

Aldosterone and *in vivo* mineralocorticoid activity in normotensive and hypertensive man¹

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Introduction

Initially the measurement of rectal electrical potential difference (PD) appeared to provide the first useful *in vivo* assay for mineralocorticoid activity in human subjects, since it was shown that the rectal mucosa responds consistently to aldosterone and other mineralocorticoids with a rise of transmucosal PD (Edmonds & Richards 1970, Edmonds & Godfrey 1970). However, it has been found subsequently that there is a poor correlation between plasma and urinary aldosterone and rectal PD (Nicholls *et al.* 1975, Beevers *et al.* 1975, Skrabal 1976), the transmucosal PD being also influenced by other factors including the autonomic nervous system (Lennane *et al.* 1975). I reported at the meeting of the Royal Society of Medicine's Section of Endocrinology, on 26 October 1977, that the subtraction of the transmucosal PD of a steroid unresponsive mucosa (oral mucosa) from rectal PD eliminates the steroid independent biological variation of rectal PD. 'Subtraction PD' therefore provides a good index of mineralocorticoid activity in normal subjects and patients with adrenocortical insufficiency and hyperaldosteronism. Some of this work has been published meanwhile (Skrabal *et al.* 1978). This paper is a further analysis of the data already published and includes additional studies in 81 patients with essential hypertension, in 20 patients with primary or secondary hyperaldosteronism and in 13 patients with adrenocortical insufficiency.

Material and methods

Normal subjects: Studies were done in 48 healthy volunteers (8 female). Eleven subjects were studied on their usual sodium intake (6–18 g/day) and after being for one week on high (>18 g/day) and low (1–4 g/day) sodium intakes. In 23 subjects studies were made after intramuscular injection of 0.25 mg tetracosactrin (Synacthen).

Patients: 81 patients with untreated essential hypertension (WHO stage 1 and 2) were studied, and were classified according to plasma renin activity as low, normal and high renin hypertensives. Studies were repeated on different days in 10 patients with primary and 10 patients with secondary hyperaldosteronism. Of the patients with primary hyperaldosteronism, 8 had adrenal adenomas which were later removed at operation, 2 had bilateral adrenal hyperplasia. Ten patients had secondary hyperaldosteronism associated with cirrhosis of the liver (4), heart failure (4), pseudo-Bartter syndrome (1) and pheochromocytoma (1). In patients with primary and secondary hyperaldosteronism, supine plasma aldosterone was above 20 ng/dl. There were 13 patients with adrenocortical insufficiency who were studied repeatedly on different days, either untreated or after being without replacement therapy for at least 40 hours: 5 had had bilateral adrenalectomy for Cushing's disease (4) or malignant pheochromocytoma (1), and 8 had idiopathic Addison's disease. Adrenocortical insufficiency was confirmed in each case by an undetectable plasma cortisol and aldosterone after tetracosactrin stimulation. All patients were studied on their usual sodium intake.

Protocol: 24-hour urinary sodium excretion was measured on the day before the study. Plasma aldosterone, cortisol and plasma renin activity (in patients with essential hypertension) were

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measured between 09:00 and 10:00 after an overnight fast and 2 hours supine bed rest. Potential differences were measured 3 hours later between 12:00 and 13:00. This is the time of maximum response to the prevailing endogenous aldosterone concentration (Skrabal *et al.* 1978). For the tetracosactrin test, 0.25 mg tetracosactrin was injected intramuscularly at 08:00, supine plasma aldosterone and cortisol were measured at 09:00 and potential differences at 12:00.

Methods: Plasma aldosterone was measured by a modification of the method of Ito *et al.* (1972), plasma cortisol by competitive protein binding assay, plasma renin activity by the method of Boyd *et al.* (1966) and oral, rectal and subtraction PD as described previously (Skrabal *et al.* 1978). All PD measurements were corrected for skin PD by the intracutaneous injection of 0.5 ml saline below the reference electrode (Archampong & Edmonds 1972). Rectal PD measurements were done after the instillation of 20 ml of 0.9% saline into the rectum immediately before the actual measurement. Rectal PD was recorded after insertion of the disposable electrode at 8 cm from the anus, the highest stable PD at this distance being taken as rectal PD. All PD measurements were done employing the instrument Adrenosonde¹.

