THE HORMONAL CONTROL OF BODY SODIUM

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OUR body cells are bathed in a fluid whose salt content resembles that of the primordial sea where we presumably had our origins several billion years ago. Since this sea probably contained about one-third as much salt as sea-water to-day (Strauss, 1957) its salt content approached that of our extracellular fluid. Among invertebrates there are wide variation in the osmotic concentration of body fluids, but the "milieu intérieur" of man and all classes of vertebrates is remarkably constant, both osmotically and volumetrically. Claude Bernard's (1878) oft-quoted idea that this stability was "la condition de la vie libre, indépendante" still appears axiomatic. When not associated with changes of the volume of body fluid compartments, quite large fluctuations of serum osmolarity are tolerable but when, as is more usual, changes in the volume of the body fluid compartments take place, then, the "freedom and independence of existence" is clearly curtailed.

Since salt balance can be preserved indefinitely on a diet as low in sodium as the dietician's ingenuity can fashion it, there must be potent renal mechanisms for the conservation of salt; without these mechanisms our aquatic ancestors presumably could never have migrated from salt to fresh water.

The volume of the extracellular fluid depends on its salt content since sodium, which is actively extruded from cells, largely determines its osmolarity. The total amount of sodium in the body is therefore very delicately controlled, and, allowing 3 to 4 days for equilibration, sodium output exactly equals sodium intake. In normal people extrarenal sodium losses are negligible if sweating is avoided, so that salt balance can only be achieved by variations of sodium output through the kidney. Looked at in another way, our very survival depends on the ability of the kidney to reabsorb the 173 litres of water and 24,000 milliequivalents of sodium filtered by the glomeruli each day. On an average salt intake about 99.5 per cent of the glomerular filtrate must be reabsorbed to

maintain a steady state. Large changes of intake induce only very small changes in sodium reabsorption; a change of only 0.75 per cent in the amount of sodium reabsorbed will maintain a steady state despite doubling or halving an average salt intake. Therefore, the mechanism(s) by which the kidney is able to detect and respond to changes in salt intake must be very sensitive indeed.

Mechanisms of Salt Excretion

Much data is now available which strongly suggests that the volume of the extracellular fluid (ECF), or rather that portion of it which is physiologically effective, is one of the main factors which determines the rate of renal sodium excretion, at least in normal individuals (Epstein, 1956, Strauss, 1957, Smith, 1957). How is the kidney able to regulate it?

The kidney can only alter the rate of sodium excretion by two mechanisms—by changes in the rate of glomerular filtration (GFR) or by changes in the rate of tubular reabsorption. GFR is determined by haemodynamic factors such as the renal blood flow and the relative resistance to flow offered by the afferent and efferent arterioles of the glomerular tuft and by the colloid osmotic pressure of the plasma proteins. The factors governing the rate of sodium reabsorption by the tubules will be tentatively assessed in this account because it is likely that hormonal, or at least humoral, factors are important.

For years there has been an earnest controversy concerning the relative importance of glomerular filtration and tubular reabsorption in mediating functional adjustments of sodium excretion. Wesson (1957) and O'Connor (1962) have been articulate protagonists of the idea that functional alterations of sodium excretion are largely determined haemodynamically by undetectably small changes in filtration rate. If, but only if, we assume that the rate of tubular sodium reabsorption is constant and unrelated to the sodium load, then a 1 per cent change in GFR for a given plasma sodium concentration could halve or double the rate of sodium excretion. By present methods of estimation, such a small change of GFR lies within the limits of experimental error, so that the hypothesis becomes rather sterile because it cannot be tested experimentally. It is certainly true, however, that all the rapid changes in the rate of sodium excretion seen with manoeuvres such as motionless standing, salt infusions and acute sequestration of blood or haemorrhage are also associated with parallel changes of the rate of glomerular filtration. Qualitatively, even slight acute changes of GFR can lead to large changes of

sodium excretion in the dog (Selkurt, 1951; Mueller, Surtshin, Rolf and White, 1951), but quantitatively the relationship between the two is quite unknown (Wesson, 1957). It cannot be assumed, as is often the case, that there is a sort of immutable, instantaneous "glomerulo-tubular balance" so that a fixed proportion of the filtered load is always reabsorbed. For example, there may well be a lag after an acute reduction of GFR before the rate of tubular reabsorption is also reduced. This, therefore, makes it impossible to decide from acute experiments whether a given fall of GFR, detectable or not, can account for an observed fall of the rate of sodium excretion. When, however, GFR and sodium excretion move in opposite directions or when there is a large and unequivocal change of GFR without a corresponding change of sodium excretion, then tubular reabsorption must be changing. This is well illustrated by the experiments of Black, Platt and Stanbury (1951). After some days of sodium deprivation in normal people, a rapid infusion of hypertonic saline increased GFR unequivocally, but the rate of urinary sodium excretion still remained low.

Many acute experiments concerning renal salt handling have been performed in the dog, which appears to use changes of GFR to regulate sodium excretion to a greater extent than humans (Chalmers, Lewis and Pawan, 1952). This should temper undue enthusiasm for extrapolation from dog to man. Finally, when trying to interpret relatively short-term experiments, we should realise that a permanent change of GFR does not appear to disrupt sodium homeostasis (Surtshin, Rolf and White, 1951). Therefore, tubular reabsorption must also change, although adjustments to altered sodium intake probably occur more slowly.

Increased tubular reabsorption of sodium is by now well-established in patients accumulating oedema fluid. The GFR is clearly below normal in cardiac failure, (Merrill, 1946) even when due allowance is made for age (Wesson, 1957), but there is now a large body of data (Davis, 1960) which strongly suggests that the rate of tubular sodium reabsorption is increased. In the hypoproteinaemic states (the nephrotic syndrome and cirrhosis of the liver) the GFR is not significantly different from normal (Wesson, 1957) and there are numerous well-documented accounts of supranormal rates, as might be anticipated when the colloid osmotic pressure of the plasma is reduced. Since the urine of these patients is often practically devoid of sodium, to conclude that increased tubular reabsorption of sodium is largely responsible for the oedema, becomes inescapable.

