \cdot 8$ mg. iodine/$1 \cdot 73$ m.^{2}/min. between the ages of 20 and 90 years. This represents a reduction of $43 \cdot 5$ per cent. The regression equation relating diodrast
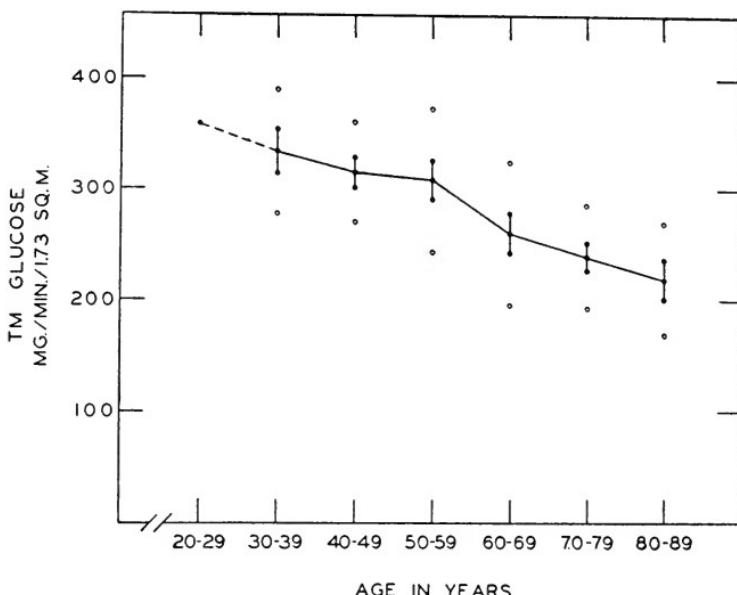
FIG. 5. Decrease in maximal tubular reabsorptive capacity with age. The slope is drawn to connect the mean values for each decade. The vertical lines represent $\pm$ one standard error of the mean, while the open circles define the limits of $\pm$ one standard deviation of the distribution.

(From: Miller, McDonald and Shock, 1952).

Tm to age is: $Tm_D = 66 \cdot 7 - 0 \cdot 40 \times$ age (in years).* The reabsorptive capacity of the renal tubular epithelium for glucose also shows a comparable diminution with age, as shown in Fig. 5. The average glucose Tm fell from 328 to 223 mg. glucose/$1 \cdot 73$m.^{2}/min. between the ages of 30 and

* The maximum excretory capacity for PAH shows the following regression on age: $Tm_{PAH} = 120 \cdot 6 - 0 \cdot 865 \times$ age (Watkin and Shock, 1955).

90 years. The regression equation is: $Tm_G = 432 \cdot 8 - 2 \cdot 604 \times$ age (in years). The maximum capacity for both a reabsorptive and excretory mechanism in the renal tubules showed approximately the same percentage decrement with age.

The average inulin clearance per unit of Tm remains constant between the ages of 20 and 90 years (Fig. 6). This finding lends support to the hypothesis that a nephron loses its function as a unit. In contrast, the diodrast clearance per
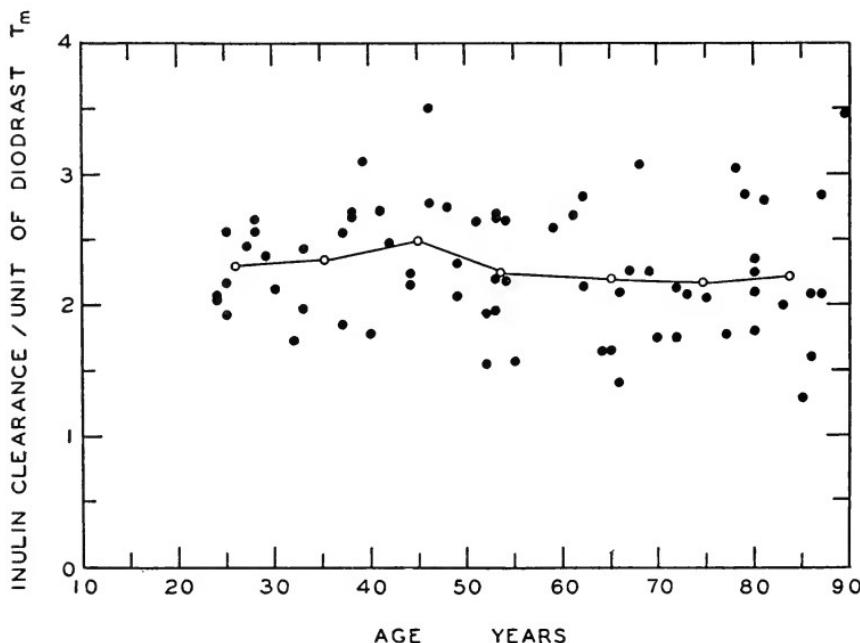
Fig. 6. Change in rate of glomerular filtration per unit of diodrast Tm.
○————○ average values.

(From: Shock, 1952).

unit of Tm decreases from an average value of $12 \cdot 6$ at age 30–39 to $9 \cdot 7$ at age 80–89 (Fig. 7). This steady decline in the effective renal plasma flow per unit of tubular excretory capacity indicates that the average amount of blood delivered to each tubule, and by implication each nephron, declines with age. Since we have been able to demonstrate a significant reduction in resting cardiac output with age (Brandfonbrener,

Landowne and Shock, 1955), as shown in Fig. 8, a portion of the reduction in renal plasma flow must be attributed to a reduction in total blood flow. However, in experiments to be reported later calculations show that the age reduction in renal blood flow is proportionally greater than the reduction in cardiac output.
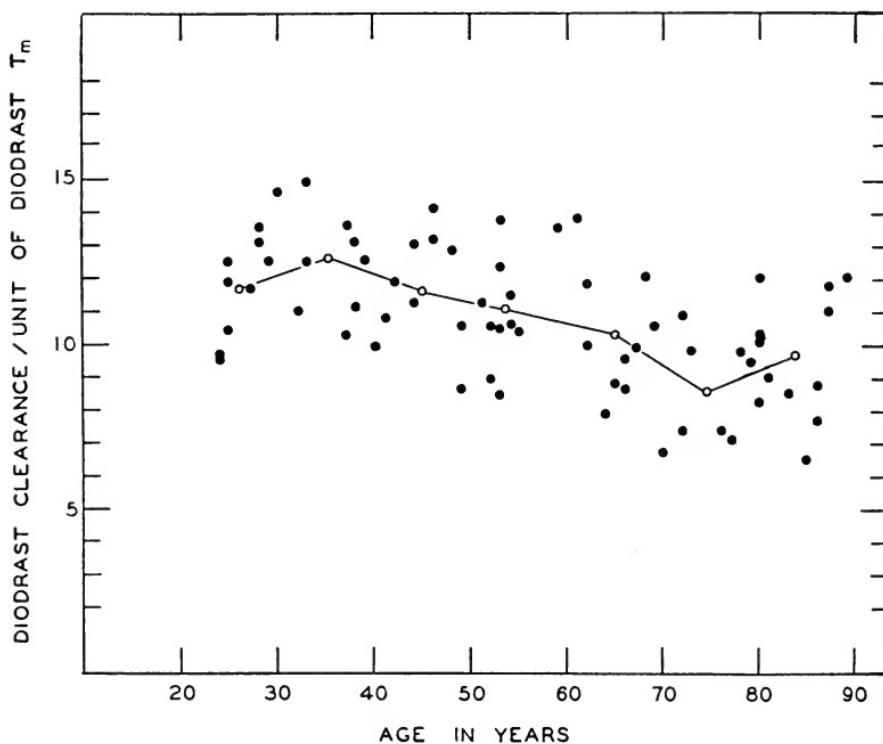
Fig. 7.   Change in effective renal plasma flow per unit of diodrast Tm.
○————○ average values.

(From: Shock, 1952).

Other experiments have shown that the reduction of effective renal plasma flow in the aged cannot be ascribed to permanent structural changes in the renal vascular bed (McDonald, Solomon and Shock, 1951). Previous studies have shown that the administration of a pyrogen to young people

results in a marked increase in effective renal plasma flow. In order to assess age changes in the ability of the renal vascular bed to dilate, glomerular filtration rate and renal plasma flow
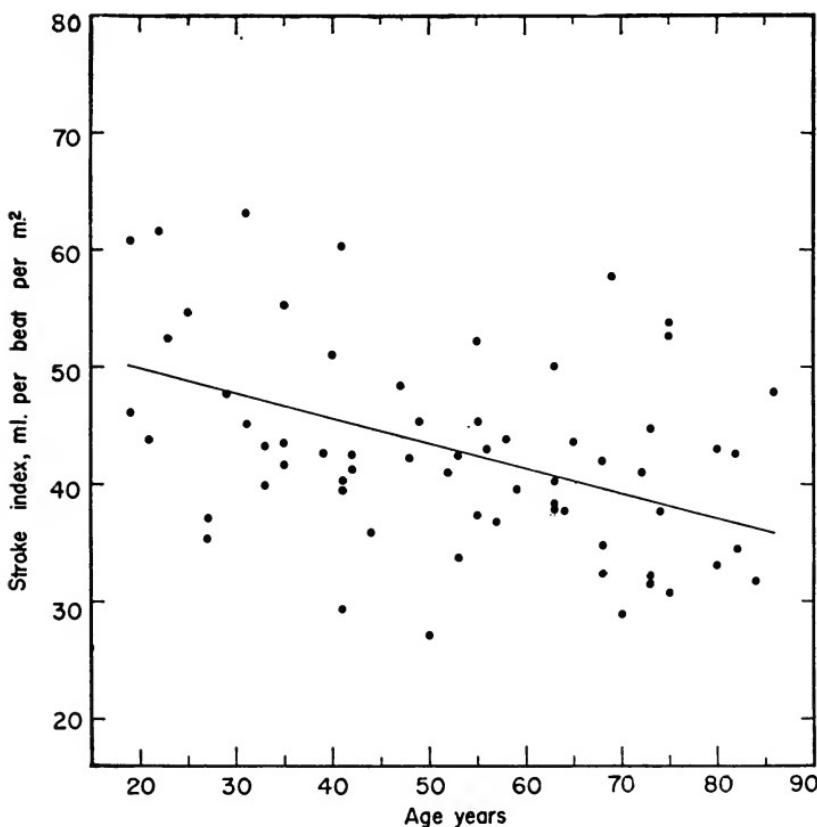
Fig. 8. Stroke output per sq. m. surface area versus age. Each point represents the average of two measurements in 49 subjects, of three measurements in four subjects, and a single measurement in 14 subjects. The line indicates the simple linear regression for the data.

(From: Brandfonbrener, Landowne and Shock, 1955).

were measured in young, middle-aged, and old subjects following the intravenous administration of 50,000,000 killed typhoid organisms (0·5 ml. typhoid-paratyphoid A and B vaccine). The results of these experiments, based on the

average of 20 subjects in each age group, are shown in Fig. 9.
From the three curves at the bottom of the chart it is clear that
although the usual age difference in glomerular filtration rate
was present, there was no significant effect of the pyrogen in
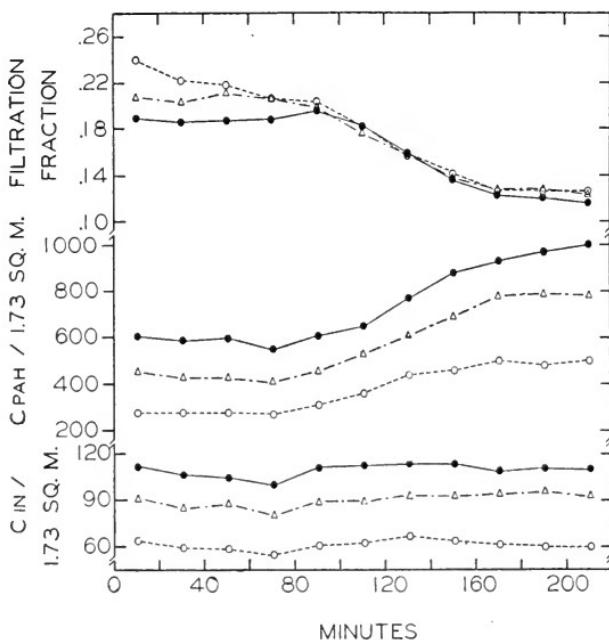
Fig. 9. Changes in glomerular filtration rate (C_{In}),
effective renal plasma flow (C_{PAH}), and filtration
fraction during the pyrogen reaction. Fifty million
killed typhoid organisms were injected intravenously
at 0 time. ○————○ mean values for 14 subjects
aged 70–85 years (O group). △————△ mean
values for 20 subjects aged 50–69 years (M group).
●————● mean values for 20 subjects aged 20–49
years (Y group).

(From: McDonald, Solomon and Shock, 1951).

either the young, middle, or old subjects. The three curves in
the centre of the graph show clearly that, beginning about 80
minutes after the administration of the pyrogen, there was a
slow continuous rise in renal plasma flow in all groups of sub-
jects. Although the mean absolute increases were greater for
the young than for the old group, where increments were

expressed as percentages of the base line values, the rise in renal blood flow for the young, middle, and old groups was 76, 86, and 91 per cent respectively. As shown by the upper three curves, the filtration fraction diminished markedly in all subjects, indicating a fall in effective filtration pressure, which would result from a greater vasodilatation at the efferent than at the afferent side of the glomerulus if there were no change in blood pressure. Actually, the diastolic blood pressure dropped slightly in the middle and old groups, but remained constant throughout the reaction in the young group. At the height of the reaction the differences in the filtration fraction, observed under resting conditions, completely disappeared. The small absolute changes in renal plasma flow in the older subjects, following pyrogen, are consistent with the anatomical findings of a progressive decrease in the number of glomeruli in the aged kidney (Moore, 1931). On the other hand, the time of onset and the percentage increase in renal plasma flow were similar in the different age groups. Consequently, it must be concluded that the responsiveness to pyrogen of the vascular elements remaining in the aged kidney is not qualitatively different from that in the young kidney. It is inferred from these experiments that the renal arterioles in the aged kidney are capable of dilating, and that in the resting state there is a functional vasoconstriction of the afferent arterioles in the aged which, under resting conditions, diverts blood from the kidney to other parts of the circulation.

To function effectively the kidney must respond to a variety of stimuli. One of the most important signals for altering the reabsorption of water by the renal tubule is the antidiuretic hormone. Age differences in the inhibition of water diuresis, following the intravenous administration of small amounts of pitressin, have been observed (Miller and Shock, 1953). In these experiments a maximum water diuresis was established by the oral administration of 500 ml. water at 6.00 a.m., followed by 250 ml. water at half-hour intervals until completion of the test. To ensure maximum urine

flows, oral fluid intake was supplemented by the intravenous administration of 5 per cent dextrose in distilled water, in which appropriate quantities of inulin and sodium amino-hippurate had been added at the rate of 8 ml./min. by a constant infusion pump. Twenty-nine adult males, ranging in age from 26 to 86 years, served as subjects. The total sample was arbitrarily divided into three age groups: young (no. = 9, age range from 26–45), middle (no. = 10, age range from 46–65), and old (no. = 10, age range from 66–86). After three control collection periods, $0 \cdot 05$ milliunits pitressin/kg. body weight was administered intravenously. Subsequently, six consecutive urine collections, each of 12 minutes duration, were made. During the control periods, the average urine flow for the young subjects was approximately 14 ml./min.; middle-aged, 11 ml./min. and old subjects, 10 ml./min. The urine/plasma (U/P) inulin ratio was calculated as an index of water reabsorption. The results of this experiment are shown in Fig. 10, where the U/P inulin ratio was plotted against the urine collection period. During the control periods, the U/P inulin ratios were approximately 10 for all three age groups. Following the administration of pitressin, prompt antidiuresis was noted in all three groups. Peak antidiuresis and peak concentration of inulin were observed in all three age groups during this period which was 12–24 minutes after pitressin. As indicated in Fig. 10, there was a marked age difference in the antidiuretic response to this standard stimulus. The young subjects showed the maximum response and the old subjects showed the minimum. In Fig. 11, the relationship between the maximum observed tubular response to the standardized dose of pitressin and age is shown. Correlation coefficient was $- 0 \cdot 73$, and the regression of the concentration on age was described as U/P inulin $= 162 - 1 \cdot 6 \times$ age (in years). Although the administered pitressin resulted in a rise of blood pressure, it averaged only 10 mm. at two minutes after injection, and fell to control levels within five minutes. These experiments indicate that, in the older individual, there is an impairment in the

functional capacities of the tubular cells to perform osmotic work on the glomerular filtrate.

The results of these observations lead to the concept that, with increasing age, there is a gradual loss of nephrons in the
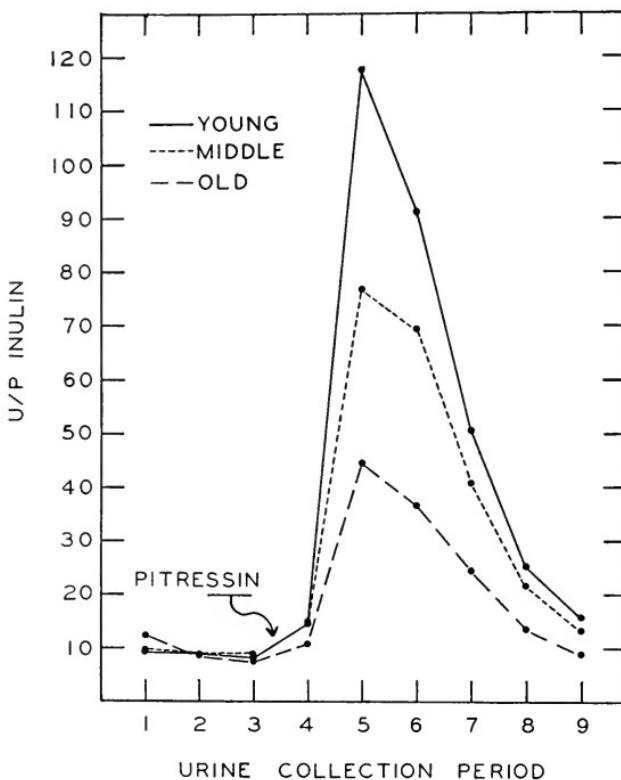
Fig. 10. Mean values of U/P inulin ratio for each of three age groups before and after the intravenous administration of pitressin. Urine collection periods 1–9 represent nine consecutive 12-minute periods. Pitressin was administered immediately after the conclusion of period 3.

