EFFECTS OF DRUGS ON SODIUM METABOLISM

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In this article an attempt is made to give an up to date account of the methods available to clinical investigators and clinical pharmacologists for the study of sodium metabolism. This is a difficult task due partly to the intrinsic difficulty of the subject itself and partly to the complexity of the methods involved.

The varied ways in which drugs can influence sodium metabolism are briefly outlined and particular reference is made to the prostaglandins. Drug effects are manifest chiefly as sodium retention or depletion.

Whole body sodium can be estimated by isotope dilution (*total exchangeable sodium*) or by neutron activation analysis. These methods are reliable but not suited to repetition whilst following a drug response. Sodium balance studies demonstrate the net results of drug effects but are restricted by the limited availability of metabolic beds and the time required to attain a steady baseline.

The effectiveness of homeostatic mechanisms makes plasma sodium concentration an insensitive index of drug action. There are similar problems with urinary sodium excretion, particularly if the base line is inadequate or the period of collection too long.

The analysis of tissue biopsy material or different blood cell types give interesting information about drug effects. There are however difficulties in correcting for extracellular fluid volume and in extrapolating to other tissues.

Drug induced changes

A wide variety of drugs influence sodium metabolism. Retention or depletion can result.

Sodium retention

The urinary output of sodium ion in health approximates to the dietary intake since extra renal losses are small. Positive sodium balance is therefore usually the result of reduced urinary excretion. This in turn can result from reduced glomerular filtration of sodium or increased tubular reabsorption.

The methods by which drug effects on glomerular filtration rate (GFR) may be measured have been reviewed earlier (Kampmann & Hansen, 1981). Adrenergic neurone blocking drugs, anti-inflammatory agents and phenothiazines produce sodium

retention partly by reducing the filtered load. The tubular reabsorption of sodium ion on the other hand is stimulated by a variety of steroid hormones notably aldosterone.

Sodium depletion

The most dramatic modes of increased sodium loss (vomiting, diarrhoea, sweating in the tropics) are outside the scope of this series. Urinary loss is however increased by many drugs. Part of the natriuretic effects of dopamine, prostaglandins and the xanthines are attributable to increased filtered load of sodium. Osmotic diuretics impair reabsorption as a secondary consequence of the retention of water in the tubular lumen. The benzothiadiazines and the loop diuretics reduce body sodium by reversibly inhibiting tubular reabsorption (Roberts & Daneshmend, 1981). Chronic renal damage secondary to pyelonephritis or to nephrotoxic drugs (Prescott, 1982) can result in permanent loss of the ability to conserve sodium.

The involvement of the prostaglandins in electrolyte handling by the kidney has become more widely appreciated as a consequence of studies on Bartter's syndrome. This rare disorder is associated with increased urinary excretion of prostaglandins and hyperplasia of the juxtaglomerular apparatus. The renin/angiotensin system is activated resulting in hyperaldosteronism, potassium wasting and hypokalaemia. Hypertension is not however a feature. Inhibitors of prostaglandin synthetase, for example indomethacin, produce a fall in urinary prostaglandin excretion and in plasma renin and aldosterone. There is a sustained rise in serum potassium concentration (Verberckmoes *et al.*, 1976; Fichman *et al.*, 1976; Norby *et al.*, 1976; Littlewood, Lee & Meadow, 1978). The determination of urine prostaglandin output may become of more general importance to the clinical pharmacologist as there is already evidence that the angiotension converting enzyme inhibitor captopril may exert part of its effect by increasing intrarenal production of prostaglandins (Swartz *et al.*, 1980).

Measurement of the direction and extent of drug induced change in sodium metabolism is readily confounded by variation in dietary intake. Only the measurement of whole body sodium can avoid this complication.

Whole body sodium

Measurement of the mass of sodium ion in the body can be achieved by isotope dilution or by neutron activation analysis.

Isotope dilution

In the 1940s several groups began to use isotope dilution methods to measure body water and solids (Kaltreider *et al.*, 1941; Moore, 1946; Forbes & Perley, 1951; Forbes & Lewis, 1956). The methods are based upon one fundamental principle: the extent to which a tracer substance is diluted in a solvent constitutes a measure of the volume of that solvent. This relationship is expressed by the equation

$$V_2 = \frac{C_1 V_1}{C_2} \quad \dots (1)$$

where C_1 represents the concentration of the tracer isotope before dilution, V_1 the volume occupied by the isotope before dilution, C_2 the concentration after dilution and V_2 the volume after dilution. Equation (1) presupposes that sufficient time has elapsed for equilibration. In the body the duration of the pre-equilibration phase is determined by the circulation time, membrane permeability, tissue mass, body temperature and other variables. In the equilibration phase, isotope concentration C_2 is dependent on the volume (or mass) in which the dilution has taken place. During the post-equilibration phase C_2 is determined also by metabolic turnover or exchange with the environment. The body is not analogous to a beaker or reservoir. At all times there is a dynamic equilibrium between liquid and solid phases. Moreover it is not homogeneous but effectively divided into multiple compartments the volume of which may be altered by homeostatic change or a disease process. All measurements by isotope dilution assume that the subject is in a steady state. If this is not so then the measurements are invalid.