Results

The relation between plasma aldosterone and subtraction PD together with concurrent plasma cortisol in normal subjects is shown in Figure 1, which gives the means of the groups according to different levels of sodium intake and tetracosactrin injection. The relation between 24-hour urinary sodium excretion, plasma aldosterone and subtraction PD in normal subjects is shown in Figures 2 and 3.

Table 1 shows plasma aldosterone, oral, rectal and subtraction PDs, blood pressure, 24-hour urinary sodium and plasma electrolytes in patients with low, normal and high renin hypertension. The relation between plasma aldosterone and subtraction PD in patients with essential hypertension, patients with primary and secondary hyperaldosteronism, and patients with adrenocortical insufficiency before and after glucocorticoid replacement (5 to 6 hours after 20 mg hydrocortisone orally), is shown in Figure 4.

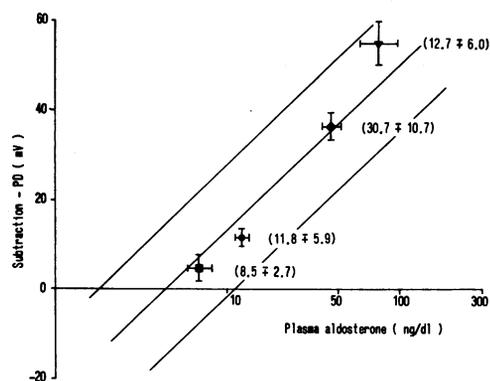


Figure 1. Relation between plasma aldosterone and subtraction PD in normal subjects on high (■), normal (●), and low (▼) sodium intakes and after tetracosactrin injection (◆) (means ± s.e.m.). In parentheses, concurrent plasma cortisol levels (µg/dl, mean ± s.d.). Regression line and confidence limits derived from all 4 groups. Salt intakes (g/day): ■ >18, ● 6-18, ▼ <4

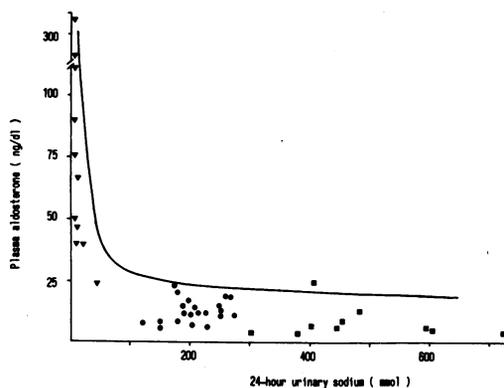


Figure 2. Relation between 24-hour urinary sodium excretion and plasma aldosterone in normal subjects on high (■), normal (●) and low (▼) sodium intakes

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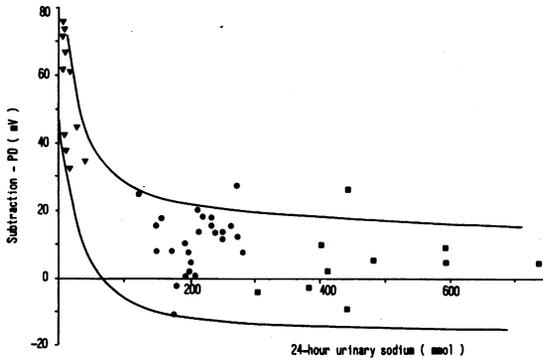


Figure 3. Relation between 24-hour urinary sodium excretion and subtraction PD in normal subjects on high (■), normal (●) and low (▼) sodium intakes

Figure 5 shows the effect of hydrocortisone and dexamethasone alone and after intravenous injection of 200 mg potassium canrenoate in a patient with adrenocortical insufficiency. As can be seen, in the absence of mineralocorticoids in adrenocortical insufficiency and then even after blockade of cytoplasmic mineralocorticoid receptors with a competitive mineralocorticoid antagonist, glucocorticoids produce a rise of subtraction PD comparable in time course to that of aldosterone (*see Skrabal et al. 1978*).

Figure 6 shows subtraction PD in patients with primary and secondary hyperaldosteronism compared with the 95% confidence limits derived in normal subjects, and Figure 7 shows the same results in patients with adrenocortical insufficiency before and after tetracosactrin stimulation.