(From: Miller and Shock, 1953).

kidney. In addition to these structural losses there are functional changes. One of these is a gradual increase in the vasoconstriction of the vascular bed of the kidney which further reduces the flow of blood through it, even in the face

of the falling cardiac output. This vasoconstriction is functional in character and can be removed by an appropriate physiological stimulus. Although the tubular epithelium responds to the stimulus of the antidiuretic hormone as quickly in the old as in the young, the functional capacity of the tubular epithelium to perform osmotic work shows a gradual reduction with age.
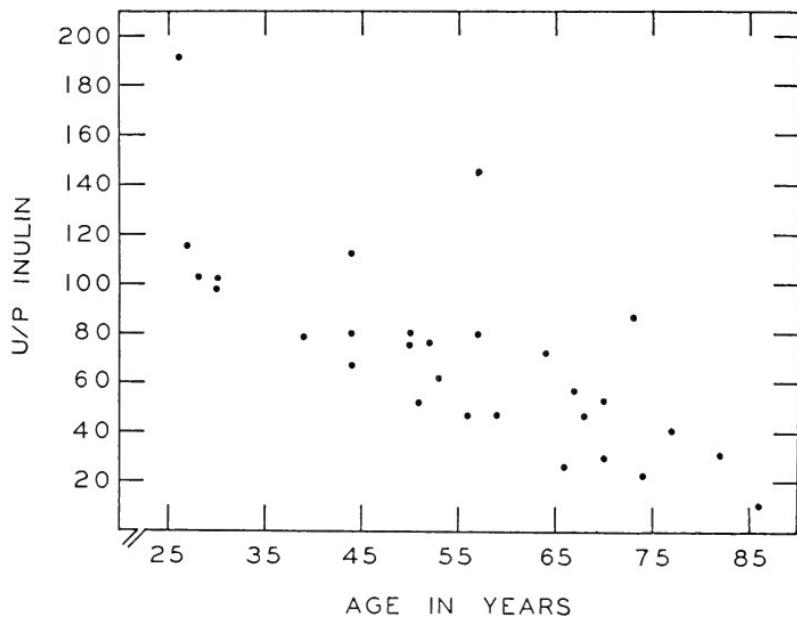
FIG. 11. Relationship between maximum U/P inulin following pitressin, and age. The ordinate is the mean U/P ratio for periods 5 and 6. (From: Miller and Shock, 1953).

Although these experiments serve to define certain limitations in renal function with increasing age, we must turn to other observations to tell us how effective the aged kidney is in maintaining volume and concentration characteristics of the extracellular fluid. With regard to electrolyte concentration of the plasma, there is no evidence of any systematic changes with age. Although Videbaek and Ackermann (1953) reported a slight rise in plasma potassium concentrations, $4 \cdot 0$–$4 \cdot 5$ m-equiv./l., between the ages of 25 and 90, the

trend was not statistically significant. The other major electrolytes, sodium and chloride, do not show any age trend (de Billis, 1954; Herbeuval, Cuny and Manciaux, 1954; Lippi and Malerba, 1955). In our own laboratory we have found no
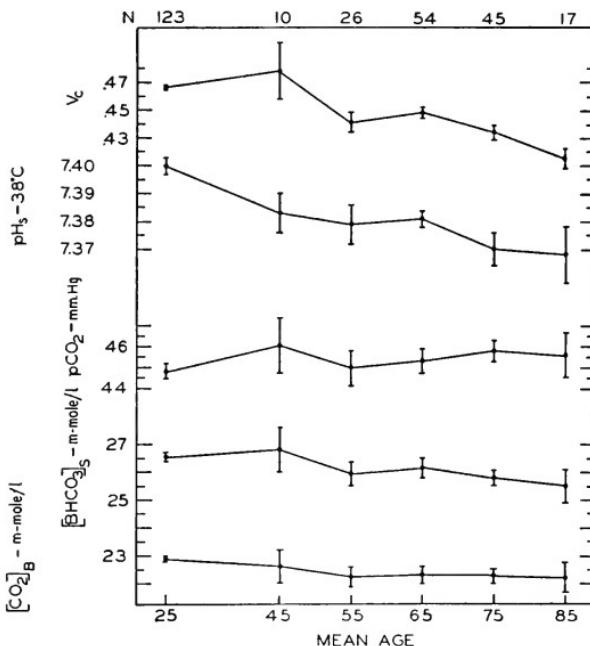
Fig. 12. Trends in the acid-base equilibrium of the blood of males with increasing age. Average curves from top to bottom include percentage of red cells, serum pH at 38°, carbon dioxide tension expressed in millimetres of mercury, serum bicarbonate and blood carbon dioxide content, both expressed in millimoles per litre. The vertical lines indicate $\pm$ one standard error of the mean. Data for the 25-year determinations taken from: Hamilton and Shock (1936).

(From: Shock and Yiengst, 1950).

systematic age changes in the total osmotic pressure of the plasma or its water content. The bicarbonate content of the plasma and the pH do not show significant age trends (Shock and Yiengst, 1950). Thus, under basal conditions the kidney is able to regulate the acid-base equilibrium of the body adequately, even to advanced ages (Fig. 12). Lewis and

Alving (1938) found some evidence that with increasing age there is an accumulation of urea nitrogen in the blood. Their data show a slight rise in the fifth decade, but no significant change during the sixth and seventh decades, with a rather sharp increase after the 70th year. Most of the total rise from a mean of 12·9 mg. urea N/100 ml. blood in the 30–40 age
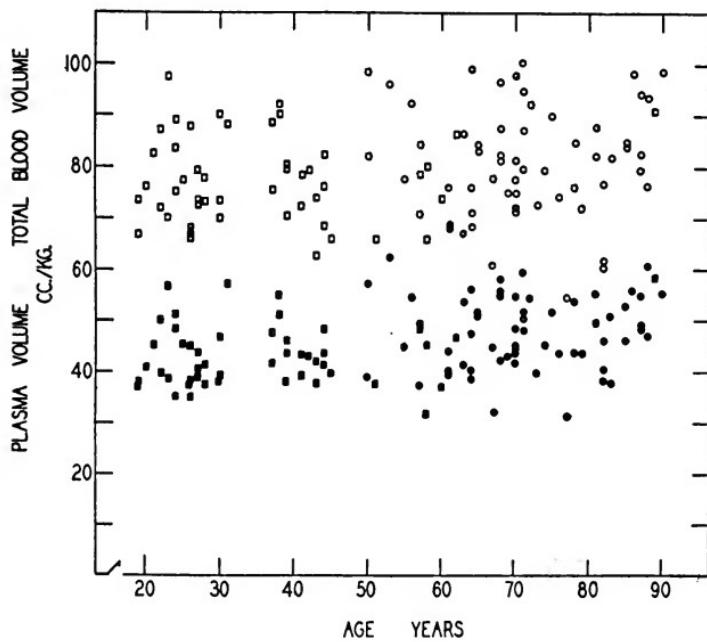
FIG. 13. Total blood volumes, ml. per kg., and plasma volumes, ml. per kg., in 105 males. □ total blood volume determinations from Gibson and Evans (1937). ■ plasma volume (Gibson and Evans), ○ total blood volume, ● plasma volume (Cohn and Shock).

(From: Cohn and Shock, 1949).

group to a mean of 21·2 mg. per cent in the 85–89-year-olds occurred after the age of 70. It therefore appears that there is some impairment in the excretion of nitrogenous substances in the aged kidney, although capacity for maintaining electrolyte concentrations under resting conditions is still adequate.

With increasing age there is a reduction in the concentrating ability of the kidney. The maximum specific gravity attained

after 12 hours of water deprivation falls from an average of 1·032 at age 20 to 1·024 at age 80–90. Although the absolute magnitude of the decrement is small, it is statistically significant (Lewis and Alving, 1938) and indicates impairment of the concentrating ability of the kidney, which is no doubt a reflection of the reduction in Tm as reported from our studies.

With regard to volume regulation, our observations on a
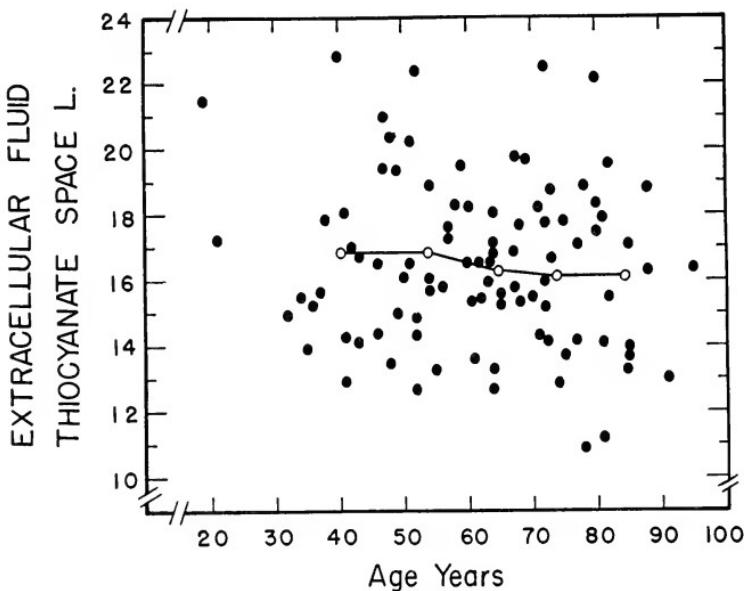
Fig. 14. Relationship between extracellular fluid space (thiocyanate space) and age in males.
(From: Shock, 1956).

series of 152 males failed to demonstrate any systematic changes in either plasma volume (Cohn and Shock, 1949) or in total extracellular fluid volume (Shock, Watkin and Yiengst, 1954) as estimated by thiocyanate determinations (Figs. 13 and 14).

Although the aged kidney has a capacity for maintaining acid-base equilibrium of the plasma under resting conditions, when an extra load is imposed upon it age differences appear. Thus, for example, we have found that a single dose of

ammonium chloride produces displacements of the acid-base equilibrium in both old and young subjects. However, young individuals are able to readjust equilibrium within a period of eight hours, following a single dose of 10 g. of ammonium chloride, whereas the older subjects require as much as 24–36 hours for the process (Shock and Yiengst, 1948). When repeated daily doses of $1 \cdot 5$ m-equiv. ammonium chloride/kg. body weight/day were administered to normal subjects for 4–14 days, readjustment of the acid-base equilibrium occurred within $5 \cdot 7$ days in the young subjects, but the aged subjects (65–73 years) were unable to attain equilibrium under this load of ammonium chloride (Hilton, Goodbody and Kruesi, 1955). It was also found that the degree of metabolic acidosis induced by a standard dose of ammonium chloride showed a greater severity in the older subjects than in the young. We have now initiated a study of age differences in the ability of the individual to regulate plasma and extracellular fluid volume following the imposition of an oncotic load.

Thus, the evidence now available indicates that in spite of the reduction in discrete renal functions with age, the kidney retains sufficient capacity to regulate both concentrations and volumes fairly closely under conditions of rest. However, when experimental displacements are produced, age differences in the speed of readjustment appear.

There are obviously many other questions, such as age differences in glomerular permeability and the activity of specific cellular enzymes in the kidney, which remain unanswered. Studies on cellular enzymes are now in progress in our laboratory, using the rat as an experimental animal. Although we have found a reduction in the total oxygen uptake for kidney tissue between the ages of 12 and 24 months in the rat, these differences disappear when an appropriate correction for cell number is introduced. There are, however, some specific enzymes, such as succinoxidase, which show an age reduction which is apparently not dependent on the number of functioning cells in the kidney preparation (Barrows *et al.*, 1957). It is our aim to extend these observations to include the

capacity for concentrating specific substances, such as PAH, in tissue slices removed from the kidneys of animals of different ages. It is thus apparent that a great deal of research remains to be done before we can interpret age changes in renal physiology.

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DISCUSSION

Zweymüller: One of the interesting things in your paper, Dr. Shock, was this tendency for the glomerular filtration rate, Tm_{PAH} and Tm_G to fall, which leads to the conclusion that the total number of nephrons is diminished. Are the nephrons which are left, and particularly the tubules, still able to elevate their function? Under normal physiological conditions we have a Tm_{PAH}, which means that under normal

conditions this function has an upper limit. If Vitamin A is fed this action is elevated, and it is called trophic action. It would be interesting to give old people Vitamin A and see if this normal Tm_{PAH} for physiological conditions could be elevated in this way.

Shock: We infused lactate in some of these older people who had low Tms and we found that this did raise the Tm by providing additional substrate; you can almost double the Tm for PAH in both old and middle-aged subjects (McDonald, R. K., Shock, N. W., and Yiengst, M. J. (1951). *Proc. Soc. exp. Biol., N.Y.,* 77, 686). In other words the tubules that are still present in the old kidney, as far as we have been able to determine, are just as good as in the young. This is all very distressing to me because I am convinced that there must be progressive changes. The tubule just cannot be working beautifully today and gone tomorrow, but unfortunately this is the way the data come out so far.

Heller: We all know that there has been a lot of difficulty in the choice of parameters when attempting to compare renal function in adults and infants. I should therefore like to ask Dr. Shock whether he has tried to express his data in terms of other parameters like, for example, total body water. That would seem important because it might reveal correlations which may have a functional significance.

Shock: Yes, we have done that, and if you refer metabolism to total body water you wipe out the age change. However, the age decrement in renal function remains, even when calculated on the basis of body water.

Hingerty: When you selected your subjects, Dr. Shock, did you exclude obese patients?

Shock: We did not use any index of body weight to exclude patients, but I would say immediately that in our population we do not see obese people over the age of 65. These patients were all males so we do not know anything about the weight of females.

Hingerty: It seems to me that the decline in kidney function sets in at about the 50-year mark, and that is about the age when you would expect a higher incidence of obesity in the general population.

Shock: Actually the body surface area decreases with increasing age in all groups of subjects we have studied. The major factor that contributes to this reduction in surface area is the body height, which goes down more than body weight. There is a wide scatter in height in our population, but there is a statistically significant linear decrement between the ages of 30 and 90. There is no significant regression of weight on age in the population of males that we have studied.

Black: Is there any serial change in the blood urea with age? It seems very odd that if you give some lactate these tubules can hypertrophy in function to twice their previous extent, and yet when you study them without any stimulus they are apparently in a fairly low state of function. If the blood urea does not go up then it looks as if the remaining tubules are perfectly able to cope with the diminished urea formation within the body.

Shock: The subjects used in our renal function studies had no elevation in blood urea because this was one of the selection criteria. Every

individual in the renal series was able to concentrate his urine at least to a specific gravity of 1·020 on a Fishberg routine. However, Lewis and Alving (1938. *Amer. J. Physiol.*, **123**, 500) have published blood urea levels in 100 subjects aged 20 to 80. They found little increment in blood urea up to the age of about 70, but from 70 on it does increase in their data.

I must make it clear that the increment in Tm following lactate infusion occurs only during the time that the blood lactate level is raised. We have not been able to show that it induced any kind of renal hypertrophy.

Fejfar: I would not expect the blood urea level to increase, because in a paper on chronic nephritis, Brod (1948. *Čas. Lék. čes.*, **87**, 711) showed that the blood urea did not rise markedly in patients with low protein intake unless the glomerular filtration rate decreased to less than 25–30 ml./min.; in your work the glomerular filtration rate was far above this figure.

In congestive failure or other situations where cardiac output is inadequate, there is usually a decrease in renal blood flow, and an increase in tubular reabsorption of water. The normal concentration test might point to a diversion of blood from the kidneys due to this insufficient cardiac output.

Shock: I did not perhaps make it clear that unfortunately we only got the cardiac output method in operation rather late in the series, so that the cardiac output results that I showed you in the average curve were not determined on the same subjects as the renal functions. We are now measuring cardiac output and renal function in the same subjects simultaneously. The crucial point to me is whether there is a change in the percentage of cardiac output that gets through the kidney, and I just cannot answer that at the moment.

Milne: I have some difficulty about this fall in glomerular filtration rate without a rise in blood urea with advancing age. It seems to me that this could only be possible if the older people were not taking in so much protein, or if the urea back-diffusion was diminishing and therefore the clearance of urea was approaching the inulin clearance. I should have thought that a fall in glomerular filtration rate of this magnitude would necessitate a rise in blood urea, although it might not of course go above some arbitrary upper limit of normal such as 40 mg./100 ml.

Black: My question on blood urea really referred to blood urea in a population and not in an individual. I think Van Slyke showed that in terms of a population, even with 80 per cent of normal urea clearance there is a detectable increase in the blood urea. All our clinical experience is that the glomerular filtration can be down to 30 per cent without the blood urea being outside the so-called normal range in that individual but if you do it in a population you then find that even with an 80 per cent clearance the level is raised.

Borst: Dr. Shock, you eliminated all diseased people, but at what blood pressure was a man eliminated as not having normal kidneys?

Shock: We excluded anyone who had a systolic pressure greater than 160 and a diastolic greater than 90 mm. Hg. Prof. Olbrich (Olbrich *et al.* (1950). *Edinb. med. J.*, **57**, 117) was doing similar renal functional

experiments at almost the same time. He did not exclude subjects with elevated blood pressures and his results on British subjects are almost identical with those we found by excluding the individuals with elevated blood pressures.

Scribner: The question posed by these data is: is the change in the kidney function, as described, a result of disease in the kidney or a wearing out with age, or is it simply a response to a decrease in the size of the living organism? This all comes back to the point raised by Prof. Heller and Prof. Borst: might not creatinine excretion, or total exchangeable potassium, be reasonable reference points?

Shock: We have done a good many creatinine determinations in balance studies under conditions of a closely regulated diet. However, I have never been able to convince myself that creatinine excretion gives a stable value that is characteristic of the individual, because we have seen some rather wide fluctuations that we have not been able to explain satisfactorily. I tried it first with adolescent children and then gave it up as I did not feel it could be determined as a characteristic constant for the individual. But I am intrigued by the potentiality of the total exchangeable potassium, and would like to study its changes with age.

Bull: The lines you showed in illustrating the decline of renal function with age are practically identical with the lines for our mortality findings in burns. By Probit analysis we can fit LD_{50}'s for the areas of burning which will produce death at different ages. It may be coincidence that you chose your ordinates on just the right scale, but the lines are almost the same in that they take off at just the same age and go down in the same way. Burning is a severe stress. We have been talking about the elderly having a reduced tolerance to stress, and burning is largely a stress affecting water and electrolytes. The burn is a convenient measurable lesion, and death occurs with a progressively smaller size of burn with advancing years, which I think probably represents an important aspect of the ageing of a regulation of water and salt.

AGE AND RENAL DISEASE

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Introduction

Senescence has sometimes been described as a deterioration in homeostasis. Dr. Shock showed us that the deterioration may be due to failing renal function, and this reopens an old question of whether the kidney cells themselves are less able to do their work in old people, or whether diseases of the kidney become more frequent with advancing age. It seems generally agreed that pathological lesions, particularly of the renal vessels, are very commonly found *post mortem* in old people in whom they were unsuspected during life. Oliver (1942) reviewed the controversy as to whether these lesions originate from a primary atrophy of the kidney, or are merely one of the results of generalized arteriosclerosis. He decided in favour of arteriosclerosis. The other view, that the kidney dies piecemeal, will be re-examined here because it seems possible to show that the death of some nephrons leads to pathological changes in the survivors, and some indirect ways in which this may happen will be suggested.

One can raise objections to any theory of ageing. The major defect of the definition in terms of homeostasis, it seems to the present author, is that the newborn animal finds it just as difficult to maintain a stable internal environment under stress as does the senile one. An older definition by Minot (1908), in more structural terms, described senescence as the gradual loss by differentiated cells, throughout life, of the ability to grow and to regenerate. This idea applies especially well to the kidney, as we shall see.

Renal growth and regeneration

Both the tentative and the definitive foetal kidneys develop from mesoderm, in intimate relation with the gonads. So it is not altogether surprising that the adult kidney resembles the other transient tissues, and its life cycle is not completely synchronous with that of the rest of the body (Kennedy, 1957). It would be disastrous to a species, of course, if kidney and body got too far out of step, but any tendency for this to happen during reproductive life would be prevented by natural selection. There is some evidence, however, that the kidney atrophies after the climacteric, and in some species such as the rat this may limit life.