The tracer isotope administered should fulfill definite criteria; it should be non toxic and neither metabolised nor utilised in biosynthesis; it should be accessible and easily measurable in samples of blood or urine. Unfortunately no tracer isotope fulfills all these criteria completely. Nevertheless the similarity of estimates obtained by the use of different tracer substances and comparison with direct tissue sampling methods using desiccation or ashing techniques, suggests that the dilutional measurements are valid.

When estimating whole body sodium ion the phenomenon of atomic exchange (or interchange) has to be taken into account. Radioactive sodium for example is diluted with its naturally occurring isotope

within the body. The dilution equation therefore gives rise to a *mass* and not a volume (see equation 3 below).

Some body constituents readily and rapidly exchange with the administered isotope. Others are incorporated into less readily accessible tissues such as brain, muscle, tendon, bone and even the red blood cell. Such tissues exchange at slow and variable rates with the injected isotope. It is therefore not possible to obtain a true equilibrium value unless the procedure is carried on for an infinite time. An arbitrary time t must be selected in practice and this will therefore give a lower mass than the true mass. This mass at time t is called the *total exchangeable mass*.

When sodium or potassium is used, the measurement of the exchangeable mass also depends upon the concept of specific activity, which is the radioactivity per unit weight of stable or naturally occurring isotope (microcuries per millimole).

$$\text{Specific activity} = \frac{\text{radioactive isotope concentration}}{\text{stable isotope concentration}} \quad \dots (2)$$

Following the injection of a dose of radioactive sodium there is first a rapid disappearance of the tracer from the blood, followed by a slower disappearance phase of three to four hours (Figure 1). The later dilution represents penetration of the isotope into the mass of body sodium principally in bone but even at 18–24 h bone and brain are not fully equilibrated. As a result total exchangeable sodium Na_e represents only about 70% of total body sodium.

In the usual method 1–3 μCi per kg body weight of sterile ^{24}Na (half-life 15.0 h) or 10 μCi ^{22}Na (half-life 2.6 years) is administered intravenously as sodium chloride. The isotope can be used orally but this tends to be less satisfactory as there is a further delay in the time to equilibrium.

The stages in the procedure are then as follows:

1. Urine is collected for 24 h following the injection and an 'equilibration' blood sample is taken at the end of this period.
2. A standard solution of the sodium radioisotope is prepared and this together with serum from the equilibration blood sample and an aliquot of the 24 h urine collection are counted in a liquid scintillation counter. The stable serum sodium concentration ($^{23}\text{Na}_s$) is determined by flame photometry.
3. Total exchangeable sodium is then calculated using the equation

$$\text{Na}_e = \frac{^{24}\text{Na}_i - ^{24}\text{Na}_o}{^{24}\text{Na}_s / ^{23}\text{Na}_s} \quad \dots (3)$$

where Na_e represents total exchangeable sodium in millimoles, $^{24}\text{Na}_i$ the dose of radioisotope (μCi) injected and $^{24}\text{Na}_o$ the amount excreted in the

Figure 1 The serum concentration/time decay curve of radioactive sodium following intravenous injection of 150 $\mu\text{Ci } ^{24}\text{Na}$ at $\uparrow$. The specific activity decays rapidly at first and slowly later. Total exchangeable sodium is calculated by dividing the residual dose (equation 3) by the specific activity at 24 h.

urine from 0–24 h; the denominator represents the specific activity (equation 2) of the serum sodium at 24 h.

Exchangeable sodium may also be calculated using the specific activity of a single urine sample taken at or around the 24 h point.

In contrast to total body water, Na_e varies little with age or sex. Patients with oedema have increases ranging from 20 to 100%. In acute disease processes 20% of Na_e may be lost and in Addison's disease as much as 50%. It should be noted that whereas ^{24}Na is suitable for repeated determinations for example before and after diuretic, ^{22}Na is not acceptable for this use in humans as a consequence of possible toxicity associated with its long half-life.

Neutron activation analysis

As can be seen from the above description, methods for total exchangeable sodium are complex and time consuming. They also can involve the exposure of the human subject to appreciable irradiation. Techniques have therefore been developed which avoid the use of injected radioisotopes. A most valuable technique is that which exposes the whole body to neutrons from a neutron generator or cyclotron. The induced body radioactivity is then measured by counting the subject in a whole body counter. This technique was originally developed for calcium but more recently has been applied to many elements including sodium (Chamberlain *et al.*, 1968; Cohn *et*

al., 1976). It has the advantages of being non-invasive, can be repeated easily after drug administration and a minimal radiation dose is sustained by the body (0.28 rem). Elaborate and expensive facilities are required but many teaching centres now possess the capability for this valuable procedure.