Once one accepts that the rate of tubular reabsorption of sodium is the major variable in the control of renal sodium output in man, then the problem becomes that of detecting the factors which determine it.

The Role of the Adrenal Cortex in Mediating Changes of Sodium Excretion

The adrenal glands are necessary to maintain life, an action parallel to the "mineralocorticoid" activity of the steroid hormones which promote the reabsorption of sodium and the secretion of potassium by the renal tubules. However, the adrenocortical hormones only increase the rate of tubular sodium reabsorp-

tion by such a small increment that sodium deficiency in adrenocortical failure can be overcome by a modest increase of salt intake. Nevertheless, without the mineralocorticoid action of steroid hormones, our capacity to curtail sodium losses in the face of a reduced intake is seriously impaired. Lipsett and Pearson (1958) showed that even when adrenalectomised patients are maintained on relatively high quantities of cortisone (50-75 mg. daily) by the fifth day on a low salt diet (when a normal person would have reached equilibrium) the rate of urinary sodium excretion was still some three to four times more than the intake. Nevertheless, the rate of sodium excretion did fall by about half, in association with a sharp reduction of GFR.

The adrenal cortex normally only secretes sufficient amounts of three known steroids (cortisol, aldosterone and corticosterone) which are important physiologically. Under normal conditions and in physiological quantities they probably exert their mineralocorticoid effects mainly on the distal tubule (Vander, Malvin, Wilde, Lippes, Sullivan and McMurray, 1958) where they promote sodium reabsorption, both in association with chloride and, more characteristically, by increasing the rate of exchange of sodium with potassium and hydrogen ions. Whether adrenocortical steroid hormones can influence sodium reabsorption by the proximal tubule is still unknown but, by implication (*v. infra*), they appear to do so.

Of the steroids involved, it can be roughly calculated from the known potency ratios in animals (O'Connor, 1962) and the estimated rate of secretion from turnover studies after the injection of radioactive tracers in man, that about 70 per cent of the "mineralocorticoid" activity leaving the gland under normal conditions is due to aldosterone, about 20 per cent to cortisol and about 10 per cent to corticosterone. Aldosterone is, then, the predominant mineralocorticoid despite the fact that the rate of cortisol secretion, which accounts for over 90 per cent of the "glucocorticoid" activity leaving the adrenal, is 100 times greater gravimetrically. By "mineralocorticoid activity" is meant either the overall effect of prolonging the life of an adrenalectomised animal or the more specific effect of reducing the rate of urinary sodium excretion. Actually, since cortisol (and its synthetic analogues) but not aldosterone or desoxycorticosterone increases the glomerular filtration rate in adrenalectomised dogs (Garrod, Davies and Cahill, 1955; Slater, Mestitz, Walker and Nabarro, 1962)

some of the "mineralocorticoid" renal tubular effects of glucocorticoids are partially annulled. Thus adrenocortical steroid hormones, particularly aldosterone, do have biological attributes which could be evoked to explain many of the changes of the rate of sodium excretion which are seen physiologically and pathologically.

Aldosterone and Sodium Homeostasis

Aldosterone was first isolated in 1953 by Tait, Simpson and Grundy by a brilliant and painstaking combination of chemical analysis and biological assay for mineralocorticoid activity of the "amorphous" fraction of adrenal gland extracts. This fraction was known to be the most potent in maintaining life after adrenalectomy. Once discovered, aldosterone soon came to be regarded as a major factor in regulating renal sodium excretion via changes in the rate of tubular reabsorption, not only in oedematous conditions, but also in physiological situations such as sodium depletion and repletion, the erect and supine posture, and hæmorrhage. Really good evidence regarding the role of aldosterone in these situations has been slow in coming despite many thousands of somewhat confusing publications. Evidence is needed that changes in the rate of sodium excretion are indeed associated with changes in the amount of biologically-effective aldosterone available to the renal tubular cells. The closest approach to this would be to measure the concentration of aldosterone in the circulating plasma and to assume that the ratio between free and protein-bound forms of the hormone found in the plasma also holds true at the cell surface. But aldosterone circulates in such minute quantities (of the order of tens of mille-micrograms or nanograms per 100 ml.) that a direct measurement of the plasma concentration is barely possible, even today. Another approach would be to try and obtain an index of the 24-hour average plasma level by measuring the rate of excretion of biologically active hormone in the urine. However, only a minute proportion of secreted aldosterone is excreted unchanged in the urine (about 0.2 per cent). Therefore, use has invariably been made of the fact that about 5 per cent of secreted aldosterone can be released from a conjugate by acid hydrolysis of the urine. This is now thought to be an 18-glucoronide (formerly Tait's 3-oxo-conjugate). The aldosterone so liberated can then be measured either biologically or, with greater precision, chemically. Quite apart from the inherent inaccuracies of measurement, the

rate of excretion of acid-released aldosterone will only reflect the plasma level if (1) the renal clearance rate of the hormone is constant and (2) the proportion of the total secreted aldosterone metabolised along this particular metabolic pathway also remains constant. For example, a 5 per cent reduction of the 95 per cent of aldosterone present in the form of other metabolites could double the amount of aldosterone which can be released by acid hydrolysis.

The more recent application of radioisotopic procedures for the indirect measurement of the secretion rate of aldosterone (Laumas, Tait and Tait, 1961; Peterson 1959) has been a great stimulus, but to infer that a change of secretion rate implies a corresponding change of plasma level makes the assumption that the rate of metabolic disposal is constant. Actually, if the rates of both secretion and metabolic clearance are known, it is possible to arrive at an indirect measure of the plasma level of aldosterone.