Most mammals develop their full complement of nephrons soon after birth, and postnatal growth of the kidney consists chiefly of lengthening of its tubules, at first by the growth of new cells and later by hypertrophy of existing ones. When a rat is about six months old, or a man about 30 years, the number of glomeruli in their kidneys begins to decrease, and it may fall to half the young adult value, without pathological change, by eighteen months old in the rat or seventy years in the man (Arataki, 1926; Moore, 1931; Roessle and Roulet, 1932). Moore and Hellman (1930) showed that removing one kidney from a rat did not slow down the loss of nephrons from the other, so that involution of the kidney is an even more relentless process than that of the ovary, where removal of one gland does delay the loss of oöcytes from the other (Mandl and Zuckerman, 1951).

Nowadays chemical analysis can be used to supplement histology in determining the number and size of the cells in a tissue. This is because one of the two forms of nucleic acids in cells, deoxyribonucleic acid or DNA, is confined to the nuclei, as the name suggests it ought to be, while the other, ribonucleic acid or RNA, is distributed with the bulk of the ordinary protein throughout the cytoplasm. So if DNA, RNA and protein are determined at different stages during the growth of a tissue, it is possible to distinguish between

an increase in nuclei, or hyperplasia, and an increase of cytoplasm, or hypertrophy. This method has shown that the principal increase in the number of nuclei in the kidney of the rat occurs during the first three months of life, *pari passu* with the main growth of the skeleton, and this agrees well with histological findings.

There is a conflict of evidence about regeneration, however. Rollason (1949) showed histologically that mitosis began in the surviving kidney within forty-eight hours of unilateral nephrectomy, whereas Mandel, Mandel and Jacob (1950)

Table I

THE EFFECT OF UNILATERAL NEPHRECTOMY ON THE COMPOSITION OF THE SURVIVING KIDNEY IN RATS AT DIFFERENT AGES

Age at Operation	Interval before Killing	Group	Total nitrogen (mg. per kidney)	RNA phosphorus (mg. per kidney)	DNA phosphorus (mg. per kidney)
One Month	Two Weeks	Control (not operated)	11·3	0·273	0·165
		Kidney removed	18·0	0·424	0·233
Three Months	Six Weeks	Control	19·4	0·378	0·183
		Kidney removed	29·4	0·488	0·227
Six Months	Six Weeks	Control	28·1	0·587	0·253
		Kidney removed	41·0	0·717	0·244

were unable to show any increase in kidney DNA even three weeks after the same operation. The difference apparently depends on the age of the animals. Table I illustrates a comparison made by the present author of the effect of unilateral nephrectomy on the composition of the surviving kidney in one-month, three-month and six-month-old rats. In the youngest group, which were about the same age as Rollason used, there was a rapid increase in DNA phosphorus. In the middle group the DNA increased less than the RNA and the nitrogen, and more slowly, as Mandel, Mandel and Jacob had found. No hyperplasia at all occurred in the

kidneys of the six-month-old rats. It may be emphasized that these findings accord very well with Minot's definition of ageing. As will be shown later, hyperplasia can and does occur in the tubules of older rats, but it does not then represent the normal primary response to loss of moderate amounts of renal tissue, and some additional stimulus, possibly endocrine in nature, is probably involved.

Renal Senescence

The compensatory changes that we have been considering are self-limiting, and once they have been achieved, the kidney undergoes no further changes for many months. A different sort of tubular change will now be considered. In rats killed after 18 months of age very active hyperplasia has been found in occasional tubules, at first widely scattered, affecting principally the proximal convolutions, and quite unlike the regular, orderly growth of cells in young rats' kidneys. At this age a lot of nephrons have already disappeared, but one would expect the surviving tubules to compensate for their loss by hypertrophy rather than hyperplasia. Further, this hyperplasia in ageing kidneys appears to be destructive rather than helpful, because the tubules are often blocked and functionless and eventually become dilated by hyaline casts. As age increases still further the kidneys become greatly enlarged and granular in appearance, and microscopically they show chronic interstitial fibrosis, generalized tubular dilatation, and hyaline or fibrotic changes in the glomeruli and smaller vessels. These histological changes have been described and illustrated more fully elsewhere (Kennedy, 1951, 1957). The terminal appearance has been studied by numerous pathologists, but since no two agree on a morbid anatomical diagnosis, there is no need to add to the confusion here. The terms chronic glomerulonephritis (Wilens and Sproul, 1938), nephrosis (Saxton and Kimball, 1941), pyelonephritis (Goldblatt, 1947) and senile nephrosclerosis (Oliver, 1942) have all been used.

The pathological renal changes in old rats are almost invariably accompanied by great enlargement of the adrenals, frequently by parathyroid hyperplasia, and in the later stages, at least, by cardiac hypertrophy and hypertension. Before considering further which is cause and which is effect, a description will be given of a number of ways in which similar renal lesions can be produced in much younger rats in association with the same endocrine and vascular changes.

"Senile" changes after renal overloading in younger rats

The first condition in which these lesions were found in fairly young rats was in experimental hypothalamic obesity. When the ventromedial part of the hypothalamus is destroyed electrolytically, the appetite of a rat may be doubled for several weeks and the animal becomes grotesquely fat. In view of the association of clinical obesity with renal disease and hypertension, it is interesting that most of these fat rats developed typical senile kidney lesions about nine months earlier than unoperated controls (Kennedy, 1951). If the animals were operated on at three months old, they survived nine to 12 months before pathological lesions appeared in the kidneys, but the kidneys became enlarged during the period of overfeeding soon after the hypothalamic puncture. Moise and Smith (1927) and Addis and Oliver (Oliver, 1945) showed that the renal enlargement produced by a high protein diet in rats could eventually cause pathological changes, and it seemed possible that this might be the way in which the kidneys were damaged in hypothalamic overfeeding. As a first step an examination was made of the chemical changes in the kidneys during the earlier stages of development of the obesity, while the food intake was very high. In Table II these are compared with the changes found previously in the surviving kidneys after unilateral nephrectomy, and they followed an almost identical pattern. This suggested a convenient way to isolate the effect of simple kidney overloading during overfeeding from any possible effect of the subsequent

adiposity and abnormal fat metabolism. If the normally-fed rats with one kidney were to develop the same pathological renal changes as the overfed rats with two, then it would be reasonable to attribute the lesions to some effect associated

Table II

COMPOSITION OF THE KIDNEYS OF OBESE RATS, OR OF RATS WITH ONE
KIDNEY REMOVED, AT THREE AND SIX WEEKS AFTER OPERATION

Time from operation	Group	Total nitrogen (mg. per kidney)	RNA phosphorus (mg. per kidney)	DNA phosphorus (mg. per kidney)
	Control (not operated on)	19·4	0·378	0·183
Three weeks	Kidney removed	27·8	0·504	0·210
	Obese	29·9	0·488	0·227
Six weeks	Kidney removed	29·4	0·516	0·289
	Obese	28·3	0·532	0·308

with overloading. They did develop the lesions at the same time as the obese rats, at an average age of 15 months. Table III illustrates the changes in composition of the kidneys 12 months after carrying out each type of operation on three-

Table III

COMPOSITION OF THE KIDNEYS OF OBESE ANIMALS, OR OF ANIMALS
WITH ONE KIDNEY REMOVED, TWELVE MONTHS AFTER OPERATION
(OPERATED AT THREE MONTHS OF AGE)

Group	Total nitrogen (mg. per kidney)	RNA phosphorus (mg. per kidney)	DNA phosphorus (mg. per kidney)
Control (not operated on)	34·9	0·749	0·239
Kidney removed	73·5	1·429	0·695
Obese	75·9	1·972	0·785

month-old animals. Note that in each case the final renal breakdown occurred quite quickly and that rats killed during the period between four months and a year old had large but otherwise normal kidneys.

The period of latency is interesting, because in subsequent experiments it became shorter with increasing age of the

animal at operation, and in fact the age at which the final breakdown occurred was almost constant. To take the extreme case, rats over a year old frequently failed to establish any new renal equilibrium after either type of overloading, but rapidly developed pathological lesions.

The age at which renal failure occurred was advanced still further by increasing the renal loading, either by a more extensive partial nephrectomy, or by combining unilateral nephrectomy with overfeeding. It is sometimes said that different species tolerate the removal of different proportions of their renal tissue. It is difficult to see how a valid comparison can be made when the critical amount of kidney depends so much on the age of the animal. We found that weanling rats recovered and survived for many months after losing five-sixths of their kidneys, while nine-month-old adults often developed acute tubular necrosis after the same operation. A probable explanation for the latent period in the younger animals is that it represents the time for the further loss of nephrons due to ageing to reduce the available kidney below the critical level. It remains to consider the part played by the associated metabolic and endocrine disturbance in destroying the kidney.

Endocrine stimuli to renal hyperplasia

A number of hormones are renotrophic. They include growth hormone (White, Heinbecker and Rolff, 1949), thyroid hormone (Korenchevsky and Hall, 1944) and testosterone (Korenchevsky and Ross, 1940). The results of treatment with growth hormone are particularly suggestive. Acute overdosage can lead to rapid kidney destruction, but treatment of a young rat for only a few days, apparently causing no damage at the time, can lead to the appearance of pathological lesions months later (Selye, 1951). From the limited descriptions and photographs available no difference can be seen between these and the spontaneous lesions of older rats or those which develop after partial nephrectomy. Interpretation is complicated because partial nephrectomy is

among the measures that Selye uses, as he says, to "sensitize" the rat to the damaging effect of hormones. Nevertheless, we have found that no overgrowth of the kidney occurs in hypophysectomized rats with hypothalamic lesions, although they still have increased appetites, and other tissues, such as the liver and gastrointestinal tract, hypertrophy (Kennedy and Parrott, 1958). We also confirmed, as White, Heinbecker and Rolff (1941) first showed, that compensatory growth after partial nephrectomy required the presence of the pituitary. However, the late renal changes in our rats were associated with a catabolic rather than an anabolic state of the body as a whole, so it seems unlikely that growth hormone was being secreted in excess.

There remains the possibility that adrenal overactivity plays a part in the final renal breakdown. Adrenal enlargement and the nephrotic character of the renal defect (Saxton and Kimball, 1941) have been mentioned. A number of workers have shown that complete or extensive partial nephrectomy is followed by increased urea production (Bondy and Engel, 1947; Persike and Addis, 1949; Persike, 1950; McCance and Morrison, 1956). This has recently been shown to be due to increased protein catabolism in the liver (Sellers, Katz and Marmorston, 1957), so it may well be a result of increased adrenal activity. Overdosage with adrenal steroids can certainly cause renal breakdown associated with extensive tubular hyperplasia, although the immediate cause may be potassium deficiency (Follis, 1948) or sodium retention (Ingle, 1958) associated with such experiments. We have learned little from the serum electrolytes of our rats, because any changes that might implicate the adrenal are obscured by the general electrolyte retention of incipient uraemia. Morrison and Gordon (1957), however, have shown that increased urea excretion during starvation occurs both in partially nephrectomized and senile rats *before* obvious renal damage and is accompanied by an increased potassium loss.

Another renoprival effect that may hasten the end of the kidney is hypertension, although again the exact relation

between cause and effect is uncertain. Wilson and Byrom (1939, 1941) showed that the production of hypertension by "clipping" one kidney could lead after a prolonged latent period to pathological lesions in the other kidney. They attributed these lesions to hypertension, because their development seemed to be arrested and the hypertension cured by removing the ischaemic kidney. Goldblatt (1947) pointed out that all the lesions Wilson and Byrom had described could occur spontaneously in rats without hypertension. More recent work, reviewed by Floyer (1957), suggests that removal of the clip, so restoring some of the lost excretory function, is a much better protective measure than removing the ischaemic kidney, which frequently increases the hypertension. The importance of extrarenal or renoprival factors in producing permanent hypertension now seems well established and certainly fits with our experience, and apparently with Goldblatt's, that hypertension and vascular changes are a late feature of the spontaneous renal disease of rats.

Much remains to be done, but it is hoped that some progress has been made towards establishing the thesis, stated at the beginning of this paper, that the essential vicious cycle of renal disease in old age, in one species at least, is the destruction of surviving nephrons by overloading, after the normal renal atrophy of old age has reached a critical stage.

Summary

The kidney of the rat, and of most mammals including man, begins to atrophy while the animal is still young. Pathological changes in the kidney become more frequent during involution. Irregular and apparently purposeless hyperplasia of tubular cells is a prominent feature of such lesions. Hyperplasia occurs in the tubules of growing rats both as part of normal development and as a response to a moderate increase in the excretory load, but it is not normally seen after the main growth of the skeleton is completed. The stimulus to normal renal growth probably arises in the pituitary gland. It is

suggested that the loss of renal tissue in excess of a critical amount leads to additional renotrophic stimuli, probably related to overactivity of the adrenal cortex and to hypertension, which hasten the end of the remaining nephrons.

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DISCUSSION

Swyer: You said that this renal damage in the obese rat might be a question of protein overloading. Did you try feeding these rats on an *iso*caloric diet, but with half the protein content?

Kennedy: I have tried it as a short-term experiment but I did not carry it to its logical conclusion. There was no renotrophic effect.

Swyer: Over-feeding is itself a stressful activity in the Selyeian sense and that alone might lead to adrenal over-activity. Certainly there is clinical evidence that it may. Obese people who give evidence of increased adrenal steroid production may cease to do so after they have been put on a diet and have had their weight brought down to normal.

Kennedy: To answer that I must challenge the question of whether in fact stress ever produces renal lesions in the rat. I can do that quickly by quoting some recent work by Crane, Baker and Ingle (1958. *Endocrinology*, **62**, 216; and Crane and Ingle, *Endocrinology*, **62**, 474), who have studied a large number of so-called stresses which sound quite barbaric, and have found that the only one which produces what Selye calls the stressed kidney is exposure to cold. Selye has always said that this is the most effective, and these workers now say that it is the only effective stress. Under those circumstances the rats eat twice as much food. If they are then fed *iso*calorically, as you suggest, with a high caloric diet made up with carbohydrate and fat, they do not develop lesions. These workers attribute renal lesions to overloading with salt; I choose protein.

Talbot: Will you take this as evidence in favour of restricting the protein intake of patients with handicapped renal function?

Kennedy: I can see that it would be a dangerous thing to press a trophic stimulus like a high protein intake too far in an attempt to get recovery. Are you thinking of chronic renal disease, or a recovery from acute damage?

Talbot: Both.

Kennedy: Purely from my own findings I would have said that I could see no point in producing additional renal growth in trying to help recovery of the kidney by giving a high protein diet; if the object was simply to replace protein lost from the body then my results, of course, are not relevant. I think the problem of a high protein intake has to be studied from this point of view on the human, and we cannot answer from the work on the rat. Moreover, there may be a totally different limitation to the structural renal reserve in the rat, which has a kidney of completely different anatomical character.

Borst: We treat all patients with a kidney function of less than 10 per cent of normal with a diet adequate in calories but very poor in protein

(less than 20 g. daily). I have no comparison with a group of patients who continued eating normal amounts of protein. I have the impression that our patients can continue longer with their ordinary work. Their nausea usually disappears, they often gain weight, and in other respects are also in better condition. We have the paradox that reducing the protein intake often results in a rise in serum albumin and sometimes in a slight rise in serum haemoglobin. The protein-poor diet does not prevent a gradual reduction of the kidney function. However this decline is usually slow and the patients may have several years of useful life. A high diastolic blood pressure is a very bad prognostic factor. As long as we have no control group we cannot produce convincing evidence that an untreated patient will not live as long as our 'maltreated' patients.

Kennedy: Have you done any liver function tests in a situation where serum albumin is falling in spite of a high protein intake, Prof. Borst? There may be a possible connexion with the increased liver protein breakdown when one removes the kidney (Sellers, Katz and Marmorston, (1957). *Amer. J. Physiol.*, **191**, 345).

Borst: No liver function tests were done, and we only have data on the serum proteins. There is no increased γ-globulin as is usually found in chronic hepato-cellular disease. We had, however, some evidence of a deleterious effect of the low protein diet. More cases of tuberculosis were seen than would be expected in similar patients on a normal protein diet, and two patients died from miliary tuberculosis. Probably the extremely low protein diet reduces the resistance against the tubercle bacillus in spite of the fact that the patients do not lose weight.

Talbot: How do you define a low protein diet?

Borst: It is less than 20 g./day. To control the diet and determine whether or not the patient adheres to it, 24-hour urine portions are regularly examined for nitrogen excretion. We also determine creatinine excretion to be sure that urine collection is complete. The 24-hour creatinine output is very constant. This output is determined for every kidney patient during clinical observation, and we use the figures for comparison with the nitrogen output when the patients are under control in the out-patient department. Many adhere to the diet and go along very well for several years.

Fejfar: We have had similar experiences in Czechoslovakia. This treatment originated in the experiments of Thomas Addis (1948. Glomerulonephritis: Diagnosis and Treatment. New York: Macmillan), who showed that partially nephrectomized rats kept on a higher protein intake could not survive as long as the animals with a low protein diet. We therefore started to use a low protein diet in all patients with chronic glomerulonephritis. Usually we give 0·5–0·7 g./kg. body weight per day in the diet (but no less than 0·5 g./kg.), plus the amount lost in the urine. Of course, children and those with the nephrotic syndrome are given larger amounts of protein. It is very difficult to judge long-term results as we have no control group for this treatment. Nevertheless we do think we can prolong the life of patients with chronic nephritis on this low-protein diet.

Richet: In populations that are said to eat a lot of proteins, for instance Eskimos, what is the state of the kidney? Do such people often die from chronic nephritis? They are generally said to eat 5,000 cal./day, mostly fat and proteins.

McCance: I think in fact the Eskimos do not eat a very high protein diet, although they may eat a great deal of fat. They certainly tend to die rather young, but mostly from accidents, I believe; an old Eskimo is a man of about 40–45.

Richet: Some work has been done by Lieb (1929. *J. Amer. med. Ass.*, **93**, 20), by Thomas (1927. *J. Amer. med. Ass.*, **88**, 1559), and by Bischoff (1932. *J. Nutr.*, **5**, 431), which seemed to demonstrate that a high protein diet was absolutely harmless.

Dr. Talbot, you mentioned the amount of protein given in cases of chronic nephritis. In Paris we put some chronic nephritic patients on an almost protein-free diet, about 10 g./day. Three or four patients whose death was not expected died after six weeks (Hamburger, J., Serane, J., and Cournot, L. (1951). *Sem. Hôp. Paris*, **27**, 2289). We therefore never gave that kind of diet again to any patients for more than ten or fifteen days. Also, we never give under $0 \cdot 5$ g./kg. in chronic cases, because under that amount we have a lot of trouble and the patients become so weak they would never live anyway; we prefer to have a patient with perhaps a shorter life, but healthy, than the other way round.

Fourman: Dr. Kennedy, why did you imply a relationship between catabolic reactions, adrenal hyperplasia and Selye's results with cortexone acetate?

Kennedy: The catabolism would require over-secretion of Compound F, of course. However, Hechter and Pincus (1954. *Physiol. Rev.*, **34**, 459) showed that in the rat the adrenal secretes chiefly Compound B anyway, and there is not in fact the contradiction there would seem to be. An over-secreting adrenal could damage the rat kidney and one would, at the same time, get a catabolic effect.