Discussion

This study confirms, with a greater number of patients, our previous observation that subtraction PD is a good index of current mineralocorticoid activity and demonstrates use of the method for the diagnosis of primary and secondary hyperaldosteronism and of aldosterone deficit in adrenocortical insufficiency (Figures 6 and 7). If the method is used to screen for

Table 1. Measurements in patients with normal, low or high renin hypertension (mean \pm s.d.)

	Normal renin	Low renin	High renin
No. of patients	53	20	8
Plasma aldosterone (ng/dl)	11.4 ± 6.0	10.1 ± 7.3	19.5 ± 12.8
Rectal PD (mV)	40.7 ± 13.1	42.5 ± 11.9	45.2 ± 14.3
Oral PD (mV)	27.1 ± 7.9	28.6 ± 12.3	27.8 ± 7.4
Subtraction PD (mV)	13.6 ± 11.3	13.9 ± 16.4	17.4 ± 13.6
Blood pressure (mm Hg):			
(a) Systolic	157.8 ± 27.4	179.8 ± 29.8	150.0 ± 21.2
(b) Diastolic	98.5 ± 16.6	106.5 ± 13.9	93.7 ± 11.6
24-hour urinary sodium (mmol)	183.6 ± 87.3	190.2 ± 78.4	183.5 ± 75.9
Plasma potassium (mmol/l)	4.3 ± 0.3	4.1 ± 0.3	4.4 ± 0.3
Plasma sodium (mmol/l)	139.4 ± 2.9	139.4 ± 4.0	139.4 ± 1.9

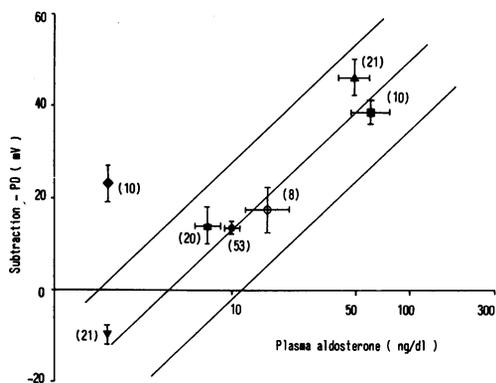


Figure 4. Relation between prevailing plasma aldosterone and subtraction PD in patients with adrenocortical insufficiency, untreated (▼) or after glucocorticoid replacement (◆); in patients with primary (▲) or secondary (■) hyperaldosteronism; and in patients with essential hypertension with low (★), normal (●) and high (○) plasma renin activity (mean \pm s.e.m.). Numbers of observations in parentheses. Regression line and confidence limits from normal subjects

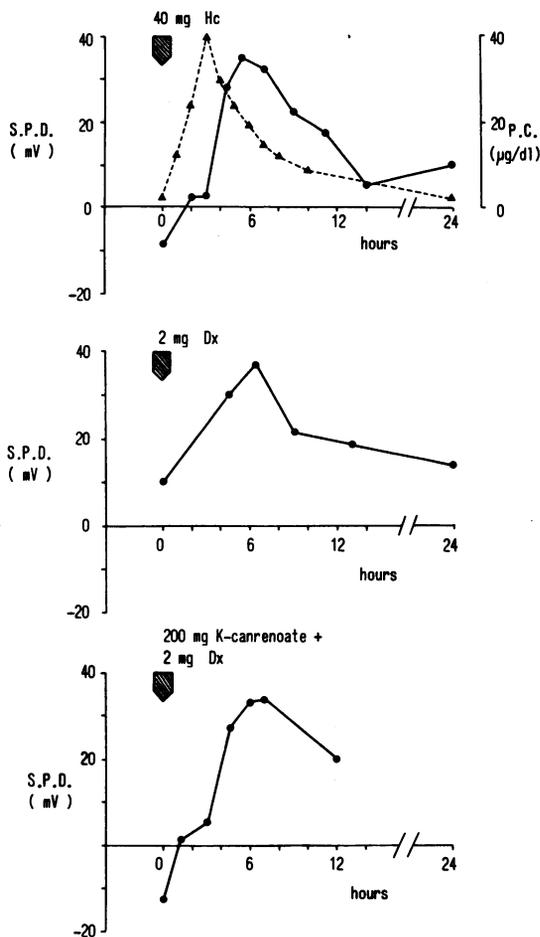


Figure 5. Effect of orally administered hydrocortisone (Hc) and dexamethasone (Dx) alone and after intravenous injection of 200 mg potassium canrenoate on subtraction PD in a patient with adrenocortical insufficiency. \blacktriangle --- \blacktriangle , concurrent plasma cortisol levels (P.C.)