With these remarks, it will be seen how the whole question of the relationship between the likely changes in plasma aldosterone concentration which could influence renal tubular activity, and the changes in sodium excretion, is bedevilled by almost insurmountable problems of measurement. It is, perhaps, all the more surprising, that there is a considerable amount of good evidence which points to an inverse relationship between the rate of aldosterone excretion and the rate of sodium excretion both in oedematous states and in the physiological response of the kidney to changes of extracellular fluid volume (Leutscher and Johnson, 1954; Davis, Goodkind, Pechet and Bahu, 1956; Duncan, Liddle and Bartter, 1956). But even if the rate of excretion of the acid-released metabolite *does* reflect the plasma level, to observe such a relationship does not necessarily imply that the one causes the other. Even though adrenalectomised patients, maintained on adequate steroid replacement therapy, cannot achieve sodium balance on a low sodium diet, this should not in any sense imply that the adrenal glands also mediate changes of sodium excretion under normal circumstances. It may mean only that *some* adrenocortical steroid activity is necessary, and that the changes of aldosterone level observed are not within the range of responsiveness of the renal tubules. Indeed, the idea that they are not so involved, but merely provide a sort of obligatory biochemical backcloth against which other factors actually mediate physiological

changes has been espoused under the term the "permissive" action of the steroids (Ingle, 1952). It is certainly difficult to demonstrate renal tubular effects when aldosterone is given to normal dogs although it is easy to do so after adrenalectomy (Barger, Berlin and Tulenko, 1958).

One approach to the problem is to follow the relationship between the rate of renal sodium excretion and some index of the plasma level of aldosterone as a function of time during the course of the renal response to sodium deprivation. This was done by Crabbé, Ross and Thorn (1958) who measured the rate of urinary excretion of acid-released aldosterone. They were unable to obtain a significant relationship between the urinary sodium output and the urine output of this particular aldosterone metabolite. In view of the foregoing remarks this is not surprising, although they were able (Thorn, Ross, Crabbé and Van't Hoff, 1957) to show an inverse relationship between urine sodium excretion and acid-released aldosterone excretion in random urines collected from normal people.

Another, theoretically more profitable line is to observe the effects of an antagonist of aldosterone at the tubular level. One such, a 17-spirolactosteroid now marketed as spiro-lactone, was synthesised by Kagawa, Cella and Van Arman (1957) who were able to show in rats that the compound, whose chemical structure was designed to resemble that of aldosterone and so inhibit its action competitively, was biologically inert unless the adrenal glands were present. In dog and man the pharmacological properties of spiro-lactone in doses which are too low to have any physiological or toxic effect are, convincingly, only those of a mineralocorticoid antagonist at the renal tubular level (Liddle, 1958; Slater, 1960). Ross and Winternitz (1961) have shown that, in a single subject taking spironolactone, sodium deprivation modifies the renal response so that after five days on a rice diet containing only 3 mEq of sodium, equilibration with intake had still not been reached; furthermore, the rate of fall of urinary sodium excretion (half-time 1.45 days) was significantly slower than the rate of fall observed in normal subjects (mean half-time 0.54 days SD 0.12 days). However, Mills (1963) in a similar experiment was unable to find evidence that the diurnal fluctuations of urine sodium output are even partly aldosterone-dependent.

The role of aldosterone in mediating changes of sodium excretion in short-term experiments

is even more dubious. Quite apart from haemodynamic factors, it is a puzzling and challenging fact that there is a delay of up to one hour before the renal tubular effects of injected steroid are apparent, even when the steroid is brought into intimate contact with the effector organ. This was clearly shown by Barger, Berlin and Tulenko (1957), and confirmed by Ganong and Mulrow (1958), who injected d-aldosterone directly into one renal artery; the characteristic effect on sodium and potassium excretion was seen simultaneously in both kidneys some 30-60 minutes later. This would appear to exclude aldosterone from mediating at least the *early* changes of sodium excretion in such manœuvres as motionless standing, hæmorrhage and acute salt loading. Furthermore, Addisonian or adrenalectomised patients maintained on an adequate and constant amount of replacement steroid can manipulate their rate of sodium excretion in an apparently normal fashion, following both quick changes of posture and sequestration of blood in the legs (Rosenbaum, Papper and Ashley, 1955; Epstein, 1956). We may reasonably conclude, therefore, that haemodynamic factors are dominant in experiments where changes of sodium excretion are seen within minutes or possibly hours, but that there is some evidence to suggest that alterations in aldosterone levels are a major variable in determining long-term adjustments to altered sodium intake. The evidence is incomplete, indirect and hardly justifies any idea that aldosterone controls renal sodium excretion in the way that the antidiuretic hormone controls urine flow.

Aldosterone and Oedema Formation

Whatever the final conclusion regarding the role of aldosterone in mediating changes of salt excretion physiologically, a reasonable case can be made, with certain assumptions (*v. infra*), for its being a major factor in the production of oedema, at least that of liver and renal disease. From a large literature, three main pieces of evidence appear to predominate. Davis, Howell and Southworth (1953) have shown that the excessive fluid retention which can be induced in dogs by constricting the thoracic inferior vena cava—an excellent stimulus to aldosterone production—could only be mimicked after adrenalectomy if desoxycorticosterone acetate (DOCA) was given in 20 to 50 times the amount needed for full replacement in otherwise untouched adrenalectomised dogs.

Similarly, Giroud and his colleagues (1961) have demonstrated that aminonucleoside nephrosis in rats is adrenal-dependent. To reproduce the fluid retention seen in animals with intact adrenal glands, ten times the amount of aldosterone necessary for replacement in normal animals was required.

Finally, in man, the effect of the 17-spiro-lactosteroids when given to patients with cirrhosis of the liver or the nephrotic syndrome can be so dramatic that all the visible oedema-fluid disappears. Usually, however, the effect is relatively small. Since, from the doses used, competition with aldosterone for receptor sites on the renal tubule is far from being on a molecule-for-molecule basis, the fact that spironolactone is usually a weak diuretic may only mean that the effect of aldosterone on the renal tubule is only partially inhibited in the quantities used. It is, perhaps, considerably more significant that an oedematous patient can be rendered "dry" by spironolactone alone. In cardiac failure the effect of aldosterone antagonism is small (Bartter, Gann and Thomas, 1960) unless cardiac cirrhosis of the liver has supervened (Slater, 1960) but then, haemodynamic factors predominating, hyperaldosteronism is usually not a conspicuous feature of cardiac failure in contrast to the intense hypersecretion of aldosterone in patients with the nephrotic syndrome or cirrhosis of the liver.