Fourman: But you would not necessarily want to relate that to the results with cortexone acetate?

Kennedy: Yes, in that cortexone acetate was the particular steroid which was used in most of Selye's experiments.

Desaulles: In our laboratories Compound B has been shown to help in inducing hypertension in the rat.

Kennedy: Does it produce renal lesions?

Desaulles: Only in enormous doses.

Milne: The recovery lesions of potassium depletion are similar in appearance to the ageing kidney, as mentioned earlier by Dr. Kennedy. The histological studies reported by Dr. Desaulles seem to show the same dilatation of the tubules that was seen in Dr. Kennedy's cases. We repeated these experiments with dietary potassium depletion and cortexone acetate injections, but we used very young rats and were unable to repeat the effects which were shown so conclusively at Cambridge. Is the ageing kidney, then, more susceptible to permanent damage from potassium depletion? This would tie up with Dr. Fourman's suggestions regarding cortexone acetate as given by Selye.

Kennedy: Dr. Fourman and I have looked at potassium-deficient kidneys together many times. We agreed then that they were closely similar to the kidneys we found in old rats of our own colony, and that this showed that the chronic potassium-deficient kidney is simply an ageing kidney. Now I am not at all sure that they are not the same thing anyway: that the ageing kidney is, in a sense, a potassium-deficient kidney and that there is an element of adrenal over-activity about it. As you say, Dr. Milne, this may really mean that older age groups are more liable to potassium-deficient states and the renal consequences of that.

Fourman: That seems to provide an explanation of why the death of some nephrons appears to lead to pathological changes in the remainder. From your studies, Dr. Kennedy, it seems reasonable to argue that in a potassium-deficient kidney some nephrons die, and as a result in the remainder there are ultimately pathological changes which are likely to be worse in older rats.

Kennedy: It is a vicious cycle and we are coming into it at different points.

McCance: Dr. Kennedy has performed a valuable synthesis in bringing together over-nutrition, age, and lesions in the kidney. No-one asked and I wish we knew what happens if these kidneys are overloaded with water and with various other test substances.

RENAL FUNCTION IN RESPIRATORY FAILURE

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With increasing age, the functional capacity of the lungs and of the kidneys declines. Respiration is embarrassed by increasing rigidity of the chest wall, and there is also an increase in the respiratory dead space of the lung itself in older subjects (Comroe *et al.*, 1955). The kidneys lose efficiency in consequence of a progressive loss of nephrons, which may reduce the nephron population to 60 per cent of the original number; the impairment of renal function is indicated by a fall in the clearance of inulin and of p-aminohippurate, and in the maximal reabsorptive capacity for glucose (Tm_G) (Shock, 1952). The blood pH in old people is a little lower, and their plasma returns more slowly to its previous level after imposed loads of either acid or alkali. These various encroachments on functional reserve are probably of no great moment in healthy old folk leading a normal life; but they are brought into prominence when respiratory function is pathologically impaired by the related changes of chronic bronchitis, bronchospasm, and emphysema. In an urban population, the incidence of chronic bronchitis in old people has been found to be 40 per cent (Sheldon, 1948); this common illness leads in time to gross respiratory failure, with the patient afflicted by anoxia, hypercapnia, and increased pulmonary vascular resistance in varying degrees. There are several ways in which advanced respiratory failure can increase the demands on the kidneys, and also diminish their functional capacity. This communication outlines the effects on renal function of chronic hypercapnia and of cardiac failure secondary to emphysema (cor pulmonale).

Hypercapnia. The effects of acute hypercapnia, usually induced by inhalation of 5–10 per cent CO_2, have been reviewed by Pitts (1953). There is a fall in plasma pH and a rise in pCO_2; the urine formed is acid, and the reabsorption of filtered bicarbonate is virtually complete, although the amount of filtered bicarbonate has been increased by the experimental procedure. Enhancement of bicarbonate reabsorption is the most striking change in renal performance induced by acute hypercapnia; and it persists when the fall in plasma pH is prevented by infusion of bicarbonate, so that in this context rise in pCO_2 seems to be the more relevant stimulus to bicarbonate reabsorption. The reabsorption of bicarbonate is also increased in subjects depleted of potassium, in whom intracellular pH is probably decreased; so it seems quite likely that the effect of raised pCO_2 on bicarbonate reabsorption is mediated by a fall in the pH of the renal tubule cells. Apart from this rather striking change in bicarbonate excretion the output of electrolytes is not significantly affected by short periods of hypercapnia, although there is a transient water diuresis (Barbour *et al.*, 1953).

It is not clear how far the information obtained from studies of acute hypercapnia can be applied to the situation of chronic hypercapnia found in emphysematous patients. Here, a steady state has been established at a new level of plasma pH and bicarbonate concentration. The electrolyte composition of plasma and red cells in emphysematous patients is different in several respects from that of normal people in whom a comparable hypercapnia has been induced acutely by CO_2 inhalation (Platts and Greaves, 1957). For example, the fall in pH is much smaller in the emphysematous patients, and the chloride content of both cells and plasma is lower than in acute respiratory acidosis.

There are few observations on the renal response to chronic respiratory acidosis in man. As part of a study on the effect of Diamox, Nadell (1953) reports observations on 24-hour specimens of urine from two patients with respiratory acidosis.

The mean urinary pH in these two patients was $6 \cdot 26$ and $6 \cdot 67$, no more acid than specimens from two 'controls' with mean pH of $6 \cdot 48$ and $6 \cdot 20$. The mean excretions of bicarbonate were $7 \cdot 5$ and $15 \cdot 2$ m-mole/day, compared with $10 \cdot 1$ and $4 \cdot 8$ m-mole/day in controls. Ammonium excretion was somewhat higher, and titratable acidity somewhat lower in the patients with respiratory acidosis than in the controls, and it has been reported that renal glutaminase is increased in experimental respiratory acidosis. There were no striking differences in 24-hour output of sodium, potassium, or chloride. These findings are consistent with the view that renal adaptation has included increased synthesis of ammonia, allowing the excretion of hydrion at a higher urine pH than in acute respiratory acidosis, without increase in urinary buffer (the excretion of phosphate was lower than in the controls).

In preliminary observations on four patients with respiratory acidosis, my colleague Dr. J. Timoner has found a pH range in urine of $5 \cdot 1$ to $6 \cdot 7$, with ammonium excretion up to 65 μ-equiv./min. and titratable acidity up to 60 μ-equiv./min. After a standard load of ammonium chloride ($0 \cdot 1$ g./kg. body weight), two patients excreted $76 \cdot 5$ and $81 \cdot 3$ μ-equiv. of ammonia, and $26 \cdot 3$ and $46 \cdot 2$ μ-equiv. of titratable acid per minute. The ammonium excretion is just above the normal range found by Davies and Wrong (1957). These two patients were aged 57 and 60, and seem to have retained the capacity of the renal tubule cells to form ammonia in response to an acid stimulus.

Renal function in cor pulmonale. In the cardiac failure associated with emphysema, the cardiac output is commonly increased, and the patient has warm extremities. Terminally, the limbs become cold, the blood pressure falls, and the cardiac output at this stage is reduced. Davies and Kilpatrick (1951) showed that even in the high-output phase of cor pulmonale the circulation through the kidneys and the glomerular filtration rate were substantially diminished. These findings have been confirmed by Lewis and his co-

workers (1952). A moderate degree of urea retention, presumably on the basis of relative renal ischaemia, is common in cor pulmonale (Simpson, 1957), as in other forms of heart failure. In patients dying from heart failure, the output of urine may be reduced to below 500 ml./day, but complete suppression of urine does not seem to have been recorded, even in the terminal stages. It is perhaps of some interest, therefore, that over the past ten years we have seen two patients, both with cor pulmonale, who became anuric (Black and Stanbury, 1958). One of them, a girl of 20 with widespread bronchiectasis and a terminal bronchopneumonia, had an eight-day period of extreme oliguria, during which her blood urea rose to 158 mg./100 ml. She was treated conservatively, urine was again formed, and the blood urea fell to 76 mg./100 ml. She continued to pass considerable amounts of dilute urine until her death a week after the end of the anuric period. The second patient, a man of 44, passed no urine for over 24 hours, and had no urine in his bladder after death. Both these patients had hypotension and cold extremities, and were presumably in the low-output phase of cor pulmonale; but cardiac output could not of course be measured. Both of them had central cyanosis, but only the second had a raised pCO_2 in the plasma. The main factor in causing anuria was probably renal ischaemia, but this may have been aggravated by arterial desaturation.

Both these patients had hyperkalaemia and low plasma sodium. This association is fairly common in patients with acute renal failure, but we have seen it also in the absence of renal failure and it may possibly represent a loss of potassium from cells, with partial replacement by sodium.

These observations in patients with terminal cor pulmonale are possibly of little more than academic interest; but they perhaps constitute yet another argument for the early treatment of intercurrent infections in patients with emphysema; such intercurrent infections may be apyrexial, and attended by little apparent reaction, but they can precipitate the patient into terminal low-output failure.

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DISCUSSION

Milne: I am not convinced, Dr. Black, that the anuria you mentioned in your two cases is in any way related to the chronic respiratory disease. During the last influenza epidemic in this country some cases of anuria were associated with influenza. I know of one case in Dundee and we ourselves have personally studied three cases. Two of those we saw recovered and one died. The one that died showed typical acute tubular necrosis; the other two showed a clinical course typical of tubular necrosis. None of these patients gave any sign of chronic respiratory disease. They were typical Asiatic influenza cases, as shown by the epidemiology and serum tests, developing in previously healthy individuals; one case was uncomplicated and two cases were complicated by a secondary staphylococcal pneumonia. A severe respiratory infection of itself in some cases seems to be able to precipitate anuria, and I myself prefer to relate your experience to infection rather than to the biochemical changes of chronic respiratory acidosis.

My other point is a personal protest: I have a tremendous respect for the work of Dr. Pitts and his colleagues, but I do think we should avoid adopting this term, 'bicarbonate-bound base'. To the chemist bicarbonate is a hydrogen ion acceptor and therefore is a base itself. Bicarbonate is the base; bicarbonate-bound base to me is meaningless.

Black: In quoting from Pitts, I used his terminology, but I do not accept responsibility for it.

When you say infection, do you mean infection leading to a fall in cardiac output and renal vasoconstriction, or do you mean an infection of the kidney?

Milne: No, certainly not an infection of the kidney. All I am stressing is that these cases occurred in young adults without any evidence

whatsoever of chronic respiratory disease, and that a severe respiratory infection, for some reason that I do not know, may cause acute tubular necrosis, for which there is autopsy proof in one case.

Black: This would really bring it into the whole group of peripheral circulatory changes.

McCance: This seems to me a matter which is wide open to experimental attack, and it might be coupled with stress tests.

Davson: The trouble is that the energy required for these active transport processes is a small fraction of the whole and when the energy supplies are interfered with to such an extent that active transport is affected, the cell will be dead long before you can obtain any useful information.

Borst: We have just had an autopsy on a very obese patient who died with bilateral cortical necrosis. I am ashamed to say that she had been under-examined. As in Dr. Black's cases she was admitted with a respiratory infection which was treated with penicillin, and in a few days the infection was under control. She was up and about until we discovered that she was producing no urine. On autopsy no abnormality in the lungs was found. The necrosis involved a great part of the renal cortex; there was no evidence of other renal disease. We thought that it was a case of Pickwick's syndrome.

It was reported about 20 years ago that giving oxygen to patients with respiratory failure resulted in an increased sodium output. In our cases there was no definite effect on sodium output in spite of the fact that the general condition of some of the patients improved markedly.

Bull: I was hoping that Dr. Black was going to bring evidence of a normal decline in respiratory function, because in our patients both renal and respiratory deaths are common, and there are many cases of the combination of the two. If someone could show that respiratory function declined in roughly the same way that renal function does that would help us to understand this situation. I believe that tissues other than the kidney must undergo a similar decline in function at the same sort of rate with age to account for this rather remarkable mortality experienced. We have now confirmed our findings on over 3,000 cases, and we get exactly the same effect as we did eight years ago.

Black: There are indeed plenty of references to the decline of respiratory function with age. A summary has been given by Stuart-Harris and Hanley (1957. Chronic Bronchitis, Emphysema, and Cor Pulmonale. Bristol: Wright & Son).

Scribner: As regards renal compensation, we had one patient with a remarkable ability to compensate for respiratory acidosis. We were interested in finding out whether high pCO_2 or low pH caused the coma-like condition that patients with respiratory acidosis may develop when treated with oxygen. Our interest began when we tried treating acute renal failure by putting a cellophan bag in the stomach, a technique first suggested by Dr. Schloerb of Kansas City. With this technique of gastro-dialysis it is possible to remove tremendous amounts of hydrogen ion, in fact usually so much that you have to put hydrochloric acid in the dialysis fluid to prevent alkalosis in the patient. We turned this around and applied it therapeutically to the respiratory acidosis patients in an

attempt to compensate them artificially by getting their serum bicarbonate levels up. We treated a 50-year-old man with acute respiratory acidosis whose initial bicarbonate figure was 40 m-equiv./l. and the blood pH, breathing room air, about $7 \cdot 28$. When he went into oxygen he became unconscious rather quickly, presumably due to the decrease in ventilation from the relief of anoxia. He was removed from oxygen and over the next 18 hours dialysed through a cellophan bag in his stomach, using a fluid containing 50 m-equiv./l. sodium bicarbonate in 5 per cent glucose. The dialysis elevated his serum bicarbonate to 64 m-equiv./l. despite a negative sodium balance of 200 m-equiv. The sodium was lost mainly in the urine. The high serum bicarbonate elevated his blood pH, breathing room air, to $7 \cdot 55$. When he again went into oxygen his blood pH fell to $7 \cdot 45$ and he did not become unconscious. His anoxia disappeared despite the fact that his ventilatory rate slowed from 9 litres per minute to 3 litres per minute. During the next 72 hours his kidneys sustained his serum bicarbonate level above 60 m-equiv./l. by excreting a normal amount of ammonia and titratable acidity.

Experience in this patient suggests that so-called "CO_2 narcosis" is actually due to the low pH rather than the high pCO_2. The results also suggest that despite the high serum bicarbonate renal compensation for the respiratory acidosis may be incomplete in this acute situation. Gastrodialysis makes it possible to treat the acidosis without resorting to sodium administration, which is contraindicated because of the heart failure from cor pulmonale.

WATER AND ELECTROLYTE METABOLISM IN CONGESTIVE FAILURE

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The rôle of the kidney in congestive failure

THE genesis of abnormal water and electrolyte metabolism in congestive failure is at present generally attributed to impaired renal function. It was previously thought that increased systemic venous pressure (and hence the imbalance of Starling forces in the capillaries) was the main factor initiating these phenomena. Warren and Stead (1944) observed in some cardiac patients an increase in body weight after the administration of salt before any significant rise in central venous pressure. This indicated that another mechanism might be responsible for the retention of salt and water in chronic congestive failure. Merrill (1946) confirmed the earlier findings of Seymour and co-workers (1942) that patients with congestive failure have a diminished renal blood flow; moreover he found that the decrease in renal blood flow was far greater than the diminution of cardiac output.

It was, however, not clear whether the retention of electrolytes and water in chronic congestive heart failure was due to a primary decrease in renal function or to the decrease in renal blood flow and function as a consequence of the increase in central venous pressure.

It appeared to us in 1947 (see Brod and Fejfar, 1949, 1950) that only observations of haemodynamic events at the time when water balance was changing could elucidate this problem. Patients with heart disease on the borderline of right heart failure usually have a low urine output during the day, but an increased urine flow at night. This spontaneous diuresis

reflects a temporary improvement of the impaired water balance. It runs its course within a few hours. It was therefore possible to follow the sequence of events and investigate the relationship between central venous pressure, systemic and renal haemodynamic changes, and renal function.

Cardiac output, right auricular pressure, water content of plasma, and renal function (renal blood flow, glomerular filtration rate and excretion of electrolytes) were studied from the early hours of the afternoon until the following morning in ten normal subjects and 25 patients with heart disease of different origin, 19 of them having congestive failure of varying degree (Brod and Fejfar, 1949, 1950; Fejfar and Brod, 1950a,b,d).

Cardiac output was measured by a direct Fick method and right auricular pressure by a water manometer attached to the cardiac catheter; changes of water content in plasma were assessed from the percentage change in plasma proteins, haematocrit and the disappearance curve of Evans blue. Renal plasma flow was estimated by the clearance of PAH (p-aminohippuric acid), glomerular filtration rate by the clearance of inulin, and chlorides by the Van Slyke and Hiller (1947) modification of Sendroy's method.

A nocturnal diuresis was observed in 11 patients with congestive failure. In none of them was it preceded by a decrease in right auricular pressure. On the other hand the increase in urine output at night started in all these patients with an elevation in renal blood flow. The decrease in urine flow at night occurred in seven decompensated cardiacs; in all of them it was associated with a diminution in renal blood flow (Fig. 1). The increase in renal blood flow was not related to a similar change in cardiac output, which increased simultaneously in only half of the investigated subjects.

There is thus evidence in dynamic observations that the increase in central venous pressure in congestive failure is not the primary cause of cardiac oedema, the main factor being impaired renal function.

A low renal blood flow with a diminished glomerular

filtration rate and increased tubular reabsorption of electrolytes was also found in patients with left-sided failure and with mitral stenosis without any clinical evidence of right-sided decompensation, the central venous pressure being normal (Fejfar and Brod, 1949; Blegen and Aas, 1950; Werkö *et al.*, 1952*a*; Himbert *et al.*, 1954; Werkö *et al.*, 1955).
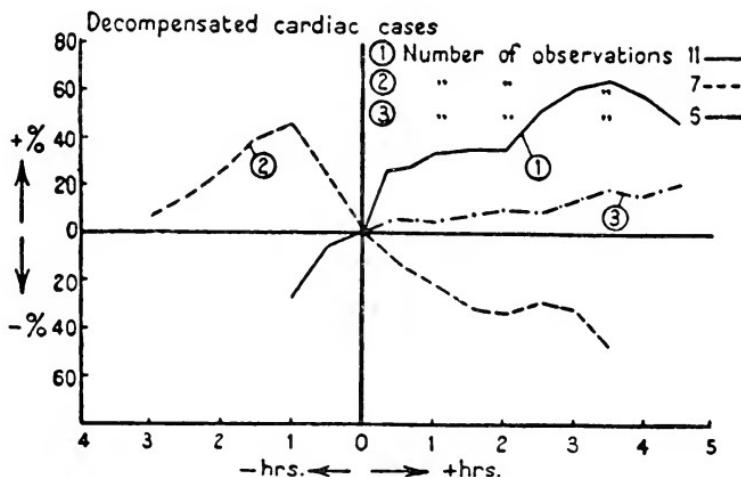
FIG. 1. Composite diagram showing percentage changes ($\triangle$%) in renal blood flow (Cl_{PAH}) from the level at 0 hrs (time at which the urine flow began to change) in decompensated cardiacs. In patients with no change in urine flow, 0 hrs was fixed arbitrarily at 7 p.m. (1) are patients with a nocturnal increase in urine flow, (2) are patients in which the urine flow decreased at night, while in (3) it did not change. See text for details. (Brod, J., and Fejfar, Z. (1950). *Quart. J. Med.*, **19**, 187.)