When facilities are available for the continuous monitoring of daily intake and excretion it is possible to measure drug responses without determination of body sodium.

Balance studies

One of the most useful techniques for the investigation of the effect of therapeutic agents on sodium handling is the use of metabolic balance studies. Initially pioneered for calcium by Albright, Drake & Sulkowitch (1937) they have been applied with success to studies on sodium and potassium.

The basic idea is simple. The patient is given a fixed intake of sodium in the diet say 20 mmol/day and this is maintained for a number of days or weeks. All excreta including urine and faeces are collected and their sodium content is determined. Eventually the sodium lost in urine and faeces will approximate to dietary intake and body weight will stabilise. The drug is then administered and the daily measurements continued. A good example is shown in Figure 2.

There are certain difficulties associated with this excellent technique:

Figure 2 The principle of a sodium balance experiment, illustrated by a study on a patient with cirrhosis of the liver. Excretion has fallen to intake level by day 5. The introduction of spironolactone (Aldactone) and later hydrochlorothiazide produces several days of negative balance. By day 12 however a new equilibrium is established at a lower body sodium level (inferred). Thereafter negative balance is seen only when ethacrynic acid is given.

1. metabolic ward facilities are required
2. in clinical conditions such as congestive cardiac failure and cirrhosis of the liver the time to equilibration may be prolonged. This is particularly true in chronic renal failure where the time to balance may be several weeks
3. even in normal subjects the time to balance may be 3 to 5 days and it is always tempting to induce the change (with drug) before proper equilibrium is attained
4. bed rest may be required in oedematous patients in order to avoid the effects of posture (and exercise) in activating the renin/angiotensin system which will result in diurnal sodium retention (Lee, 1981b).

Nevertheless, in spite of the difficulties associated with this time consuming technique, it remains the final touchstone against which other simpler, less sophisticated techniques must be judged.

Plasma and urine sodium concentration

Both these variables are measured routinely by flame photometry—a technique that has held pride of place for 30 years. However it has certain disadvantages

notably the necessity, in the case of blood, to separate the red cells by centrifugation and the employment of inflammable gas. It is likely therefore that flame photometry will be replaced by methods using the sodium electrode, thus avoiding centrifugation and a naked flame. Moreover the sodium electrode technique can process samples more quickly, allowing minute by minute, or hour by hour, monitoring of plasma or urine sodium concentrations (Ladenson, 1977).

There are two major caveats which limit the usefulness of plasma and urine sodium determinations:

1. Plasma sodium concentration is affected by many different variables including the renin/angiotensin system, adrenal mineralocorticoids and antidiuretic hormone (Lee, 1981a, b). Plasma sodium may therefore be relatively well maintained in spite of severe depletion of total body sodium. As a result plasma sodium estimations have proved less useful than would at first sight appear likely although they are usually part of the standard clinical chemical evaluation of the patient (or normal volunteer).
2. Measurement of urine sodium output can be widely variable in patients (or volunteers) who have not come into the steady state on a standard

oral sodium intake. Stamler and his colleagues (Liu *et al.*, 1979a, b) have demonstrated recently that in such circumstances three or even four sequential 24 h urine collections must be analysed before any reasonable idea can be obtained of the sodium status of the individual. For the short term experiment 4 hourly urine collections may be preferable provided that the subject is well hydrated, that precautions are taken to ensure complete bladder emptying and urine creatinine is used as an internal control.

Too much emphasis has been placed in the past on one single 24 h urine collection for sodium as for example in renin/sodium profiling and this has led in my view to much of the disagreement in the literature. In the case of diuretic drugs, particularly of the loop or high ceiling variety which are often short acting, the initial natriuresis is often followed by rebound activation of the renin/angiotensin system and sodium retention—all within the 24 h collection period. This can give rise to considerable confusion unless shorter collection periods are employed.

Fractional analysis of tissue sodium content and flux

In view of the difficulties of whole body techniques for the determination of sodium status in the individual, investigators have sampled tissues such as muscle or blood. The sample is analysed for electrolyte content by standard methods and in the case of blood the flux of ions into cells is measured by radioisotope techniques.

However as we proceed from plasma through interstitial to intracellular fluid, determination of precise ionic composition becomes progressively more difficult. The main problem is the accurate definition of intracellular and extracellular fluid volumes.