But, if inappropriate overproduction of aldosterone is really important in oedema formation, prolonged administration of aldosterone or other mineralocorticoid steroid hormones such as desoxycorticosterone might be expected to lead to sodium retention and ultimately the appearance of oedema. This does not happen (August, Nelson and Thorn, 1958). By some mechanism which may be haemodynamic or may involve a process of tubular antagonism, an "escape" from the effect of mineralocorticoids on ion transport occurs. The timing of this "escape" will vary with the salt intake and the dose of steroid used, but in normal people and in animals it always occurs. It may have its pathological counterpart in the syndrome of primary aldosteronism when oedema is strikingly absent and hypokalaemia prominent. This contrasts with the fluid retention and normal serum potassium levels seen in the secondary hyperaldosteronism of cirrhosis and nephrosis.

It is therefore clear that, for fluid retention to take place, there must be some other important factor operating apart from an increased

circulating level of aldosterone. Whatever its nature, whether humoral or circulatory, it would appear likely that its main effect is to increase the rate of proximal tubular sodium reabsorption. If this is greatly enhanced it can be reasonably argued that insufficient sodium is delivered either to the site of free-water formation or to the site of exchange between potassium and sodium further down the nephron. Impaired free-water excretion is a characteristic feature of oedematous states (Ralli, Leslie, Stueck and Laken, 1951) and sodium depletion (McCance and Widdowson, 1937). It is unlikely to be due to excess anti-diuretic hormone (Lamdin, Kleeman, Rubini and Epstein, 1956; Schedl and Bartter, 1960). In contrast to normal people, the infusion of mannitol into patients with cirrhosis of the liver or cardiac failure increases the free-water clearance (Bell, Schedl and Bartter, 1964; Schedl and Bartter, 1960). Similarly, potassium depletion is conspicuous by its absence in the secondary hyperaldosteronism of untreated patients with cirrhosis and nephrosis. Since tubular potassium secretion largely occurs in exchange for sodium (Berliner, Kennedy and Hilton, 1950), a reduced delivery of sodium to the distal tubule will reduce the urinary loss of potassium that would presumably otherwise occur in response to high levels of circulating aldosterone.

From the fact that 85 per cent of filtered sodium is reabsorbed proximally, it is clear that an influence which even slightly changed the rate of this erstwhile "obligatory" sodium reabsorption would have a profound influence on the rate of renal sodium excretion. Since the fluid retention of cirrhosis of the liver appears to be largely aldosterone-dependent, and since proximal tubular sodium reabsorption is enhanced in this condition, it can be argued that aldosterone must be acting on the proximal tubule.

Non-Adrenal Factors Influencing Tubular Sodium Reabsorption

Others have sought to discover evidence for non-adrenal factors affecting tubular reabsorption. The posture and thigh-cuffing experiments of Rosenbaum, Papper, and Ashley (1955) in adrenalectomised patients were continued for several hours; the rate of urine sodium excretion remained low despite a clearly rising rate of glomerular filtration. Since the quantity of steroid replacement was constant, the authors felt compelled to postulate the existence of some other non-adrenal factor which promotes

tubular sodium reabsorption. But this interpretation cannot be accepted until we know whether the circulatory level of biologically-active steroid was, in fact, constant. Changes of posture are known to change the rate of liver blood flow which may well be a critical factor in determining the steroid plasma level.

Ingenious cross-circulation experiments with correction for fluid displacements have been performed by de Wardener, Mills, Clapham and Hayter (1961) in an attempt to avoid the vexed question of whether saline infusions increase renal sodium excretion haemodynamically or by changes of tubular reabsorption. In 3 experiments, when supramaximal quantities of anti-diuretic hormone (with a mineralocorticoid to suppress aldosterone production) were given to both dogs, the saline-infused animal nevertheless excreted much more sodium than the other despite apparently equivalent changes of filtered sodium load. It was therefore concluded that changes in the concentration of an ephemeral hormone, other than aldosterone, was responsible.

The Control of Aldosterone Secretion

Whatever the final conclusion about the precise rôle of aldosterone in the preservation of salt balance, its import cannot be denied, particularly in oedema formation. We have seen how renal sodium excretion appears to be largely a function of the extracellular fluid volume and that under most circumstances the rate of renal sodium excretion varies inversely with the rate of excretion of the acid-released urinary conjugate of aldosterone. Assuming this reflects the plasma level and assuming that the plasma level is largely a function of the rate of secretion, it would appear reasonable to predict that changes of extracellular fluid volume would be a major factor influencing the rate of aldosterone secretion.

Changes of extracellular fluid volume

The excellent series of experiments by Bartter and his colleagues in man (Bartter, Mills, Biglieri and Delea, 1959) clearly showed that an expansion of the extracellular fluid (ECF), whether associated with an increase, no change, or a decrease in the volume of intracellular fluid, reduced the rate of urinary excretion of the acid-released aldosterone metabolite and increased renal sodium excretion. Conversely a contraction of ECF volume, whether associated with a decrease or an increase in the volume of the intracellular fluid, increased the rate of aldosterone excretion

and reduced renal sodium excretion. Finally, it was shown, by infusing salt-poor albumin, that expansion of the intravascular fluid alone would still produce a fall in the rate of aldosterone excretion. Since there was a net decrease in the total ECF volume, this is the most convincing demonstration that it is intravascular and not interstitial cell volume that matters. If we assume that the excretion of aldosterone, as the acid-released conjugate, mirrors its secretion, then changes of blood volume are at least one factor likely to have a profound influence on the latter. But how is the adrenal able to sense these changes?