Fig. 2 presents the individual values of renal blood flow in normal subjects and in patients with heart diseases. All patients are divided into five groups according to the clinical degree of heart failure.

In the first group are clinically compensated patients. The second group includes patients with a slight to moderate dyspnoea on effort; in the third are those with marked dyspnoea on effort, orthopnoea or attacks of nocturnal dyspnoea and acute pulmonary oedema. The fourth group covers patients with signs of right-sided decompensation who responded well

to digitalis, and in the fifth group are patients refractory to the usual methods of treatment.

It may be seen that patients without right-sided failure have a decreased renal blood flow in comparison with the values in normal control subjects.

On the other hand increase of pressure in the renal vein brought about by a partial occlusion (Selkurt, Hall and
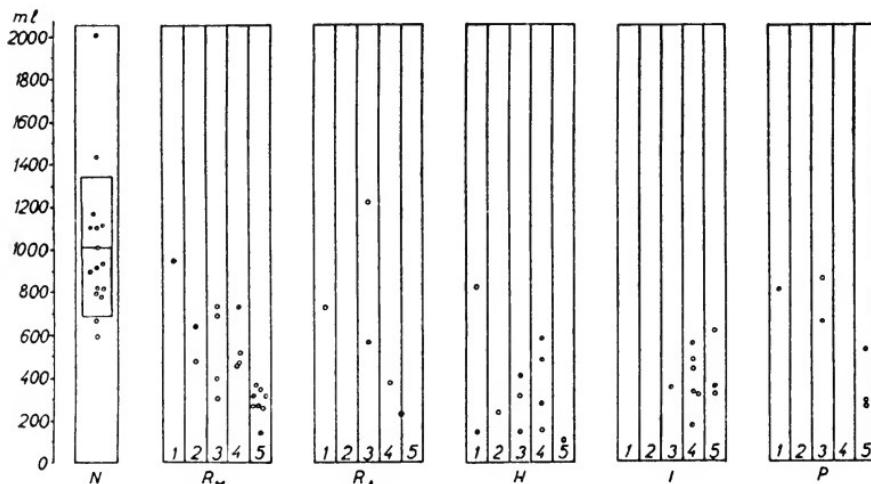
Fig. 2. Renal blood flow in normal subjects and in patients with rheumatic (R_M and R_{A_0}), hypertensive (H), ischaemic (I) and pulmonary (P) heart disease. All patients are divided into five groups according to the clinical degree of heart failure. See text for details.

Spencer, 1949), or by an increased abdominal pressure (Bradley and Bradley, 1947), is followed by only a small diminution of the renal blood flow.

Maxwell, Breed and Schwartz (1950) measured pressure in the inferior vena cava in 17 healthy subjects and ten patients with congestive failure. The mean pressure in healthy subjects was $15 \cdot 2$ cm. H_2O, and in patients with congestive failure 27 cm. H_2O. From the measured values of pressure they calculated that the increase of renal resistance due to the elevation of pressure in renal veins would reduce renal blood flow by about 14 per cent. The actual decrease in renal blood flow in congestive failure is far greater (see Fig. 2).

Farber and co-workers (1951, 1953) studied in man the effect of an increase of pressure in the vena cava produced by means of a balloon above and below the orifice of the renal veins. In both procedures there was a diminution of renal blood flow, glomerular filtration rate and excretion of water and electrolytes.

The increased central venous pressure in congestive failure may, of course, contribute to reduction in renal function (Briggs *et al.*, 1948; Bradley and Blake, 1949; Earle *et al.*, 1949). It determines the distribution of retained water and electrolytes, which in left-sided failure is in the lungs and in congestive failure mainly in the lower part of the body.

The nature of renal changes in congestive failure.

The nocturnal increase of diuresis and renal blood flow in our investigated patients with congestive failure was also associated with an elevation of glomerular filtration rate and with a decrease in tubular reabsorption of water and electrolytes. This may be seen in Fig. 3, which covers 20 spontaneous changes in urine flow in 14 patients with congestive failure. The lower urine output was always taken as the initial value (100 per cent).

The mean increase in diuresis was 187 per cent (range from 44 to 672 per cent). This increase was associated in all instances (as seen in Fig. 1) with an elevation in renal blood flow. This latter increased on the average by 55·5 per cent (from 6 to 146 per cent). Only three times was the increase in renal blood flow smaller than 20 per cent. In 14 subjects in whom it was measured cardiac output (CO) rose significantly in six instances, fell in three and did not change in five. It is clear that the increase in renal blood flow could not depend on the primary increase in CO. This is confirmed by an increase in the renal fraction of cardiac output in all instances except one, in which the renal fraction did not change.

Glomerular filtration rate at high urine flow was elevated 15 times, and unchanged five times. The average increase was 27·1 per cent, with the range − 4·5 to + 82 per cent.

The elevation of renal blood flow was effected in the great majority by a decrease in postglomerular resistance, the filtration fraction diminishing 17 times and increasing in only three instances.

The increase in chloride clearance was of the same order as
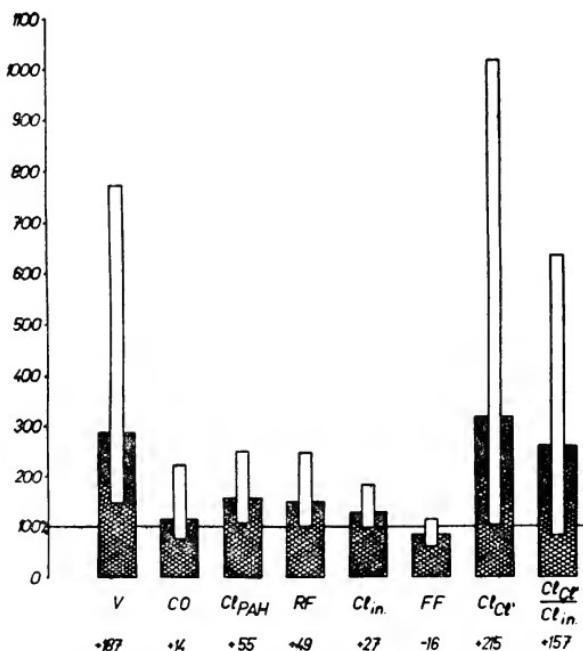
FIG. 3. Percentage nocturnal changes in urine flow (V), cardiac output (CO), renal plasma flow (Cl_{PAH}), renal fraction of cardiac output (RF), glomerular filtration rate (Cl_{in}), filtration fraction (FF), chloride clearance ($Cl_{Cl'}$) and in the ratio of chloride clearance to glomerular filtration rate $\left(\dfrac{Cl_{Cl'}}{Cl_{in}}\right)$. The mean percentage change (▨) and range (☐) in 14 patients with heart failure are presented. The lower urine output was taken as 100 %.

the elevation of urine volume. The mean increase was 215 per cent, range 2 to 910 per cent. The ratio of chloride clearance to glomerular filtration rate rose on an average by 157 per cent (range $-17 \cdot 6$ to $+530$ per cent).

According to Wesson, Anslow and Smith (1948) some 85 per cent of the filtered sodium and chloride is reabsorbed by an active mechanism in the proximal tubule, irrespective of the amount filtered. The reabsorption of the remaining 15 per cent of sodium and chloride is limited by a fixed maximal rate at which the distal tubular cells are able to reabsorb these electrolytes. Whenever the tubular chloride load decreases with a fall in glomerular filtration rate in the presence of this maximal reabsorption capacity, almost all of the filtered chloride is reabsorbed. Merrill (1949), Mokotoff, Ross and Leiter (1948), Selkurt, Hall and Spencer (1949), Stead (1951) and others are of the opinion that in congestive failure this mechanism leads to the maximum reabsorption of electrolytes and water; that is to say that the diminution of glomerular filtration is such that with a normal unchanged tubular reabsorption, water and electrolytes are retained.

Our results are not in accord with the hypothesis of Wesson, Anslow and Smith. In patients with severe congestive failure, glomerular filtration rate did not rise towards normal levels at the time of nocturnal diuresis; in spite of this, the amount of excreted chloride was far greater than the quantity of chloride excreted at night in healthy subjects with a normal glomerular filtration rate. Fig. 4 demonstrates that the tubular reabsorption of chloride can vary markedly with a constant tubular chloride load. It is clear, of course, that at a given chloride load less chloride is reabsorbed at a high than at a low urine flow.

The concentration of chloride in urine exceeded its plasma level in only seven out of 24 observations at high urine flow. The increased urine flow, therefore, cannot be explained on osmotic grounds by an increased excretion of chloride.

The lower elimination of electrolytes and water in congestive failure is, according to these findings, not caused only by decreased glomerular filtration rate. Tubular reabsorption of water and electrolytes increases as well. The same conclusion is stated by Briggs and co-workers (1948), Kattus and co-workers (1948), Davis and Shock (1949), Newman (1949),

Himbert and co-workers (1954), Cort (1955b), Cort and Fencl (1957), and others.

Doyle and Merrill (1957) studied renal function in 18 patients with congestive failure in a supine position and tilted in a passive erect posture. The changes were qualitatively similar to those in normal subjects. There was a further depression of renal plasma flow, glomerular filtration rate and also a decreased urine flow and a fall in the excretion of the
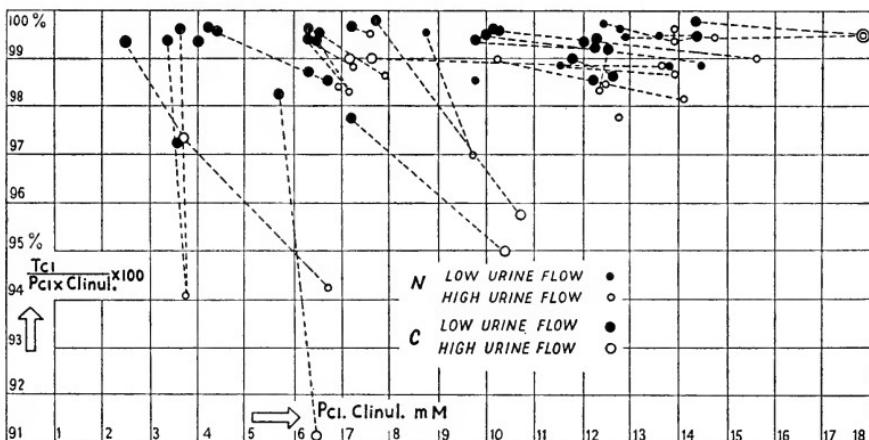
FIG. 4. Relationship of the amount of the chloride filtered ($P_{Cl} \times Cl_{inul.}$) and reabsorbed $\left(\dfrac{T_{Cl}}{P_{Cl} \times Cl_{inul.}} \times 100 \right)$ in individual subjects at high and low urine flows. Values in individual subjects are connected with dotted lines. N—normal control subjects; C—patients with heart disease. See text for details.

electrolytes. In accord with our previous findings, with nocturia the decreased urine flow in the erect posture was closely correlated with changes in renal plasma flow. There was, on the other hand, a very poor correlation between changes in glomerular filtration rate and sodium excretion.

In these observations there was an indirect relationship between the tubular reabsorption of electrolytes and water and renal blood flow. The tubular reabsorption increased when renal blood flow fell and vice versa.

This finding does not characterize congestive failure alone.

Bucht and co-workers (1953) studied the haemodynamic changes together with the excretion of sodium in eight healthy human subjects during muscular exercise of varying degree. As long as the effort was small (oxygen consumption not above 500 ml./min.), an increase of CO was found without significant effect on renal blood flow, glomerular filtration rate or excretion of sodium. A greater muscular effort (oxygen consumption about 1,000 ml./min.) was character-ized by a marked increase in CO (almost double) and a simultaneous fall in renal blood flow and the renal fraction of CO. The excretion of sodium and water fell. Glomerular filtration rate and pressure in renal veins did not change significantly. Similar results were observed in patients with heart disease (Judson *et al.*, 1955; Himbert, Scébat and Théard, 1956). Increase of tubular reabsorption was there-fore responsible for the diminished excretion of sodium and water.

The close relationship between renal blood flow and excre-tion of electrolytes in congestive failure is striking. We have expressed the opinion (Brod and Fejfar, 1950) that decreased renal blood flow directly impairs the excretion of water and electrolytes. A smaller glomerular filtration rate diminishes tubular electrolyte load and, owing to a slower flow of tubular urine, a greater proportion of the filtered amount is reabsorbed. We could not, of course, exclude another possibility: that increased reabsorption of water and electrolytes in the renal tubules could occur parallel with, but independently of the diminished renal plasma flow; i.e. the stimulus for the renal vasoconstriction could directly influence the function of renal tubules, leading to an increased reabsorption of salt and water.

Humoral and neural regulatory mechanisms in congestive failure.

Some known humoral and neural factors can alter the function of renal tubules. In the urine of patients with con-gestive failure renin (Merrill, Morrison and Brannon, 1946), VEM (vaso-excitor material) and VDM (vasodepressor

material) have been found (Edelman *et al.*, 1950). Extracts of urine from patients with congestive failure contain anti-diuretic materal (Bercu, Rokaw and Massie, 1949, 1950) with a great sodium-retaining activity (Deming and Luetscher, 1950*a*,*b*), which disappears when the patients become compensated (Luetscher, Deming and Johnson, 1950, 1951). The substance responsible for this is aldosterone (Luetscher and Johnson, 1954). An increased excretion of aldosterone is not characteristic only of congestive failure, but accompanies nephrotic and cirrhotic oedema as well. A permanent increase of aldosterone under these conditions is called secondary aldosteronism (Conn, 1955; Bartter, 1956; Milne and Muehrcke, 1956; Thorn *et al.*, 1956; Liddle, Duncan and Bartter, 1956; Wolff, Koczorek and Buchborn, 1957).

The increased secretion of aldosterone in congestive failure may be important in some patients, as can be seen from the favourable effect of bilateral adrenalectomy (Thorn *et al.*, 1956).

Buchborn (1956) estimated the activity of plasma anti-diuretic hormone (ADH) by a sensitive biological method on the toad, together with serum osmolarity. He found a close indirect correlation between the plasma ADH and serum osmolarity in 14 normal subjects, in patients with hepatic cirrhosis, in compensated cardiac patients, and also in patients with congestive failure. The increased plasma level of ADH in congestive failure is not therefore primary, being an expression of the homeostatic function of ADH, regulating osmotic pressure in the organism (Buchborn, 1956).

Neither ADH nor aldosterone significantly influences circulation in the kidneys. Their main effect is on renal tubules, where they increase the reabsorption of water (ADH), or sodium (aldosterone). In addition we have already indicated that the vasoconstriction in the kidneys, together with diminished elimination of sodium, occurs during a short muscular effort (10 minutes, Bucht *et al.*, 1953). The effect of aldosterone would be slower. According to Bartter (1956) the excretion of sodium in a patient with Addison's disease did

not start to fall until more than an hour after intravenous injection of 40 μg. aldosterone.

It would appear to us, therefore, that neither of these humoral substances is the primary cause of the retention of salt and water in heart failure.

The results of haemodynamic changes in human subjects following intravenous injection of Dibenamine called our attention to the importance of reflex (neurohumoral) regulation in the genesis of haemodynamic changes in congestive failure.

Blockade of adrenergic impulses by Dibenamine in patients with heart failure caused a diminution of a high peripheral vascular resistance and central venous pressure. Cardiac output increased. Renal blood flow rose in a great majority of investigated subjects, suggesting that this was independent of the increase in CO. These changes were not produced by blocking the adrenergic impulses in the heart or by an increased secretion of adrenaline (Fejfar and Brod, 1950c, 1951, 1954; Brod, Fejfar and Fejfarová, 1951, 1954) (Fig. 5). The increase in renal blood flow in seven out of nine patients in congestive failure was accompanied by a rise in urine flow and an increased elimination of sodium or chloride.

We were able to conclude from our results that, with the onset of congestive failure, reflex (neurohumoral) vasoconstriction develops in both arterial and venous circulation. The function of this selective vasoconstriction may be to secure a sufficient supply of oxygenated blood to working tissues such as the heart and other muscles.

A haemodynamic pattern resembling chronic heart failure (i.e. unequal distribution of blood supply to various organs, increased utilization of oxygen in tissues, and an insufficient CO) may also be found in clinical circumstances with a diminished return of venous blood to the heart (e.g. mitral stenosis, constrictive pericarditis), or when the amount of circulating blood and oxygen decreases, as well as in acute heart failure or peripheral circulatory failure (see Fejfar, 1958). A similar haemodynamic picture can be seen in severe

muscular effort in healthy subjects. It differs from that found in heart failure by an increase in CO and by vaso-dilatation in the skin due to increased temperature.

Haemodynamic changes in heart failure therefore do not represent a new and special adaptation of the organism to the
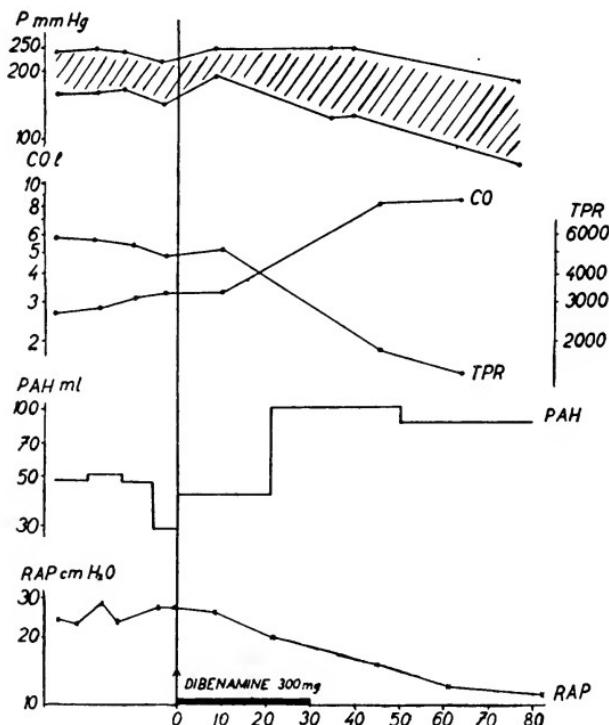
FIG. 5. Changes in cardiac output (CO), peripheral vascular resistance (TPR), blood pressure (P), right auricular pressure (RAP) and renal plasma flow (PAH) after Dibenamine in a subject with heart failure. See text for details. (Fejfar, Z. (1957). *Acta cardiol. (Brux.)*, **12**, 13.)

diminishing performance of the heart. They are a typical reaction which appears in every situation in which CO is inadequate for oxygen requirement in the tissues. This reaction becomes a chronic feature during the development of congestive failure and leads to retention of water and sodium.

A high central venous pressure and a secondary excretion of humoral substances like aldosterone complicate the response.

Werkö and co-workers (1955), in a study of systemic and renal haemodynamic changes in 146 subjects with different cardiac disorders, came to a similar conclusion. Their results suggest that "the adrenergic impulses could contribute to the diminished renal blood flow in severe heart disease before any signs of congestion are apparent". They think one of the factors causing the release of adrenergic impulses may be a decreased stroke volume.

The origin of the afferent impulses of this functional haemodynamic reflex is not known. There are, of course, several pieces of evidence on the influence of nervous impulses on diuresis. Viar and co-workers (1951) demonstrated an increase in urine flow and excretion of sodium as the result of a rising venous pressure in the head (following the compression of neck by a manometer cuff). Cort (1953), in agreement with these results, found an increased diuresis with higher elimination of sodium in subjects with the head lowered (Trendelenburg position of 15°). The changes in renal blood flow were not reported. Cathcart and Williams (1955) did not confirm this.