primary hyperaldosteronism in hypertensive patients it is recommended that 80 mmol of potassium chloride be given orally for 2 days before the investigation, to eliminate false negative results in patients with primary hyperaldosteronism and profound potassium depletion. In patients with primary hyperaldosteronism, subtraction PD will then be found to be higher than 40 mV. For the diagnosis of adrenocortical insufficiency it has been found necessary to give tetracosactrin intramuscularly, since mineralocorticoid activity is also completely suppressed in normal subjects at the usual level of sodium intake (Figure 3).

Figure 8 illustrates a possible explanation why the subtraction of oral PD from rectal PD (subtraction PD) gives a good index of *in vivo* mineralocorticoid activity. The rectal mucosa not only responds through specific receptors to mineralocorticoids with a rise of transmucosal PD (Figure 8, upper half) but also to changes of adrenergic tone probably mediated through cyclic AMP (Figure 8, lower half). Apparently the oral mucosa is not equipped with mineralocorticoid receptors but otherwise responds to the same influences as the rectal mucosa. Therefore the subtraction of oral PD from rectal PD much improves the correlation coefficient between plasma aldosterone and transmucosal PD (Skrabal *et al.* 1978). Measurement of oral PD alone, changes of which are closely associated with sleep-

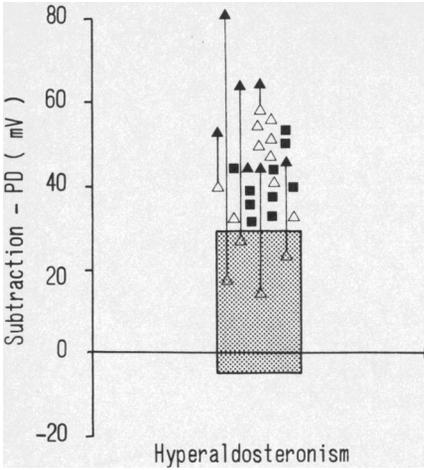


Figure 6. Subtraction PD in patients with secondary (■) and primary hyperaldosteronism before (△) and after (▲) potassium repletion. Shaded area corresponds to 95% confidence limits in normal subjects

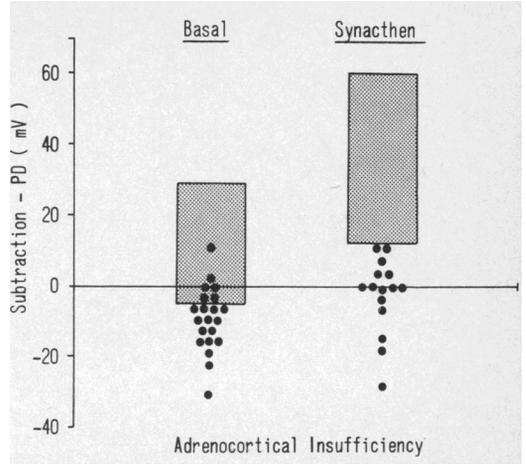


Figure 7. Subtraction PD in patients with adrenocortical insufficiency untreated and after tetracosactrin (Synacthen) injection. Shaded area corresponds to 95% confidence limits in normal subjects

wakefulness cycle (Skrabal 1977), could possibly serve as a simple tool to investigate autonomic nervous system function.