Muscle

One representative technique employs needle biopsy of the quadriceps femoris or vastus lateralis (Bergstrom, 1962). Apart from the errors in weighing and counting radioisotopes the major difficulty stems from the assumption that the chloride ion is totally extracellular. As a result of the penetration of chloride into the cells the estimate for extracellular fluid volume will be too high and that for intracellular volume too low. If on the other hand inulin is used the extracellular volume may be underestimated. Results from these techniques can therefore only be regarded as approximations. Moreover the painful and invasive nature of the technique makes it unsuitable for repeated determinations as may be required for the estimation of therapeutic activity.

Red cell

This cell is easily obtained in multiple samples and has been much studied both in relation to ion content and ion flux (Canessa *et al.*, 1980; Garay *et al.*, 1980). In 1957 Skou described an enzyme found in the leg nerve of the shore crab which appeared to have an important relationship to active transport of sodium and later came to be known as sodium potassium ATPase. The enzyme is present in an enormous number of cell membranes, can be inhibited by cardiac glycosides, and its activity can be shown to vary with the sodium transport taking place in the tissue (Sweadner & Goldin, 1980). Tosteson, Moulton & Blaustein (1960) have found a strain of sheep with an inherited low concentration of potassium in their red blood cells; the membranes of these cells have a low Na/K ATPase activity.

The same enzyme also exists in the gills of euryhaline fish where it plays an important part in adaptation to salt water (Epstein, Katz & Pickford, 1967). Other sodium pumps have been described, for example in the kidney there is one type which couples with bicarbonate reabsorption and there is also evidence of a third system inhibited by cooling (Whittembury & Proverbio, 1970; Besarab, Silva & Epstein, 1976). The subject is well reviewed by Whittembury & Grantham (1976).

Recently interest in the red cell transport systems has been restimulated by the work of Meyer's group (Garay *et al.*, 1980) who have suggested that there is an inherited sodium-potassium ion cotransport defect in essential hypertension. This particular transport mechanism is blocked by frusemide but not by ouabain.

White cells

White cells can be obtained from blood by dextran sedimentation of red cells. They are then concentrated by centrifugation. Residual red cells are removed by hypotonic lysis and then the white cells are incubated in tissue culture medium (TC 199) containing ^{22}Na ($0.9 \mu\text{Ci}/\mu\text{l}$). The rate constants are then determined for ouabain sensitive and ouabain insensitive sodium efflux. ^{125}I -polyvinyl pyrrolidone is used to measure the extracellular fluid space and unlabelled sodium and potassium are measured by flame photometry.

This technique has been used in the study of electrolyte distribution in normal health (Baron & Ahmed, 1969), in essential hypertension (Edmondson *et al.*, 1975) and in pre-eclamptic toxemia (Forrester & Alleyne, 1980). It would lend itself to the *ex vivo* study of drug action in a manner similar to studies made on the red cell.

A note of caution should be sounded in relation to

the use of red or white cells for *in vitro*, or *ex vivo*, studies of electrolyte distribution and transport. It has been assumed, rather too lightly, that the red (or white) blood cell systems will reflect the activity of the sodium pumps at other strategic sites such as the peripheral arteriole or the renal tubule. This assumption may not necessarily be true. The red blood cell is highly specialised and its drug responses may not reflect changes elsewhere. Studies on the white cell have proved difficult to reproduce and standardise particularly in relation to extracellular fluid contamination. Moreover the changes seen may be non specific occurring in diverse conditions, as for example the changes seen in red cell sodium content and flux in Bartter's syndrome (Gardner *et al.*, 1972).

Nevertheless useful information has been generated. For example digoxin inhibits Na/K ATPase. Consequently intracellular sodium increases which in turn is thought to stimulate sodium/calcium exchange and increase intracellular calcium. Measurement of intracellular sodium may thus provide additional evidence of digoxin toxicity (Gervais *et al.*, 1977).

Conclusion

The length and complexity of this review of methods in the study of sodium metabolism serves to illustrate

the fundamental message that none is satisfactory. The whole body methods are tedious; expensive and in some cases limited by the restraints on the use of radioactive isotopes. The fractional methods are selective; open to error and can be difficult to interpret. When drugs are studied the situation is made doubly difficult. The whole body methods are usually not suitable for repeated determination on the same subject before and after treatment. On the other hand the fractional methods may be suitable for *ex vivo* measurement of drug effects but will require also measurement of drug concentration to make sure that this is not varying widely and is within the therapeutic range.

The benzothiadiazine diuretics were introduced in the late 1950s and shown shortly afterwards to lower arterial blood pressure. The fact that there is still dispute over the mechanism of their long term hypotensive effect is largely because of the unsatisfactory nature of present methods. Until rapid, accurate and reproducible methods for the study of sodium handling in the body become available, it is likely that our present difficulties with the interpretation of the action of therapeutic agents on the distribution and handling of this ubiquitous ion will continue.

Figures 1 and 2 are reproduced from Maxwell and Kleeman, Clinical Disorders of Fluid and Electrolyte Metabolism 1980 by kind permission of McGraw Hill Ltd, New York.

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