Gauer and co-workers (1954) described an increase in urine flow in anaesthetized dogs during the negative pressure breathing period. This was also found in healthy human subjects (Sieker, Gauer and Henry, 1952, 1954). The rise in diuresis was not accompanied by increased elimination of electrolytes (Na^+ or K^+). This water diuresis was thought to be caused by stimulation of volume or stretch receptors localized in the cardiovascular system in the thorax (left atrium or pulmonary veins). The values of renal plasma flow were not measured in these experiments. We do not know, therefore, if the changes reported were produced by a direct influence on the renal tubules without any change in renal haemodynamics.

It is also difficult to use these findings to explain the electrolyte and water imbalance in heart failure. We have produced

evidence (see above) that renal blood flow and a decreased
excretion of electrolytes occurs in left ventricular failure and
mitral stenosis without right-sided decompensation, when
there is an increased pressure in the venous side of the pulmon-
ary circulation.

On occasion, however, a sudden increase of pressure in this
part of the pulmonary circulation may be associated with a
rise of urine flow in patients with a heart disease. We have
followed haemodynamic changes in nine patients with acute
pulmonary oedema (Fejfar *et al.*, 1958a); in three of them we
also studied renal haemodynamics and the excretion of
electrolytes. At the onset of recovery from pulmonary
oedema there was a depressed renal blood flow and the renal
fraction of CO started to increase before any significant changes
in cardiac output occurred. In two of these three patients the
rise in renal blood flow was accompanied by an increased
excretion of chloride (Fig. 6). A rise of pressure in the left
auricle and pulmonary veins is typical for acute pulmonary
oedema in patients with mitral stenosis or left ventricular
failure. It is therefore possible that this elevation of pressure
could influence renal blood flow, diuresis, and the excretion
of electrolytes. The diuresis was not, however, a water
diuresis as described by Sieker, Gauer and Henry (1952,
1954).

Gömöri and co-workers (1954) studied renal circulation in
dogs with crossed circulation under hypoxaemia. They
found a decrease in renal blood flow in a dog whose head was
perfused from the other body by hypoxic (venous) blood.
Following denervation of the kidneys, this vasoconstriction
either disappeared completely or was insignificant.

Földi and co-workers (1955) found in hypoxaemic dogs a
decrease in renal blood flow, excretion of water and electro-
lytes. In healthy subjects breathing a mixture of 10 per cent
oxygen there was also a decreased renal blood flow and elimi-
nation of electrolytes. On the other hand a low renal blood
flow, glomerular filtration rate and excretion of sodium
significantly increased in patients with congestive heart
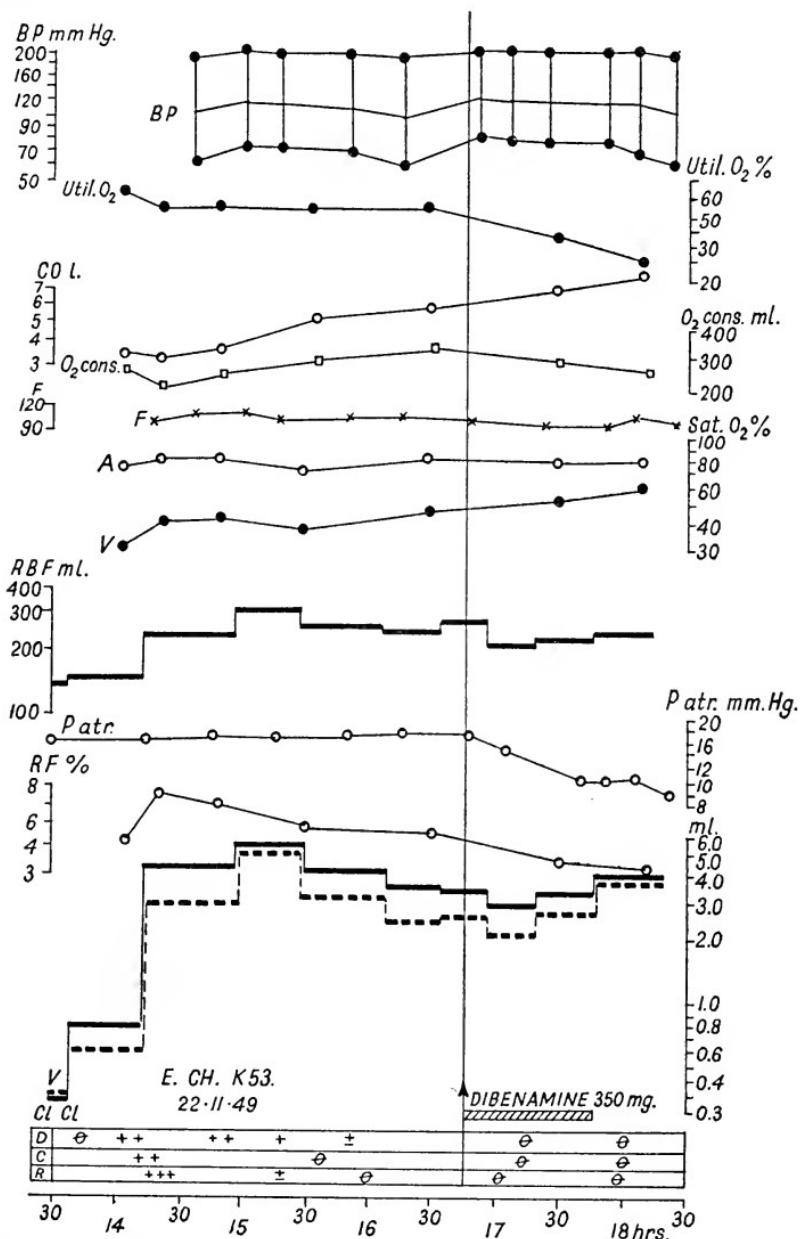
FIG. 6. Haemodynamic changes and renal excretion of chloride in a patient with acute pulmonary oedema. BP—blood pressure; F—pulse frequency; Util. O_2—oxygen utilization in tissues (in percentage of O_2 supply); O_2 cons.—oxygen consumption/min.; CO—cardiac output; Sat. O_2—arterial (A) and mixed venous (V) oxygen saturation as a percentage; RBF—renal blood flow; RF—renal fraction of cardiac output; V—urine flow in ml./min.; Cl_{Cl}—chloride clearance; P atr—right auricular pressure; D—dyspnoea; C—cough; R—râles.

failure inhaling 50 per cent oxygen plus 4 per cent carbon dioxide for 30 minutes (Földi *et al.*, 1956). According to these authors renal changes are brought about by hypoxia in the brain.

It is improbable, however, that every case of heart failure is accompanied by cerebral hypoxia. The renal changes are manifested, as shown above, in left-sided failure. The results of Scheinberg (1950) indicate a decreased blood flow through the brain in heart failure together with a rise in cerebral vascular resistance. If the cerebral supply of oxygen is really insufficient, we might expect quite the reverse: a diminution of cerebral vascular resistance and an increase in cerebral blood flow. This was actually demonstrated in man during experimental hypoxaemia by Kety and Schmidt (1948).

We are of the opinion that the heart itself may be the starting point for the haemodynamic functional changes in heart failure, and in all situations in which CO is inadequate for the requirement in tissues, i.e. where oxygen utilization in tissues increases (Fejfar, 1956, 1957, 1958). The basis for this hypothesis will be briefly summarized:

(a) Myocardial utilization of oxygen is, even with physical inactivity in healthy subjects, greater than that by the other important organs of the body. Every rise in oxygen consumption or utilization in tissues (muscular effort, anaemia, mitral stenosis, etc.) is associated with coronary vasodilation, an increase in the coronary fraction of CO, and vasoconstriction in the kidneys.

(b) We have demonstrated that during the inhalation of oxygen a normal CO in a healthy subject, or in compensated patients, either does not change or decreases, while a low cardiac output in heart failure increases (Fejfar, 1957; Fejfar *et al.*, 1958*a*).

(c) Gömöri and co-workers (1954), in experiments cited above, did not find an elevation of CO during isolated hypoxia of the brain. On the other hand, when the isolated head of a dog was perfused by arterial blood and the trunk supplied with hypoxaemic blood (the dogs inhaled a mixture with a low

concentration of oxygen), CO rose in a similar way to the rise observed in hypoxaemic hypoxia in intact animals.

(d) Harrison and co-workers (1927) concluded from their studies on experimental hypoxaemia in dogs that the oxygen tension in the myocardium is the most important factor determining the rise in CO.

A direct efferent nervous influence on the kidneys was demonstrated by Kaplan and Rapoport (1951) and Blake (1952) in dogs with unilateral renal denervation. Tubular reabsorption of sodium was less in the denervated kidney. Bykov and Alexejev-Berkmann (1930, 1931) (see Bykov, 1952) found that a conditioned "water" diuresis in dogs may be partly inhibited by denervation of the kidneys.

Renal blood flow was measured only in the experiments of Kaplan and Rapoport (1951), where the increased renal excretion of water and electrolytes after splanchnicotomy was independent of changes in renal blood flow. Our experimental results in patients with heart failure (see above) demonstrated a close relationship between changes in renal blood flow and tubular reabsorption of water and electrolytes.

A partial answer to this question can be found in the experiments of Cort and Kleinzeller (1956) on isolated kidney tissues of rabbits. Changes in transport of cations and water were studied during two hours' exposure of kidney slices to unoxygenated physiological saline at 0°, and then after 10 and 30 minutes of incubation in Krebs' phosphate saline with oxygen at 25°. One kidney was decapsulated and denervated 14 days before the actual experiment. It was shown that there was a greater influx of sodium into the denervated slices during leaching at 0°, and a slower expulsion of sodium from the denervated kidney slices during the incubation period. The changes in water content of the slices were in the same direction as the shifts of sodium. The difference between denervated and innervated kidney was, however, not marked. Potassium loss during the two-hour leaching period was greater, and its reaccumulation during subsequent incubation slower, in the denervated kidney.

In six rabbits with bilateral denervation the resting clearances of inulin and PAH were practically the same as in the rabbits without renal denervation (Brod and Sirota, 1949). Cort and Kleinzeller (1956) therefore conclude that the differences described are due to a direct nervous effect on tubular cells rather than to a change in renal blood flow.

It is difficult to compare results obtained from experiments with tissue slices or in anaesthetized animals, with results from human subjects, in which every disturbance of homeostasis is immediately compensated for in several ways. Neural and humoral regulation act simultaneously and it is practically impossible to differentiate them. It seems, nevertheless, that even in subjects with chronic heart failure, retention of electrolytes and water is the result of haemodynamic changes parallel with increased tubular reabsorption of sodium and water. These changes may be initiated by a reflex mechanism acting through adrenergic nerves. Increased secretion of aldosterone and ADH is a secondary manifestation. This secondary aldosteronism may, however, prevail in the long run, dominate the whole picture of chronic congestive failure, and close the vicious circle.

Further consequences of retention of salt and water in heart failure.

The retained sodium and water in congestive failure does not enlarge the volume of extracellular fluid only. In patients recovering from heart failure the reduction of body weight was greater than the reduction in the amount of extracellular fluid (Seymour *et al.*, 1942), chloride output (Schroeder, 1950) or sodium loss (Miller, 1950, 1951). This surplus water must come from cells. In the development of congestive failure, the water accumulates in both extracellular and intracellular compartments.

At the same time changes begin in the concentration of extracellular and intracellular electrolytes. The loss of cellular potassium in congestive failure was described in 1930 by Harrison, Pilcher and Ewing. It has been ascertained by

balance studies, and by analyses of muscle biopsies, that in addition to the cellular loss of potassium there is an increment of sodium in cells (Iseri, Boyle and Myers, 1950; Iseri *et al.*, 1952; Squires, Crosley and Elkinton, 1951a; Warner *et al.*, 1952; Cort and Matthews, 1954; see also Elkinton and Danowski, 1955; Cort and Fencl, 1957). Particularly important is the fact that potassium depletion occurs in subjects treated by repeated injections of mercurial diuretics (Squires *et al.*, 1951b; Cort and Matthews, 1954). In some of these severely ill cases hyponatraemia and hypochloraemia with an elevated concentration of bicarbonate may be observed.

Clinical diagnosis of potassium depletion in chronic congestive failure is difficult to prove. Decompensated cardiacs excrete negligible amounts of sodium and the stronger acid radicals are excreted neutralized by potassium. Therefore the typical finding of a far higher concentration of potassium than sodium in the urine in congestive failure is not alone sufficient proof of cellular loss of potassium.

Plasma levels of Na^+, K^+, and Cl^- are usually within the normal range in decompensated cardiac patients.

Table I presents the relationship between plasma levels of Na^+, K^+ and HCO_3^- and concentration of Na^+ and K^+ in muscle biopsy specimens in 13 patients with various degrees of heart failure. Concentrations of total muscle Na^+ and K^+ are expressed in m-equiv. 100 g. of fat-free dry solids (FFDS). Normal values given by Cort (1955b) are about 13 ± 2 m-equiv. of Na^+ and 45 ± 3 m-equiv. of K^+.

It will be seen that all the patients had a decreased amount of potassium in skeletal muscle. This K^+ depletion was very marked, although not all were treated with mercurial diuretics. Patient M.E. was not yet in right-sided failure. In all patients, with the exception of A.Z., the plasma concentrations of Na^+ and K^+ were within the normal range. In the majority the concentration of bicarbonate was slightly elevated. None of them showed ECG changes typical of potassium depletion.

The lowest figure of muscle potassium (11·2 m-equiv./100 g.)

Table I

RELATIONSHIP BETWEEN PLASMA LEVELS OF Na^+, K^+ AND HCO_3^-
AND CONCENTRATION OF Na^+ AND K^+ IN THE SKELETAL MUSCLE

MS—mitral stenosis; MI—mitral incompetence; Tri S—tricuspid stenosis; Tri ins.—tricuspid insufficiency; H. + I.H.D.—hypertensive and ischaemic heart disease. See details in text.

Name	Sex	Diagnosis	Age years	Degree of heart failure	Muscle		Plasma			Note
					Na^+ total	K^+ total	Na^+	K^+	HCO_3^-	
					m-equiv./ 100 g. FFDS		m-equiv./l.			
E.Č.	M	MS>MI Tri S	33	5	10·23	18·8	137	4·47	28·3	
A.S.	F	Atr. sept. def.	48	3–4	19·75	27·6	141	5·15	29·4	0 mercurial diuretic
M.B.	F	MS	38	4	13·9	28·3	150	4·54	28·0	
M.E.	F	MI>MS	37	3	15·04	28·64	137	4·5	26·4	0 mercurial diuretic
I.D.	F	MS>MI postcommis.	40	4	23·39	21·52	145	5·75	28·1	0 mercurial diuretic
M.D.	F	MI, bacterial endocarditis	35	3	12·51	29·92	143	3·9	30·2	0 mercurial diuretic
E.K.	M	MS, Tri S.	46	5	14·3	37·2	131	5·34	28·5	
P.U.	M	MS, Tri ins.	43	5	27·1	25·46	145·1	4·56	28·1	
A.Z.	M	MS, postcommis.	49	5	22·5	11·2	126·5	4·02	14·8	8th day post-operative
A.V.	F	MS	51	3	10·3	21·76	148·5	5·2	31·1	
H.Ch.	F	MS, postcommis.	37	4	19·57	33·61	143·3	4·97	28·5	
V.B.	M	H. + I.H.D.	60	4	16·91	39·88	143·5	4·98	29·4*	* not at the same time
M.V.	F	MS, postcommis.	37	3	20·78	34·06	141·5	4·44	26·8	

was found in patient A.Z., with suppuration in the thoracic wound one week after mitral commissurotomy, 24 hours before death. He was by this time in severe metabolic acidosis. The loss of about three-quarters of the muscle potassium was

probably not just a consequence of postoperative suppuration; it must already have been present before the operation.

Experiences with two other patients with mitral stenosis and congestive failure, who died within a week after operation with a picture of combined peripheral and cardiac failure, led us to the conclusion that a greater operative risk with mitral commissurotomy in patients with congestive failure (group IV in the usual classification) is associated with potassium depletion and intracellular acidosis with increased retention of sodium (Fejfar *et al.*, 1958a).

Negative nitrogen balance following surgical operations is connected with potassium depletion (Moore and Ball, 1952), and it is clear that in patients with potassium depletion in chronic congestive failure a further loss of potassium after operation brings about various complications (shock, acute heart failure, infection, slow recovery, etc.).

It follows that the laboratory diagnosis of potassium depletion in chronic congestive failure is not easy to make. A low serum concentration of Na^+, as an indirect indicator, is present only in very advanced stages. One should suspect potassium depletion if there is a decrease of serum chloride and a rise in HCO_3^- accompanying the usual urinary pattern in heart failure (negligible concentration of Na^+ and a marked excretion of K^+).

Analysis of a muscle biopsy specimen or balance studies, which, together with measurement of total exchangeable K^+, are at present the only methods for detecting early stages of a metabolic imbalance of electrolytes, are both rather complicated for practical use.

It is therefore more useful to assume potassium depletion in every patient with chronic congestive failure. The treatment of every patient should be supplemented by a diet rich in potassium. In more severe cases potassium salts are useful, being particularly important in all patients treated with mercurial diuretics. Cort (1955c) demonstrated in 12 patients with congestive failure that potassium chloride, given some days before the injection of mercury, potentiated its diuretic

effect more than ammonium chloride and simultaneously compensated the potential loss of potassium. As the loss of potassium from the cells is probably connected with a breakdown of cellular glycogen and protein, it is advantageous to add N hormones (methylandrostendiol) to the treatment.

It is not easy to correct completely a severe potassium deficiency in chronic congestive failure. Even with a high potassium intake it may be several weeks before cells become saturated (Cort and Matthews, 1954).

There remain many unanswered questions. It is customary to treat patients with congestive failure with a low sodium diet. It has been shown, however, that a low sodium diet in healthy subjects increases aldosterone excretion in the urine (Luetscher and Axelrad, 1954; Liddle, Duncan and Bartter, 1956; Wolff *et al.*, 1956*a, b*), while a diet rich in sodium has led to a decrease of aldosterone activity in the urine (Luetscher and Curtis, 1955*a, b*; Gordon, 1955; Bartter *et al.*, 1956; Garrod, Simpson and Tait, 1956).

Potassium administration also increases the excretion of aldosterone (Laragh and Stoerk, 1955; Luetscher and Curtis, 1955*a, b*; Falbriard *et al.*, 1955; Bartter *et al.*, 1956).

Laragh and Stoerk (1957) recently demonstrated that no sodium-retaining activity was found in the urinary extracts from dogs on a diet low in both sodium and potassium. When the amount of potassium was increased, hyperkalaemia developed and sodium-retaining activity appeared in the urine. Similar results were observed in one patient suffering from rheumatic heart disease with congestive failure. As long as he was kept on a diet low in sodium (about 12 m-equiv. daily) and a rather high potassium intake (140 m-equiv.), the excretion of aldosterone was high (about 300 μg./24 hr.). After the marked reduction of serum potassium to $2 \cdot 7$ m-equiv. by an injection of 2 ml. of Mercuhydrine together with a low potassium diet, the excretion of aldosterone fell to 35 μg. Restoration of a normal serum potassium level by administration of potassium was again followed by a very marked excretion of aldosterone in the urine (630 μg./24 hr.).