Changes of electrolyte composition of the extracellular fluid

Concomitant alterations of the plasma concentration of sodium or potassium were explored particularly by the Australian workers (Blair-West, Coghlan, Denton, Goding, Munro, Peterson and Wintour, 1963; Coghlan, Denton, Goding and Wright, 1960). They transplant the one remaining adrenal gland of a sheep into its neck so that local changes in composition of the blood supplying the gland can be made without disturbing the rest of the animal. The rate of steroid secretion can then be measured directly, and in selected animals the transplant retains apparently normal responsiveness for years; it may even grow medullary tissue. Local infusions of glucose to lower the plasma sodium concentration by clinically large amounts are associated with only a small increase in the rate of aldosterone secretion. A relatively large increase in the potassium concentration, however, is a sharp stimulus to the secretion of aldosterone, especially when accompanied by decrease of sodium concentration.

It is now clear that local changes of plasma potassium concentration can also be an important stimulus to aldosterone secretion in the dog (Urquhart, Davis and Higgins, 1962), but, in man, very large changes of potassium balance only change the rate of aldosterone excretion to a relatively small extent when appropriate compensation is made for the fact that potassium loss causes sodium retention and vice versa. Profound hyponatraemia following water loading in man is associated with a low rate of aldosterone excretion, even when sufficient potassium is given to reduce sharply the plasma sodium-to-potassium ratio (Bartter, Liddle, Duncan, Barber and Delea, 1956), which again emphasises the physiological insignificance of changes of plasma sodium and potassium concentration in man.

Arterial receptor areas

If, then, it is the volume of the blood rather than its composition which is the major variable in regulating aldosterone secretion, what function of it provides the afferent stimulus? Under- or over-filling of the venous side is probably not important because a reduced amount of blood in the central veins (as in constriction of the thoracic inferior vena cava) and also distension of the central veins (as in cardiac failure) are both associated with hyperaldosteronism. With each, however, there is a tendency for inadequate filling of the systemic arterial tree which, it has been suggested, is a major influence on the rate of renal sodium excretion (Epstein, Post and McDowell, 1953). Therefore, localised receptor areas have been looked for in the arterial system. Already it appears that constriction of at least two major arteries, the carotid and the renal (*v. infra*), will stimulate aldosterone production. Constriction of the carotid arteries was discovered by Bartter, Mills and Gann (1960) to be a strong stimulus to the secretion of aldosterone in normal dogs stressed by surgery. This was confirmed by Carpenter, Davis and Ayers (1961 a and b) but, in their hands, the stimulus was a very weak one. Both this effect and the intense aldosterone-stimulating effect of *acute* constriction of the inferior vena cava in normal dogs stressed by surgery was found by Bartter and his colleagues to be prevented by prior removal of all visible nerves from the thyrocarotid arterial junction, but not by denervation of the carotid sinus. The significance of these exciting results was questioned when Carpenter and his colleagues showed that hyperaldosteronism persisted in dogs with *chronic* constriction of the thoracic inferior vena cava despite extensive stripping of all visible nervous tissue from the central arterial tree. Moreover, hypophysectomy greatly weakens the aldosterone-stimulating effect of acute, but not chronic, constriction of the inferior vena cava, and Biglieri and Ganong (1961) showed that after hypophysectomy, carotid artery compression failed to increase aldosterone secretion, although it would do so in normal animals. It could be argued that some ACTH is necessary "synergistically" and it certainly seems unlikely that *all* the acute effects described by Bartter, Mills and Gann (1960) can be explained on the basis of changes of ACTH production, but since these are extremely difficult experiments with a high biological and chemical variability, adequate interpretation must await further data. Certainly the more recent discovery that

FIG. 1.—The effect of constriction of both renal arteries on the production of aldosterone, corticosterone and cortisol in 5 dogs hypophysectomised 6 to 14 days previously, plotted as a difference from the mean of the control data (Slater, Barbour, Henderson, Casper and Bartter, 1964). Each point is the secretion rate over a 20 to 30 minute period. Blood collections were started 35, 70 and 95 minutes after constriction was applied. The constriction was then released and 2 final samples collected 60 to 75 minutes later. (By permission of *SURGERY, GYNECOLOGY & OBSTETRICS*).

constriction of the renal arteries (Fig. 1) and hence the renin-angiotensin system (*v. infra*) will stimulate the adrenal cortex in hypophysectomised dogs has temporarily eclipsed further work on other areas of the arterial tree. On the efferent side, the regulation of aldosterone secretion has been shown to be hormonally, or at least humorally, mediated both in the sheep (Denton, Goding and Wright, 1959) and dog (Yankopoulos, Davis, Kliman and Peterson, 1959). This situation is also com-

FIG. 2.—The rate of adrenocorticosteroid production before and 18 hours after hypophysectomy in 6 conscious dogs deprived of dietary sodium. Secretion rates have been calculated with allowance for the uptake of aldosterone by erythrocytes (Slater and others, 1964). Data plotted on a log-scale to emphasise *proportional* changes.

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plex but has been partially clarified by the recent discovery of the direct steroid-stimulating effects of angiotensin. Before considering this, two other potentially relevant hormonal mechanisms should be mentioned.