The present investigation also demonstrates that supine plasma aldosterone and (probably more significant) electrical asymmetry across sodium transporting epithelia are almost completely suppressed at sodium intakes of 100–300 mmol per day (Figures 2 and 3). This is the range of sodium intake encountered in most developed countries at present (Meneely & Battarbee 1976, Morgan *et al.* 1978). As can be seen from Figures 2 and 3, there is little further suppression of plasma aldosterone and aldosterone action at higher levels of sodium intake. Only below a sodium excretion of 50–80 mmol per day, corresponding to an intake of 3–4 g sodium chloride, can a stimulation of plasma aldosterone and subtraction PD be observed. From the function between sodium excretion and mineralocorticoid action, it appears that only at sodium intakes of below 3–4 g salt per day can sodium balance be sufficiently regulated by the adrenal gland. At this range of sodium intake small variations are followed by large

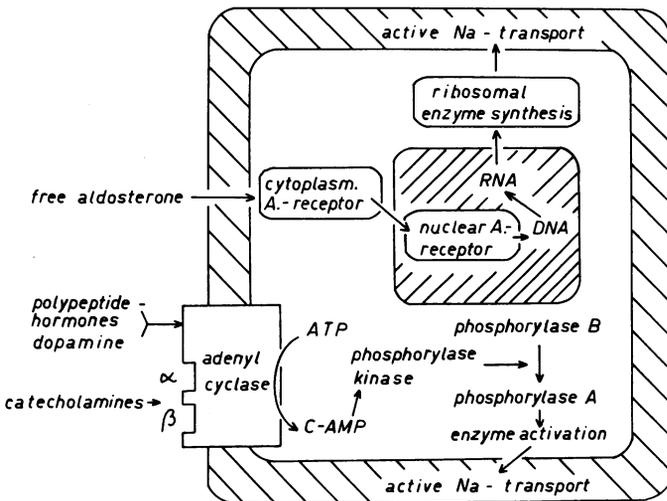


Figure 8. Mechanisms that may alter transmembrane PD. It is proposed that the rectal mucosa is equipped with both mechanisms, whereas the oral mucosa lacks mineralocorticoid receptors

variations of electrical asymmetry of epithelia involved in external sodium homeostasis. Especially, I envision the method also as a model of the distal tubules of the kidney.

This provides an experimental explanation for the high incidence of hypertension observed in communities with sodium intakes higher than 3–4 g salt per day, and may offer a biochemical argument in support of those workers who advocate a reduction of sodium intake for the prevention of hypertension on the basis of anthropological (Maddocks 1967, Prior *et al.* 1968, Oliver *et al.* 1975) or experimental studies (Ambard & Beaujard 1904, Kempner 1948, Dahl 1972, Tobian 1972, Freis 1976, Meneely & Battarbee 1976, Morgan *et al.* 1978). Opponents of the theory linking salt intake to hypertension (Corcoran *et al.* 1951, Chasis *et al.* 1949, *Lancet* 1975, Kincaid-Smith *et al.* 1975, Pickering 1978) may need to take into account the data in Figure 3 showing the levels of sodium intake with which adequate regulation of sodium balance through transepithelial transport processes may be expected. The low critical value of 3–4 g salt per day could also explain why modest salt restriction has little effect upon blood pressure (Corcoran *et al.* 1951). Additional mechanisms for sodium elimination through the kidney, such as pressure natriuresis (Molhuysen *et al.* 1950, Selkurt 1951, Borst & Borst-De Geus 1963), apparently prevent a detectable cross sodium overload, but by definition only work at some level of sodium retention, so that some degree of chronic sodium overload must remain. The haemodynamic consequences of (however small) a sodium overload would then, in the susceptible part of the population, initiate and perpetuate hypertension in a well documented way (Borst & Borst-De Geus 1963, Molhuysen *et al.* 1950, Coleman *et al.* 1972, Guyton *et al.* 1974). If a natriuretic hormone exists (De Wardener 1977), at least in this model it does not alter electrical asymmetry across sodium transporting epithelia at high levels of sodium intake.