During the whole course, the serum sodium level did not change significantly. Laragh and Stoerk (1957) concluded from these results that the higher serum potassium level is probably a stimulus for the secretion of aldosterone.

If patients with heart failure respond to a low sodium and high potassium intake in the same way as normal subjects, our customary therapeutic procedure would assist in the creation of secondary aldosteronism.

Reduction of body water increases the excretion of aldosterone in normal subjects (Luetscher, Deming and Johnson, 1951, 1952; Beck et al., 1955; Falbriard et al., 1955; Bartter et al., 1956; Garrod, Simpson and Tait, 1956). When the volume of extracellular fluid rises, the urinary elimination of aldosterone diminishes (Beck et al., 1955; Liddle et al., 1955; Muller, Riondel and Mach, 1956).

In patients with congestive failure and other oedematous states there is on the contrary an expanded extracellular fluid volume associated with a rise in the urinary excretion of aldosterone. The explanation of this reversed reaction is at present difficult. Wolff, Koczorek and Buchborn (1957) argue that in congestive failure there must be a disturbance of, or a new regulatory mechanism for the secretion of aldosterone.

Increased elimination of aldosterone in the urine was found in the first week following surgical intervention (Llaurado, 1955; Wolff, Koczorek and Buchborn, 1957) or acute myocardial infarction without signs of congestive failure (Wolff, Koczorek and Buchborn, 1957). This may be explained by a diminution of extracellular fluid volume. But one must not neglect the fact that in all such stressful situations there is a raised adrenergic activity; and the same stimulus may perhaps also lead to an increased production of aldosterone, irrespective of the level of extracellular fluid volume, as seems to be the case in congestive failure.

Summary

Retention of salt and water in heart failure is caused by disturbed renal function. The main factors are a decreased

renal blood flow and an increased tubular reabsorption of salt and water. High venous pressure in the systemic circulation is not the primary cause of this disturbed water balance. It may, however, contribute to it.

In congestive failure there is not merely a simple retention of extracellular electrolytes and water. Serious metabolic changes may also occur. Great clinical significance should be attached to cellular potassium depletion. The laboratory diagnosis of the latter is difficult, the best method at present being chemical analysis of muscle biopsy specimens. One must consider this disturbance in every patient with heart failure, and consequently treat all such patients with sufficient potassium in the diet, or by administering potassium salts, particularly when mercurial diuretics are used.

Consideration was given to the significance of regulatory mechanisms responsible for renal dysfunction in congestive failure. The primary rôle of reflex changes was stressed and the present knowledge of the rôle of aldosterone and ADH was discussed.

Acknowledgements

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DISCUSSION

McCance: Prof. Borst, can you bring together these discoveries about nocturnal diuresis, reflex activity and aldosterone excretion?

Borst: The rôle of aldosterone should not be exaggerated. Heart failure and nocturia can be seen in patients with Addison's disease; therefore in the disturbance in water and electrolyte excretion of heart failure and of nocturia the effect of aldosterone cannot be the only factor. We believe that the evidence is in favour of the theory that salt retention in the presence of normal kidneys is always largely effected through the same pathways. The same mechanism is responsible for the retention after haemorrhage, in nephrosis, in cirrhosis and in heart failure. On the other hand we assume that salt diuresis is also always effected through the same pathway. The characteristics of this mechanism can best be studied in the excellent experimental conditions provided by patients with paroxysmal tachycardia accompanied by polyuria. The attack of tachycardia elicits the typical 'salt diuresis', though blood volume and extracellular fluid volume remain constant. The diuretic stimulus must therefore result from the change in heart action. The pulse rate acutely rises from 80 to 160 and after a certain period falls suddenly to the original rate. The consecutive portions of urine in patients who are on a standardized diet show a brisk water diuresis followed by a gradual increase in sodium output. The excretion pattern is very characteristic and is in

every respect similar to that following the rapid intravenous injection of saline. These facts point to a dependence of the sodium and water output on blood pressure or on blood flow; there is no direct relation to volume. A fact worth remarking is that the diuresis may continue several hours after the tachycardia stops. This suggests that the effect of the abnormal circulation on the renal tubules is mediated by a slowly acting mechanism, possibly a renal hormone. Experiments in animals in which the functions of the two kidneys have been compared also prove that the adrenal is not essential and that the receptor must be in the kidney. When one renal artery is gradually narrowed the sodium and water excretion of the corresponding kidney may fall sharply before a fall in PAH and creatinine clearance can be demonstrated. Probably the kidney responds even to the slightest reduction in intrarenal blood pressure by an increased tubular reabsorption of sodium chloride and water.

Fejfar: I quite agree with you in all points. It is also my personal view that this reaction might start in the heart itself. In all these types of circulatory disturbances (mitral stenosis, pericarditis, acute heart failure, hypoxaemia, anaemia), and in muscular effort, the only common factor is a very low oxygen content in the central venous blood. When we gave oxygen to patients with normal cardiac output, the cardiac output did not change. When oxygen was given to patients with a lowered cardiac output, the output increased; there is, therefore, indirect evidence that if more oxygen is given to the heart muscle in congestive failure the performance of the heart improves.

Milne: Have you any observations on a similar correlation, or the reverse, in other conditions besides congestive heart failure associated with nocturnal diuresis? In starvation and cortisone overdosage, particularly, a similar reversal of the normal diurnal rhythm may be seen.

Fejfar: No, I have no comments to make.

Milne: I would agree with all the points you make regarding the diagnosis of potassium deficiency in heart failure, but I think that most clinicians are now using a very useful clinical method of diagnosis—excess sensitivity to digitalis. Of course, as you say, it can be checked by balance or exchangeable potassium if necessary.

Fejfar: You are right about digitalis, but of course this usually occurs in advanced stages of potassium deficiency. When patients are in potassium deficiency it takes weeks and weeks to restore the balance. This is not just an academic question because we had three deaths due to these metabolic changes shortly after mitral valvulotomy. When a patient already has a negative balance with loss of potassium, the added operative trauma and hypotension will easily lead to so-called metabolic death.

Olesen: I have had the opportunity of studying the problem of the diagnosis of potassium depletion in congestive heart failure with the dilution methods used in Boston (McMurrey *et al.* (1956). *Metabolism*, **5**, 447). I would say first that the diagnosis is not very easy; in fact it is probably impossible to make it by the dilution methods alone. We found, however, that there were very marked changes in the body composition of these patients with congestive failure. There was a relative decrease in the total intracellular mass, as expressed either by total

intracellular water or total intracellular potassium. This is a change which may also be seen in severe weight loss without congestive failure, and the situation is very difficult to evaluate because patients with congestive failure will often have lost weight in the late stages. An interesting finding was that although there were almost equal degrees of congestive failure the average intracellular potassium concentration appeared normal in the males but was low in the females. We have no explanation for this finding.

The question to us, however, is whether a low average intracellular potassium concentration means a reduction in the relative amount of potassium or too much water in the cells. We cannot answer this. In tissue analysis results we are faced with the same question: when there is a low intracellular potassium concentration related to the intracellular water, is there too little potassium or too much water? The relationship of potassium to nitrogen or phosphorus does not seem to change very much. This might suggest that it is as much an increase in water as it is a decrease of potassium in the cells.

There are conflicting opinions on the balance studies. Most American studies demonstrate a positive potassium balance during recovery from congestive failure. However, most of these studies have been carried out on low sodium/high potassium intake, and the high potassium intake may explain the positive potassium balance. In a study made in Switzerland a medium-sized intake of potassium was used and no positive potassium balance during recovery from congestive failure was seen.

Milne: There seem to me to be two sides to this question of assessing the cause of secondary aldosteronism in relation to the expansion and contraction of body fluids. There is the physiological stimulus in haemorrhage, shock, etc., where, as you say, there is contraction; and there is the pathological stimulus in the nephrotic syndrome, cardiac failure, and hepatic cirrhosis, where there is expansion. All this is really tied up with the philosophy of volume receptors. It always seems to me to be impossible for the body to have a true volume receptor. The only way we know of measuring volume is to pour fluid into a graduated cylinder. I feel the only possible explanation is that the body is relating tension to volume, and that the receptors are tension receptors for either static or pulsatile tension. I think the stimulus is the same in all forms of secondary aldosteronism and that the receptors must be on the arterial side of the circulation.

Fejfar: I agree with you about volume and stretch receptors. I would like to add that if one gives sodium to patients with congestive failure, the aldosterone excretion decreases (Gordon, 1955); these people therefore react in the same way as normal persons, although their actual levels of aldosterone may be higher.

A CASE OF MAGNESIUM DEFICIENCY

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Our knowledge of the effects of magnesium deficiency in man is so meagre that we feel warranted in presenting the data from a single case and, though these data are not as complete as one would wish, we believe they are sufficient to allow useful though tentative conclusions to be drawn.

The state of magnesium deficiency in animals whether experimentally produced or occurring as a natural state has been recognized for some time (Kruse, Orent and McCollum, 1932; Greenberg and Tufts, 1938). In animals such as cows the syndrome goes under various names (Blaxter, Rook and McDonald, 1954); it can be cured by the injection of magnesium salts and prevented by using magnesite dressings on the pasture. In man there seems to be no clearly recognized picture. There have been reports of various states associated with lowered blood magnesium which have responded to magnesium sulphate injections, and it is recognized that various excitable states such as delirium tremens may be associated with a low serum magnesium and may improve with magnesium therapy (Flink *et al.*, 1954; Martin, Mehl and Wertman, 1952). A case described as tetany and associated with low blood magnesium has been reported in a child (Miller, 1944).

Such observations are not wholly satisfactory since the fraction of magnesium which exists in the plasma is so minute that it must necessarily be a very imperfect reflection of the state of magnesium in the body. The only satisfactory evidence for a magnesium deficiency is clearly some measure of the actual body store of magnesium. Fitzgerald and Fourman (1956) have shown how very difficult it is in man, owing to

the conserving action of the kidney, to deplete the body of magnesium to any serious extent by taking a diet low in magnesium. The opportunity occurred to us some four years ago of treating a patient with an ileal fistula from which extensive fluid and electrolyte losses occurred, and in whom a magnesium-deficient state ultimately appeared.

For the purposes of this paper the precise clinical details are irrelevant; it is sufficient to say that the patient was a woman aged 34, suffering from ulcerative colitis, who had had performed a proctocolectomy with ileostomy. The immediate postoperative course was satisfactory but it became necessary to refashion the ileostomy a fortnight later, and this was followed by intestinal obstruction for which a further operation was performed. An ileal fistula then developed. Such a fistula results in large fluid and electrolyte losses.

It is not of course possible in clinical practice to measure electrolyte balances on all patients postoperatively, but it is clearly necessary to have sufficient knowledge of their losses in order to replace them effectively. The routine ward procedure, which was followed in this case, is as follows:

A fluid balance chart is kept on which the amounts of all fluids given orally and by intravenous infusion are noted, as well as all losses whether urinary, faecal, by aspiration or by any other route. In patients such as this woman, where the intake of food is important, the food taken is recorded on a slip of paper, so that the dietitian may make some estimate of caloric or protein intake. From the fluid balance chart, with, if necessary, the estimation of electrolytes in any aspirated fluid, the necessary amounts of fluid, water, sodium, chloride, and potassium, are prescribed for the next 12 or 24 hours. Serum electrolyte concentrations are measured, daily if necessary, as in this case.

This procedure was carried out with this patient so that she was kept in water, sodium, potassium, and chloride balance. The CO_2 combining power remained within normal limits. There was no rise in her blood urea and judging by the urinary specific gravity reached the kidneys functioned well. Calcium

gluconate was given intravenously but in insufficient amounts, and in retrospect it is clear that she was in negative calcium balance. No thought was given at this time to the possibility or the significance of any magnesium loss.

In such an ill patient adequate nutrition and the replacement of protein is very difficult to achieve and her oral food intake was augmented by intravenous feeding. The fluids
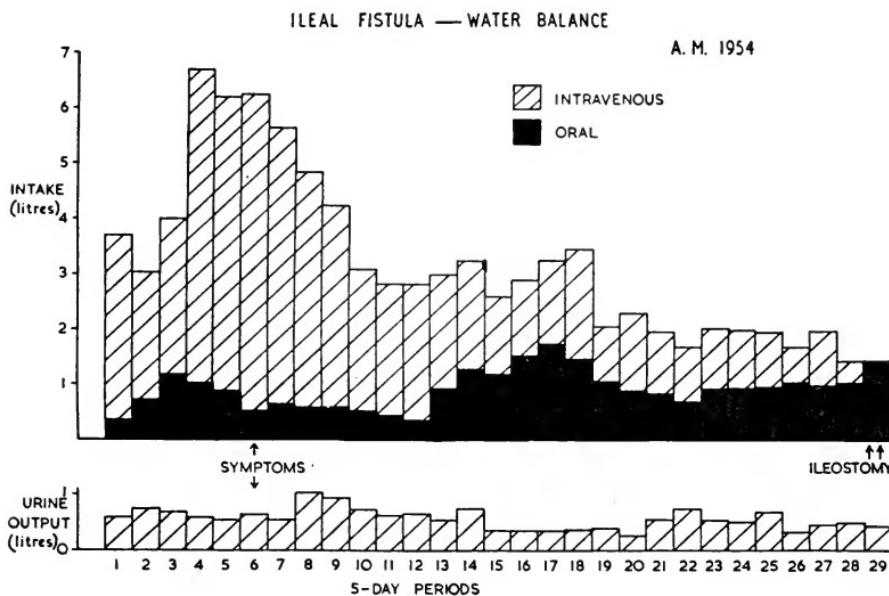
FIG. 1. Chart showing fluid intake and urinary output over a five-month period, with the appearance of symptoms one month after the onset.

given were glucose solutions, sodium lactate, and alcohol, while a casein hydrolysate supplied nitrogen. Loss of blood was replaced by blood transfusions. Despite all these measures she undoubtedly lost weight.

Fig. 1 shows the extent of the fluid replacement necessary over nearly five months, plotted in five-day periods, and it will be seen that the losses were very great. At their maximum, calculation shows that the fistula losses were of the order of five litres a day. Since the patient at this time weighed less

than 35 kg. she was losing the equivalent of about 15 per cent of her body weight daily through the fistula.

The patient during this time was, of course, extremely ill with consistently rapid pulse and occasional fever. Towards the end of a month, however, an entirely new symptomatology appeared. It was noticed that the patient became excitable, apprehensive, and required doses of sedatives some three or four times what would ordinarily be adequate. It was indeed difficult to procure sleep. This excitable mental state was an entirely new clinical picture to us and we finally wondered whether it might not be due to magnesium deficiency. Signs of tetany, in the sense of peripheral neuromuscular irritability, were lacking. An electrocardiogram was within normal limits. Her serum calcium was 8·1 mg. per cent.

Arrangements were therefore made for serum magnesium estimations and magnesium sulphate was given intravenously. In 24–48 hours the state of the patient altered very considerably, the excitement disappeared and the ordinary doses of sedative were able to induce sleep. Magnesium therapy was therefore continued to repair the deficit, and balance studies were started and continued for some three weeks. All magnesium therapy was given intravenously and the magnesium ingested orally was not increased. This is important in the light of subsequent calculations.

Table I shows how the deficit prior to the institution of therapy was calculated. It should be made clear that the loss of fluid by fistula could not be measured directly, since a complete collection was quite impossible. It was calculated as follows:—

Fistula fluid loss = (Oral + Intravenous) Intake +
    Metabolic water − (Urinary output + Extrarenal loss).

Calculated in this way the total volume of fistula loss over the period was 109·4 litres. The magnesium content of the fistula fluid before therapy was started was never measured. We have therefore made the assumption that intravenous

magnesium therapy does not alter the output of faecal magnesium (McCance and Widdowson, 1939) and that this is also true of the magnesium content of ileal fluid.   If this assumption is true, then we can calculate the magnesium content before therapy by measuring it in the fistulous fluid after therapy had started.   On 18 days a sample of ileal fluid was measured and the mean magnesium concentration was

Table I

MAGNESIUM DEFICIENCY—A.M.

18 April–19 May, 1954.

Volume of fistula loss = (Oral + Intravenous) Intake + Metabolic water
— (Urinary output + Extrarenal loss)
= 109·4 l.

Magnesium loss

Fistula = 109·4 × 4·1 = 447 m-equiv.
Urinary =   19·4 × ? 1 =   19 m-equiv.

Total = 466 m-equiv.

Magnesium intake

Oral = 105 m-equiv.
Intravenous =   15 m-equiv.

Total = 120 m-equiv.
Balance = −346 m-equiv.

Body weight 17.4.54   = 34 kg.
less fat 7% = 31·6 kg.
Body Mg at onset = 31·6 × ·45 = 14·2 g. = 1180 m-equiv.
Deficit = 29 %

4·1 m-equiv./l.   The total loss of magnesium through the fistula can now be calculated and is 447 m-equiv.

The urinary loss of magnesium cannot be measured in this way since the infusion of magnesium salts has been reported to increase the amount put out by the kidney (McCance and Widdowson, 1939) and this was certainly true in this patient. Since the kidney was functioning well as judged by its concentrating power, the urinary concentration in the period before symptoms occurred probably never rose above 1 m-equiv./l. This gives a total urinary loss of 19 m-equiv.

The food intake of the patient over this period was small and at times negligible. The magnesium content of the food taken has been calculated from food tables and amounts to 105 m-equiv. She had no drugs containing magnesium and no toothpaste was used. Of the intravenous fluids given none appeared to contain magnesium. The makers (Bengers) kindly sent us an analysis of the casein hydrolysate (Casydrol) given which contained only negligible amounts of magnesium. The only magnesium given intravenously was that given in whole blood. The total negative balance over this period therefore amounted to some 346 m-equiv.

The weight of the patient at the beginning of the period was 34 kg. and, if we assume that the body at this stage contained 7 per cent fat, the total magnesium content of the body according to the data of Widdowson, McCance and Spray (1951) was $14 \cdot 2$ g. or 1,180 m-equiv. The patient therefore over this period lost something like 25–30 per cent of her total body magnesium. This calculation makes the assumption that she was normal at the onset, but it is quite possible that she was already depleted since she had had an ileostomy for a month with an episode of intestinal obstruction needing suction and fluid replacement.

The balance studies which followed the institution of therapy are shown in Fig. 2. The magnesium content of a sample of the fistulous fluid and of the urine was estimated daily and the output of magnesium calculated as described. The serum magnesium was estimated every few days.

The results show that with the therapy, the patient passed into positive balance over this period and that in all she retained some 279 m-equiv. of magnesium before the observations were discontinued. The results are in general accord with the previous conclusions.