The role of the pituitary

By analogy with the ACTH-cortisol system the role of the pituitary gland has been extensively studied. Large doses of ACTH will certainly increase aldosterone excretion but the effect is short-lived in man even when sodium retention is prevented. Furthermore, suppression of the pituitary with cortisone does not reduce the rate of aldosterone excretion (Liddle, Duncan and Bartter, 1956). The pattern of adrenocortical stimulation with decreasing doses of ACTH in dogs shows quite clearly there is no dose of ACTH which stimulates aldosterone production without also stimulating the production of cortisol (Slater, Barbour, Henderson and Casper, 1963). A patient without his adrenal glands dies of sodium deficiency if deprived of salt, whereas a patient without his pituitary gland conserves salt normally (MacLean, Lipsett, Li, West and Pearson, 1957) and dies eventually of cortisol deficiency without gross electrolyte imbalance. After hypophysectomy the zona glomerulosa of the adrenal gland, where aldosterone is produced, does not atrophy, whereas the zona fasciculata, where cortisol is produced, does. Even from the evidence available in 1940, Swann was able to postulate the existence of a separate non-pituitary mechanism for the control of mineralocorticoid activity of the adrenal

glands. Nevertheless, more recent work concerning the acute effects of hypophysectomy in dogs appeared to implicate the pituitary (Davis, Bahn, Yankopoulos, Kliman and Peterson, 1959; Slater, 1963). Most of this work was done in heavily anaesthetised dogs acutely stressed by surgery. To avoid this the effect of hypophysectomy by estimating the adrenocorticosteroid secretion rates has been assessed before and some 18 hours after surgery, when the animal was calm and conscious (Fig. 2). As was expected, aldosterone secretion does not change despite sharp falls in the rate of cortisol and corticosterone secretion.

Role of the pineal

Since, therefore, the pituitary can be excluded as a major factor, Farrell produced the phylogenetically fascinating idea that the function of the pineal gland was concerned with the control of aldosterone secretion (Farrell, 1958). He found that extracts of beef diencephalon stimulated the secretion of aldosterone in decerebrate dogs and that a large part of this activity could be accounted for by that present in the pineal gland (Farrell, 1959). But pinealectomy does not reduce aldosterone secretion and the usual increase in aldosterone secretion following salt-deprivation is seen in pinealectomised dogs (Farrell, 1960). Unfortunately, experimental confirmation of the adrenoglomerulotrophic effects of pineal extracts and compounds therefrom is still awaited so that at the present time it is virtually impossible to assess the importance of the pineal-hypothalamic system. Nevertheless, it seems highly likely that the central nervous system is somehow involved, possibly in mediating fine adjustments of aldosterone production, but it is clear from the work of Davis and his colleagues that hyperaldosteronism in dogs can persist and be produced despite chronic extensive hypothalamic lesions or even decapitation.

The role of the kidneys

The finding of Deane and Masson (1957) that, in the rat, renal extracts produce adrenal hyperplasia, the discovery by Biron, Kiow, Nowacynski, Brouillet and Genest (1961) that the adrenal glands are stimulated by angiotensin II in man and Bartter's description of hyperplasia of the juxtaglomerular complex in a patient with hyper-aldosteronism due to bilateral adrenal hyperplasia (1962), all suggested that the kidneys may be important in the control of aldosterone via the renin-angiotensin system. Three separate groups in the United States studied this simultaneously and independently

FIG. 3.—Adrenocortical response in dogs without pituitary or kidneys, to saline extracts of kidneys from (a) a hypophysectomized dog deprived of salt and (b) a normal dog loaded with salt.

in recently hypophysectomized dogs (Davis, Carpenter, Ayers, Holman and Bahn 1961; Ganong and Mulrow, 1962; Slater, Barbour, Henderson, Casper and Bartter, 1963, 1964). Each group found that nephrectomy uniformly reduced, if it did not abolish, the adrenocortical response to acute hæmorrhage, although the gland was still easily stimulated by ACTH. They each also showed that saline extracts of the kidney would stimulate the adrenal cortex. That this effect is not due to renal sequestration of ACTH but, rather, is related to the salt balance of the animal whose kidney is used, is indicated in Fig. 3. The effect of nephrectomy is, however, not so clear-cut. Performed as an acute experiment in recently traumatised, sodium-depleted, and hypophysectomized animals under heavy anaesthesia, nephrectomy sharply reduced the rate of aldosterone secretion to basal levels. There was, however, a relatively slight fall in aldosterone secretion seen in conscious or lightly anaesthetized hypophysectomized animals where one remaining kidney was removed with minimal trauma

some 18 hours after major surgery (Slater, 1963). Similarly, Blair-West and his colleagues (1963) found that the rate of aldosterone secretion often remained well above basal in sodium-depleted sheep despite hypophysectomy and bilateral nephrectomy. More data on this important question is needed, but it is already clear that the kidneys do not produce a trophic substance affecting aldosterone secretion similar in importance, for example, to ACTH in the control of cortisol secretion. Nevertheless, the recent finding of extractable renin in the blood vessels (Gould, Skeggs and Kahn, 1964) may, if the amounts are sufficient, prove to be the explanation.

The Renin-Angiotensin System

As long ago as 1898 Tigerstedt and Bergman discovered that extracts of the kidney would raise the blood pressure. Following Goldblatt's demonstration in 1934 (Goldblatt, Lynch, Hanzal, and Summerville) that renal ischaemia produces hypertension, interest in the mechanism of this hypertensive effect of renal

FIG. 4.—The renin-angiotensin system.

extracts was stimulated, chiefly with regard to the cause of hypertension. From the pioneer work of Page, Pickering, Braun-Menéndez and their collaborators (Page and Bumpus, 1961), it has now become clear that renal tissue contains a proteolytic enzyme, renin, which splits specifically a leucyl-leucine bond in a circulating α -globulin of hepatic origin (renin substrate or angiotensinogen) to produce a largely inert decapeptide, angiotensin I. A circulating enzyme then splits off two amino acids to produce angiotensin II, an octapeptide which is the most potent pressor substance known. Angiotensin is destroyed in a short time by potent enzymic systems, collectively known as "angiotensinase", which are present in all tissues and circulating blood. It is now almost certain that the renin liberated from the kidney originates in the granular cells of the juxtaglomerular apparatus (Cook and Pickering, 1958; Bing and Kazimierzak, 1962; Hartroft, Sutherland and Hartroft, 1964) and possibly even in the granules themselves (Skelton, Chandra and Bernardis, 1964). First described by Ruyter (1925), these cells were studied by Goormaghtigh (1945) as an example of the endocrine function of the renal arterioles. The granular or afibrillar cells are found in the media of the afferent arteriole, near the glomerular tuft. They always lie in contact with a group of specialised cells, the macula densa, in the

FIG. 5.—Pattern of adrenocortical response to angiotensin II in a hypophysectomised, nephrectomised dog.