The serum magnesium showed a low figure at the time of symptoms and rose with therapy but the estimations are perhaps chiefly of value in emphasizing how little use can be made of them as an index of magnesium deficit in the body.
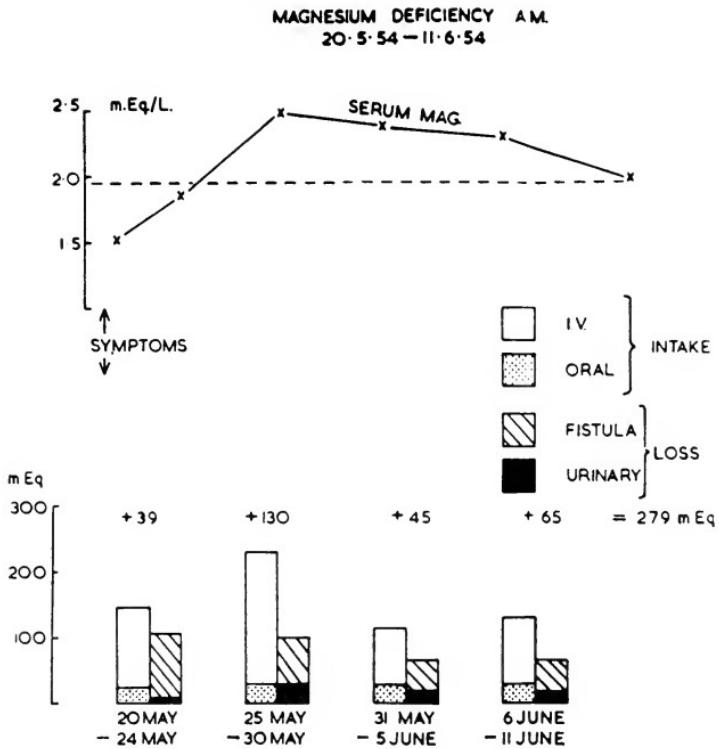
FIG. 2. Chart showing the effect of magnesium therapy in producing a positive magnesium balance, and its effect on the serum magnesium.

Discussion

When first seen the symptomatology of the patient in this state was extremely puzzling. The clinical picture was quite unusual and something we had not encountered before. The patient was apprehensive, "on edge", and proved extremely difficult to sedate. She was very ill at the time and there may well have been earlier manifestations which passed unnoticed. The animal behaviour as described by Greenberg and Tufts (1938) in rats, and in particular the apprehensive state described in induced magnesium deficiency in calves by Blaxter, Rook and MacDonald (1954), strongly recall the clinical picture we saw. Magnesium deficiency in man may ultimately proceed to a condition of tetany and even convulsions as it

does in animals, but the state we observed bore no resemblance to low calcium tetany as seen clinically.

The other point worth discussing is the level of depletion at which these symptoms appeared. It seems likely from this one case, and we have failed to find a comparable example in the literature, that symptoms of what might be called moderate severity appeared when something like 25–30 per cent depletion of the total body magnesium had occurred. If we may adduce evidence from animal experimental work, Blaxter, Rook and MacDonald (1954) calculated that in calves on magnesium-deficient diets symptoms appeared when a deficit of about 25–30 per cent magnesium had occurred, while at death it was estimated that 35 per cent of the magnesium in the body was lacking. If this general conclusion is true, it follows that the small deficits of 50–100 m-equiv., which have been described by various authors (Nabarro, Spencer and Stowers, 1952), are unlikely to produce clinical manifestations and in themselves hardly call for treatment. In man, the conditions necessary to produce magnesium depletion sufficiently severe to result in a recognizable clinical state are unusual and can hardly be expected to occur with any frequency.

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DISCUSSION

Fourman: When Dr. Fitzgerald and I started to produce an experimental depletion of magnesium we had in mind to do what I had done with potassium (1956. *Clin. Sci.*, **15**, 635). But we got nowhere near a significant depletion: only some 70 m-equiv. of magnesium were lost from the body in the course of a month's efforts. Afterwards we realized that this was partly because the urinary and faecal losses became very small when the intake was low.

Duckworth, Godden and Warnock (1940. *Biochem. J.*, **34**, 87) found that the magnesium of bone makes up one-half of the body magnesium. This forms a mobilizable store, which is probably why it is so difficult to produce symptoms of a deficiency of magnesium (Blaxter, K. L., Rook, J.A.F., and McDonald, A.M. (1954). *J. comp. Path.*, **64**, 157). A depletion of magnesium seems to bear little relation to what is called a clinical magnesium deficiency by some workers, who have attributed the condition of tremors in patients with alcoholism to a low serum magnesium (Flink *et al.* (1957). *Ann. intern. Med.*, **47**, 956). The plasma magnesium must depend on more than the stores of magnesium in the body.

Dr. Card, what were the urinary losses of magnesium when you gave the intravenous injections of magnesium? In our experiments, even with the small deficits we had, we found that the urinary losses after injection were less than when the subjects had no deficit.

Card: I have not got the figures for the amount of magnesium in the urine in the early days of treatment. When you give intravenous magnesium some does come through the urine, but these amounts were variable (McCance and Widdowson, 1939). The lowest magnesium we have ever got, without magnesium therapy, was down to 1 m-equiv./l., and we have taken that as the concentration of the urine prior to magnesium therapy. Even that may be too high when a patient is in a deficient state.

Fourman: It would be very convincing if the injection of magnesium produced little rise in the urinary magnesium, while in normal people it is known to produce a large and prompt rise in the urinary excretion of magnesium.

Davson: McCance established that the concentration of magnesium in the cerebrospinal fluid was considerably higher than that in the blood plasma. It may be that it is necessary to have a high concentration surrounding the nerve cells to maintain a low level of excitability, in much the same way as there is a low concentration of potassium which also decreases with excitability.

Card: In the experiments where the calves ultimately died, with a big deficit, the tissue magnesium was normal. The whole deficiency appears to occur in the bones, and I think that, as Dr. Fourman suggested, there must be states in which the magnesium is not available. There is one example of magnesium tetany in the literature which is obviously not a case of deficiency, in a child with osteochondritis; so there may be bone diseases in which this interchange is impossible, and acute states in which magnesium deficiency can occur, entirely different from this chronic deficiency loss. Greenberg and Tufts (1938) went to a good deal

of trouble to find out which part of the brain was particularly affected; they thought it was the mid-brain, and pointed out various differences from low-calcium tetanus.

Black: Was there any tremor in your patient, and what was the state of the reflexes?

Card: There was no obvious tremor, but of course she was extremely ill. She had a very rapid pulse, up to 160, which may have been partly due to magnesium deficiency as the animals showed that too. The deep reflexes were probably gone, but they might have gone in any case.

McCance: What do you mean by 'gone in any case', when the magnesium deficiency was raising the excitability?

Card: I simply mean that in a patient in this extremely wasted state, with very little muscle tissue remaining, we may not be able to elicit reflexes, quite apart from any electrolyte disturbance. We did an ECG and it was normal.

Hingerty: Were there any noticeable symptoms of muscular dysfunction when the plasma magnesium was above normal, Dr. Card? In animal experiments we tried to reproduce some of the symptoms of adrenal insufficiency by raising the plasma magnesium by injecting magnesium sulphate. When we got the plasma magnesium and muscle magnesium up to the level seen in adrenal insufficiency, we got very similar disturbances in the levels of the hexose esters, phosphocreatine and adenosine triphosphate (Hingerty, D. J. (1957). *Biochem. J.*, **66**, 429).

Card: Again, she was extremely ill, and I would say there was nothing detectable. Only gross changes in the clinical state would have been noticed. I would repeat, the clinical condition itself was most striking.

CONCLUDING REMARKS

Adolph: It is easier, I find, to mention some of the things we have omitted in this colloquium than to dwell on some of the things that we have gone into. We are all concerned with studies of regulation, some of us as observers of normal individuals and some of us by trying to cut in on the mediators by administering hormones. Perhaps the most important element in metabolic events, particularly in respect to water and electrolytes, may be the detection by the body and the cells themselves of departures from the normal. In other words, we must recognize that for each one of the constituents which we have been talking about as having a constancy, there is some sort of a detection machine. The fact that there are so many machines all in one small body or cell is something to bear in mind. Since regulation involves intrinsic detections both for the body as a whole and for each constituent compartment, how is it that we had nothing to say about the cell's own assessment of its state? I suppose it is entirely because nobody so far has found a method of cutting in on messages which are being transmitted from the surface of a cell to the interior of a cell, or the kinds of excitation which occur to produce the response within a cell. If we could find out whether these detectors and transmitters, if there be such, differ at differing ages, then we would have a more intimate picture of physiological changes with age. So far we have mainly had to content ourselves with seeing whether we could show some morphological or biochemical change with age. As I see it we have not yet got down to what a physiologist could be really proud of in the measurement of age changes. In my estimation we do not need to wait until we know what the nature of these detectors and transmitters may be before we can tackle these problems of assessment of the state of the responding system. We can study many a responding system without having any knowledge of the kinds of gadgets which are in it. Our ignorance of cell excitations is well founded, I suppose, and yet it is disappointing. I hope the future physiology of cells will develop a knowledge of these detectors, and of the way they change with age.

Next I want to try and needle you into thinking of age changes not as changes of immaturity and senescence but as states in the organism which are perhaps optimal for each of the age groups. A man of 80 years of age need not necessarily be considered inadequate in any particular respect. If he has not got as high a clearance at 80 as he had at 30, can that mean that he has no use for it? This point of view may lead to a slightly different kind of evaluation of what we

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find, and certainly to a revision of the kind of language in which we express our results. I think that if we adopt a more descriptive terminology, and do not imply that one type of organism is inferior to another, the physiologist, at least, can feel a little satisfaction.

My third point is that we have not done much in this conference with the description of the intake side of metabolism; we have talked about water and electrolytes almost entirely from the point of view of output. I realize that we all think that we know a little more about output than we do about intake, but perhaps we should have made up our minds before we began the meeting that we knew enough about outputs to feel semi-comfortable and that we knew sufficiently little about intakes to feel distinctly uncomfortable, so we might plan to see what we can find out about them. Lots of people think that a regulation consists in an organism taking in everything in sight and then getting rid of what is excessive. In my experience this is a distinct misconception because where intakes have been studied, we find that they are at least as accurately regulated and controlled as outputs. If you give an animal a water deficit of 5 per cent of the body weight and see how much water it takes in the first half-hour of recovery from that deficit, you will find that its accuracy of intake is equal to its accuracy of output when it has an excess of water from the body of 5 per cent. This accuracy, then, is of a kind that must be assessed when we talk about intakes. The intakes are, so far as we know, specific in a number of instances. We have not been able to recognize specific ways in which the organism responds to each of its deficiencies, but we know that there are specific recognitions for sodium, and there may be more specific recognitions for some of the other components. If we can see how the organism relates its intake to its deficits, and how specific those relations are, we shall have made the sort of quantitative progress that we have already been able to recognize with respect to excretion.

Davson: Prof. Adolph has spoken as a physiologist, and there is very little left for me to do, except to re-emphasize what he has said. The organism is most dependent upon the reactions of certain critical cells which respond to minute changes in their environment, such as changes in magnesium concentration. It seems quite miraculous that the cell could respond in these circumstances; we know that it can respond to a large jump in its external potassium, and we think we know the theory of that, but we are usually concerned with barely measurable changes in the cell's environment. Consider, say, the olfactory organ. There you have a concentration of gas which is quite undetectable by any chemical means and yet one can detect the presence of this gas; that means that your cell is responding to some infinitely small change in its environment and, as Prof. Adolph has

emphasized, that is the way in which we regulate both output and input.

The Chairman created a precedent by quoting from a minor poet last night, and I would like to quote from a major poet. Shakespeare was, I think, a very good physiologist, and he described age by saying "when age hath drunk his blood and filled his brow with lines and wrinkles". Now those are two aspects that we have ignored. We have been told about the extracellular volume but not whether the blood volume has changed in age; the wrinkles of the brow I think must be determined partly by extracellular water, and also by the state of the collagen under the skin.

Swyer: As one of those who have something to do with hormones I have been struck by one or two points more forcibly than by others in this conference. When hormones are considered in relation to electrolyte metabolism in ageing and with regard to sexual differences it seems to me that we have two sets of data, both incomplete. One of them relates to changes in hormone production with age and sex, and the other to changes in water and electrolyte metabolism with age and sex. For example, we have the data on body compartments that Dr. Olesen gave us, which were very interesting indeed, and I wish I had known more about that side of the problem before I set about my own task. We have, too, the experimental evidence on the development of hormonal responses with age and sex, and on this point I feel there is something very fascinating which was touched upon in the discussion but not sufficiently elaborated. I feel that we need to determine more precisely the exact effect of sex, whether it is indeed hormonal or genetic. I would like to suggest to Dr. Desaulles that an interesting extension of his experiments might be to carry them out on rats which had been castrated *in utero* by the technique of Jost, and subsequently had their sex determined by the cytological techniques which are now so readily available.

Another point which I thought was brought out very well by Dr. Fourman was this question of the differential action of cortisol and aldosterone, the one liberating potassium in the cells as a result of protein catabolism, and the other altering the renal exchange of sodium and potassium. The importance of taking this into account in attempting to use urinary Na/K ratios as a measure of these salt-retaining hormones was emphasized.

I feel I should say a little about some of the things which were not quite left out but almost so: calcium seems to have come in for remarkably little attention during this colloquium, and I think the only mention of the parathyroid glands was made by Dr. Kennedy this morning. It is true that the parathyroids have no effect on water metabolism except in highly abnormal states, but like some

other hormones which receive little attention I think their hormone deserves more thought than we have given it. Among these other hormones I would like to mention perhaps the thyroid. In myxoedema there is a profound alteration in water metabolism, and that might have exercised our thoughts too. Growth hormone is another one which may be very important in the development of some of the responses which vary with age, particularly in the younger organism.

Finally, the data which Dr. Shock described to us and on which Dr. Kennedy's experiments also have a bearing, raise the question, not completely solved, of whether the variations in renal function which occur in senescence are entirely due to the age changes in the kidneys themselves, or whether they might also be partly influenced by the changes in hormone levels at that age. I have in mind particularly the altered relationship between the adrenal anabolic and catabolic steroids, which apparently moves in favour of the latter.

CHAIRMAN'S CLOSING REMARKS

McCance: On the opening day of this meeting Prof. Adolph discussed the capacity of the infant kidney to maintain the composition and volume of the extracellular fluids, and he gave us a picture of its responses to water, salt, and various other kinds of loading as it developed. He was really discussing the ability of an "end organ" to maintain the composition of the body. He said nothing about the fact that the composition of the infant's body differed from that of adults. We heard nothing about why such differences existed and how they were maintained, yet they are the very essence of electrolyte metabolism at that age. But the next day differences in the composition of the body were considered when Dr. Olesen told us that the extracellular fluids are comparatively very much larger at the time of birth, and at the age of which Prof. Adolph was speaking, than they are in the adult. Prof. Heller then brought up the question of whether this large volume of extracellular fluid in the infant was of any value or had any function. Nobody took up this challenge or discussed how the volume was normally maintained.

Prof. Kerpel-Fronius's paper, which was read by Dr. Young, introduced some rather novel ideas which were discussed to some extent but we missed the originator of them, and I would prefer to leave you to make your own interpretation of them. However, I was interested in the point he made that the infant's water reserves and fluid volumes were *small* relative to its normal requirements even for the circulation and metabolic rate, quite apart from losses through the skin. Dr. Davson brought the matter to a head, I felt, in insisting that size must be clearly separated from immaturity in their effects on somatic function.

Dr. Shock showed that in advanced old age, even apart from disease, the end organ begins to respond in the same kind of way that it does in very early life. In both cases the end organ seems quite capable of doing the work which nature intended it to do in a healthy person of that age, but when one subjects it to the stresses which it is capable of correcting in the young adult, one can pick out signs of weakness. He did not discuss the composition and volume of the body fluids in old people. Are there any steady states, normal or abnormal, due to senility, either in the cell or in the body as a whole? Something like this may be the basis of senility. The inability of senile kidneys to maintain internal acid-base control as perfectly as those of young adults was an interesting point to me.

Dr. Fourman gave us a good account of an abnormal steady state in the body, maintained and religiously guarded by the end organ and the sensitive organs, but we did not have time to discuss the effect of this on the function of the body as a whole, or how the abnormality had been created.

Dr. Davson gave a clear exposition about the way in which the cells maintain their electrolyte metabolism and their internal structure. In other words he discussed the cellular steady state as distinct from bodily steady states. He pointed out, which is very important of course, that the cellular steady state is maintained by the metabolism of the cell itself.

Dr. Křeček, Dr. Desaulles and Dr. Swyer put my fears to rest about the hormone balance of the colloquium. They demonstrated both well-known and hitherto unknown ways in which the hormones can be shown to affect the end organ, and something about how this effect varies with age and with sex.

Dr. Thaysen gave what was to me a most interesting paper about the way in which various glands elaborate and deliver their secretions and particularly the electrolytes in them, and the way in which their mode of action can be interpreted in the light of their final product. The glands as a group are certainly worth further study for no two seem to do the same thing. If we could only isolate them and compare their metabolism with their secretions in relation to the level of sodium, potassium, oxygen, etc., in the serum and blood, how interesting it would be!

Dr. Karvonen's paper about the genetic control of electrolyte metabolism in the erythrocytes was the only major contribution on this general subject, but of course there are plenty of ways in which we know that genetics and inheritance can affect electrolyte metabolism. There are abnormal steady states in the body well known to be under genetic control, such as the "hyperelectrolytaemia" of infants. We have recently had male infants (brothers) under observation, in whom there has been a breakdown in acid-base control and an abnormal steady state in the body fluids, due among other things to a failure of the kidney to make and excrete ammonia. Genetic aspects of electrolyte metabolism are going to become more important as time goes on, and indeed a discussion of the hereditary transmission of abnormal steady states and electrolyte metabolism would be a very interesting one.

Prof. Wallace discussed the ability of the organism to maintain its normal cellular steady states under various nutritional conditions. He came to the conclusion that wide variations in specific intakes did not affect the composition of the cells but they may apparently greatly affect the amount of calcium and phosphorus in the bone.

Dr. Talbot gave us a practical paper on the tolerance of the body, particularly the developing body, to stresses caused by the administration of too large and too small amounts of the electrolytes normally present in the body. In dealing with the responses of the body as a whole rather than with the end organ responsible for the restoration of the steady state he was showing us the results of tests which had been discussed before in relation to the kidney.

Dr. Kennedy summarized and synthesized the information about the effect of over-nutrition, age, and so on, on the kidney, and the points have been thoroughly discussed. Dr. Black gave us a good illustration of the way in which the end organ, again, can break down and thus allow an abnormal steady state to develop, but why and how it breaks down he did not decide.

Dr. Fejfar gave us a glimpse of some of the interesting work going on in the Institute for Cardiovascular Research in Prague. His subject was congestive heart failure, and he discussed the renal and extrarenal reasons for the retention of water and salt. This consideration of the production of an abnormal steady state and the potassium deficiencies which might follow from it gave rise to a discussion which will be fresh in your minds.

Dr. Card kept the subject of his paper secret till the last moment, but in the end he had to come out with it. He gave us a fascinating description of a patient with severe magnesium deficiency, which as far as I know has never been described before. The results of his metabolic studies made us realize how difficult it would be to reproduce the state of this patient experimentally, and we certainly know more about the functions of magnesium than we did when I made my opening remarks.

We could have had more about the body as a whole. We have not heard as much as I should have liked about what maintains the electrolyte make-up of the body. Why is it different at birth, maturity and in old age? What maintains these steady states, which together make up the composition of the body? What causes departures from them, and how are the abnormal ones maintained?

One could go on asking questions for ever. Let us be satisfied; we have had a good colloquium. Thank you all for coming to it, and let us all thank the Ciba Foundation for entertaining us so hospitably.

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