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distal convoluted tubule of the same nephron. Teleologically this arrangement is dangerously seductive—what better anatomical basis for sensing the filling of the arterial tree? Fig. 4 shows the overall picture of the system today though, if this is anything like the broadly analogous kinin-forming system, it will soon require considerable modification.

The importance of angiotensin in the control of sodium metabolism has become evident in the past few years. Indeed, it appears possible that the real physiological rôle of angiotensin is on renal salt excretion rather than on the control of blood pressure.

Angiotensin (or rather the synthetic aspartic β -amide) is a confusingly versatile molecule. Apart from being a senior member of the growing band of polypeptides which affect smooth muscle (so that it may influence sodium excretion haemodynamically) it stimulates the production of adrenocortical steroids, liberates catecholamines from the adrenal medulla (Feldberg and Lewis, 1963) and inhibits distal tubular reabsorption of sodium (Vander, 1963). To make the all-important separation of pharmacological from likely physiological effects is extremely difficult since we know so little about the circulating concentration of angiotensin. In any case, the local concentration at the site of

renin liberation in the juxtaglomerular complex may be quite different, and yet it is just here that angiotensin is in a strong position to influence the rate of both glomerular filtration and distal tubular sodium reabsorption. There are at least two obvious ways already known by which the renin-angiotensin system *can* influence renal sodium excretion. It can operate directly on the kidney or indirectly via the stimulation of production of aldosterone. The real question is whether angiotensin *does*, in fact, and if so, under what circumstances.

The role of the renin-angiotensin system in the control of aldosterone secretion

The first problem concerns the dose-response relationship between angiotensin action and adrenocortical response. The critical criterion for the designation of angiotensin, or any other potential candidate, as an aldosterone-stimulating hormone, is that it should affect the secretion of aldosterone almost exclusively. However, in the hypophysectomised, nephrectomised dog (Carpenter, Davis and Ayers, 1961; Mulrow, Ganong, Cera and Kuljian, 1962; Slater, Barbour, Henderson, Casper and Bartter, 1963) corticosterone secretion is stimulated to a comparable degree whatever the dose of angiotensin given, whereas Davis finds that constriction of the inferior vena cava barely alters corticosterone secretion in the conscious, minimally stressed dog (Davis, Carpenter, Ayers and Bahn, 1960). Slater and colleagues (1963) found, in addition, that whenever aldosterone secretion is clearly stimulated, so also is that of cortisol (illustrated in Fig. 4). But the rate of corticosterone and cortisol secretion reached is relatively modest compared with the effect of ACTH, which has quite a different pattern of adrenocortical stimulation (Slater and others, 1963; Mulrow and others, 1962). In the intact dog, the rate of equilibration of the ACTH-cortisol (and corticosterone) feed-back system *may* be sufficiently rapid to obscure changes of cortisol and corticosterone secretion in response to angiotensin. However, small amounts of angiotensin II infused locally into the transplanted adrenal gland in the neck of a sheep, (Blair-West and others, 1962) usually do not increase the secretion of cortisol or corticosterone. Therefore, providing the responsiveness of these adrenal transplants can be validated, either there is a species difference or intravenous angiotensin injections promote cortisol secretion indirectly. With man, hypertensive quantities of angiotensin II do stimulate the urinary

excretion of cortisol metabolites but this appears trivial compared with the considerable increase of excretion of aldosterone metabolites (Biron and others, 1961). In smaller, non-hypertensive, quantities an exclusive increase of aldosterone excretion, as the acid-released metabolite, is seen despite suppression of ACTH production with dexamethasone (Fig. 6). It is therefore possible that the pattern of adrenocortical response to angiotensin is compatible with that caused by salt-deprivation and other stimuli, though intense hyperaldosteronism is seen in many oedematous patients without any apparent change of the rate of cortisol secretion, which implies the existence of some other variable. This may be the pattern of responsiveness of the adrenal zona glomerulosa. The Melbourne group have shown that in the salt-depleted sheep small doses of ACTH given locally stimulate the secretion of much more aldosterone in relation to cortisol than in the salt-repleted sheep (Blair-West, Coghlan, Denton, Goding, Wintour and Wright, 1963).

There is now little doubt that in secondary hyperaldosteronism, whether experimental or clinical, there is a considerable elevation of circulating renin-like activity (Brown and others, 1964; Fasciolo and others, 1964; Davis, Higgins, and Urquhart, 1964) which is often beyond the possible range of the likely error of the method. It has been known for some time that the amount of renin which can be extracted from rat kidneys (Gross and Lichtlen, 1958) is both reduced by salt-loading and mineralocorticoid administration and increased by salt deprivation or adrenalectomy. The granularity of the juxtaglomerular cells (Pitcock, Hartroft and Newmark, 1959; Tobian, 1960) varies similarly. If the renin-store diminishes as the rate of renin discharge diminishes (a somewhat surprising feature which, on indirect evidence, nevertheless appears to be true), then it implies that the rate of renin discharge varies inversely with the volume of the extracellular fluid and hence directly with the rate of aldosterone secretion.

It is still uncertain whether the circulating renin concentration rather than the concentration of renin substrate is the rate-limiting factor for the production of angiotensin physiologically, but, in dogs, sodium depletion, which Brown and his colleagues find increases the circulating renin concentration, does appear to elevate circulating angiotensin levels (Bartter, 1963; Scornik and Paladini, 1964). It must be said, however, that the whole question of the accurate and reliable assay of

ANGIOTENSIN INFUSION IN HEALTHY PEOPLE

0.25 μgm . per minute

1962

FIG. 6.—Adrenal and metabolic effects of a 58-hour infusion of small amounts of angiotensin II in 7 healthy people, 2 of which (the lower 2 points under "urine 17-OHCS") were given dexamethasone.

renin and angiotensin in biological fluids on an analytical rather than preparative scale, is still overshadowed by methodological difficulties, although Boucher, Veyrat, le Chamlain and Genest (1964) have considerably simplified a very difficult method. That we are still on the threshold of our understanding of the role of the renin-angiotensin system in the control of aldosterone secretion is suggested by some recent findings. Van Brunt, Biglieri and Ganong (1964) find in renin-immunised dogs that the rate of aldosterone secretion is normal and that it increases with salt-depriva-

FIG. 7.—Acute renal effects of angiotensin II in a patient with Addison's disease.

tion although the animals are unresponsive to the pressor action of injected renin. This may only mean that there is still enough circulating angiotensin to mediate changes of aldosterone secretion, but it also may imply that other mechanisms for aldosterone control are dominant in the normal, conscious dog. Another apparently incompatible finding is that of

METABOLIC EFFECTS OF ANGIOTENSIN

FIG. 8.—Comparison of the long-term metabolic effects of small quantities of angiotensin II in 5 normal people and a patient with well-controlled Addison's disease.

Marieb and Mulrow (1964) who cannot stimulate aldosterone or corticosterone secretion from the rat adrenal gland with pressor quantities of angiotensin II. Yet, in the rat, which secretes increased amounts of aldosterone after hæmorrhage (Singer and Stack-Dunne, 1955), there is a positive correlation between the width of the zona glomerulosa and (a) the administration of renin (Deane and Masson, 1951), (b) granularity of the juxtaglomerular cells (Tobian, 1960), and (c) the extractable renin content of the kidneys (Gross, Schaechtelin, Brunner and Peters, 1964). There

is also, of course, a negative correlation between the width of the zona glomerulosa and the salt intake (Deane, Shaw and Greep, 1948). If increased quantities of angiotensin circulate in the blood of patients with secondary hyperaldosteronism, one might expect hypertension to occur. But the blood pressure is usually normal in oedematous states whether clinical or experimental. In man it is possible that subpressor quantities of angiotensin stimulate aldosterone production (*v. infra*) but, in any case, oedematous states are associated with a remarkable resistance to the pressor effects

of angiotensin and noradrenaline (Johnston and Jose, 1963; Laragh, Cannon, Bentzel, Sicinsky and Meltzer, 1963). Whether this is inherent or merely a result of diuretic therapy is controversial. Hypertensive patients treated with chlorothiazide are relatively resistant to the pressor effects of noradrenaline (Freis, Wanko, Schnaper and Frohlich, 1960), and this appears to be a direct effect of the drug on peripheral resistance vessels (Jackson and Duff, 1963).

Whatever the final outcome, we now know that aldosterone secretion will increase whenever the circulating angiotensin concentration increases; whether the former is actually controlled by the latter under normal circumstances must await further study. However, the idea that the renin-angiotensin system controls aldosterone secretion is particularly attractive because it provides, at least potentially, both effector and effector mechanisms.

More direct effects of angiotensin on sodium excretion

Even in amounts which have little or no effect on the blood pressure, angiotensin produces an immediate sharp fall in the rate of sodium excretion when infused into normal people (Bock, Dengler, Krecke and Reichel 1958; Gill, Barbour, Slater and Bartter, 1964) which does not depend on the presence of the adrenal glands (Statius Van Eps, Smorenberg-Schoorl, Zürcher-Mulder, de Vries and Borst, 1962). This is illustrated in Fig. 7. However, over a longer period of time the indirect effect of angiotensin on aldosterone may outweigh the "direct" effects of angiotensin on the kidney. Fig. 6 shows the adrenal and metabolic effects of sub-pressor amounts of angiotensin II infused into normal people. Despite an average weight gain of just over 1 kg. the rate of aldosterone excretion increased when it would otherwise have been expected to decrease. In the one patient with Addison's disease treated with cortisone who was in sodium balance, a 4-day angiotensin infusion had no metabolic effect, although pressor quantities were used (Fig. 8). This serves to emphasise that in small sub-pressor quantities such as are likely to be found physiologically, the effect of angiotensin on sodium excretion is likely to be largely mediated by its effect on aldosterone production.

In hypertensive patients, angiotensin infusions often produce an immediate increase rather than a decrease in the rate of sodium excretion (Brown and Peart, 1962). This reversal of the normal response also occurs in patients receiving salt and large quantities of mineralocorticoid and we have observed it over a longer period in a normotensive Addisonian patient overtreated with desoxycorticosterone. In short-term experiments it is impossible to disprove the hypothesis that, under these conditions, angiotensin constricts the efferent arteriole to a relatively greater extent than the afferent arteriole. For example, Brown and Peart report that the GFR increases rather than decreases in patients with hypertension. In cirrhosis of the liver, however, Laragh and others (1963) had shown that such a massive outpouring of salt can occur in response to large quantities of angiotensin, despite minimal changes of GFR, that a tubular effect seems probable; and it appears from the stop-flow experiments of Vander (1963) that angiotensin in normal dogs inhibits distal tubular sodium reabsorption directly. This may also occur in the rat (Peters, 1963; Gross and others, 1964).

These are some facts which will need to be considered in formulating a hypothesis for the mechanism of action of angiotensin but in view of the already numerous biological effects of the polypeptide, it is perhaps invidious to attempt one until we know more about which effects are likely to be relevant physiologically.

Conclusion

This is a necessarily incomplete account of the present state of our knowledge about the hormonal mechanisms controlling body sodium. An attempt has been made to stress principles and to keep an exceedingly complex field uncluttered with unresolved detail except when the problems themselves are likely to be stimulating rather than confusing. One important field, for example, which has not been discussed (largely because methodological problems still abound) is the study of the precise factors which control renin release from the kidneys. Nevertheless, the discovery of the likely importance of the renin-angiotensin system in the control of body sodium is perhaps one of the most exciting new developments in the realm of fluid balance since Verney's discovery of the physiological role of anti-diuretic hormone.

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