Figure 5 demonstrates that hydrocortisone and even dexamethasone (which is supposed to be without any mineralocorticoid effect: Feldman *et al.* 1972, Wiederholt *et al.* 1966), in the absence of mineralocorticoids in adrenocortical insufficiency, also lead to a rise of subtraction PD. This rise is comparable in time course to that induced by aldosterone (*see* Skrabal *et al.* 1978), and can be observed even after blockade of cytoplasmic mineralocorticoid receptors by intravenous potassium canrenoate. If this is a potential difference generated by activation of sodium transport, and if it represents true 'illicit' mineralocorticoid action (Edelman 1977), the following explanation is proposed. If nuclear mineralocorticoid receptors are unoccupied due to lack of cytoplasmic aldosterone-mineralocorticoid receptor complexes, these could have sufficient high affinity for cytoplasmic glucocorticoid-glucocorticoid receptor complexes, which would then trigger off the mineralocorticoid effect. From the time course of glucocorticoid effect on transmucosal potential difference, which is comparable to that of aldosterone, it appears unlikely that this effect is mediated through inhibition of phosphodiesterase with consequent increase of levels of cyclic AMP (Manganiello & Vaughan 1972). As has been shown, hormones which act through cyclic AMP, such as catecholamines, act on transepithelial PD within a considerably shorter period (Koefoed-Johnson *et al.* 1952, Wood & Tomlinson 1974). That glucocorticoids indeed act on transmucosal PD only when aldosterone is absent is also shown in Figures 1 and 4. After tetracosactrin stimulation a marked rise not only of aldosterone but also of cortisol is observed (Figure 1). Despite the fact that plasma cortisol levels are three times as high in the tetracosactrin group as in the other groups of normal subjects shown in Figure 1, this group fits exactly into the overall regression between plasma aldosterone and subtraction PD, without any evidence of deviation from the regression line. In contrast, in patients with adrenocortical insufficiency the administration of hydrocortisone is followed by a marked rise of subtraction PD, with a consequent deviation from the regression line between plasma aldosterone and subtraction PD (Figure 4). In accordance with an 'illicit' mineralocorticoid effect of glucocorticoids in adrenocortical insufficiency are the data (but not their interpretation) of Dingman *et al.* (1958) and of Edelman (1968), and also a clinical observation of ours: a few patients with adrenocortical insufficiency have developed a tendency towards hypokalaemia and hypertension on glucocorticoid replacement alone.

Figure 4 also demonstrates that the groups of patients with untreated adrenocortical insufficiency and hyperaldosteronism fit exactly into the regression between plasma aldosterone and subtraction PD.

sterone and subtraction PD derived in normal subjects. Therefore in these conditions there is no evidence for any deviation of mineralocorticoid receptor function from normal. Patients with essential hypertension likewise have on average an undisturbed relation between plasma aldosterone and *in vivo* mineralocorticoid activity, regardless of their renin subgroup (Figure 4). In only 1 patient with normal renin hypertension and in only 3 with low hypertension, out of a total of 81 patients, was subtraction PD inappropriately high for the prevailing plasma aldosterone. One of these patients had low renin hypertension with suppressed plasma aldosterone and intermittently low serum potassium, and responded exceptionally well to treatment with spironolactone. In this patient deoxycorticosterone excess (Brown *et al.* 1972) or another mineralocorticoid syndrome appears possible. Since in only 4 out of 81 patients with essential hypertension (4.9%) was subtraction above the 95% confidence limits derived in normal subjects, and since in only 2 patients (2.5%) was it above 40 mV, whereas all patients with primary hyperaldosteronism after potassium repletion had a subtraction PD above this value, this measurement appears to be useful as a screening test for primary hyperaldosteronism in hypertensive patients. The data presented in Figure 4 obviate the need for a search for another unidentified mineralocorticoid excess syndrome in the great majority of patients with essential hypertension.

The measurement of subtraction PD adds another to the methods available for quantifying mineralocorticoid action (Dorfman *et al.* 1947, Crabbe 1961, Porter & Kimsey 1971, Feldman *et al.* 1972, Feldman & Funder 1973, Baxter *et al.* 1976, Lohmeier *et al.* 1976) and has the advantage of being applicable *in vivo* in human subjects. In combination with integrated steroid hormone measurements in plasma employing the exact time course of mineralocorticoid action, it could probably be used to study the *in vivo* interaction of steroids at the mineralocorticoid receptor. Since the first and the last steps of mineralocorticoid action are measured concurrently, the integrity of function of all intracellular metabolic steps can be assessed, which might be an advantage as compared to *in vitro* steroid receptor binding studies.

Summary

In vivo mineralocorticoid activity as measured by subtraction potential difference (Skrabal *et al.* 1978) and preceding plasma aldosterone were measured in 48 normal subjects on varying sodium intake, in 20 patients with primary or secondary hyperaldosteronism, in 13 patients with adrenocortical insufficiency and in 81 patients with untreated essential hypertension.

This study confirms that the measurement of subtraction PD could facilitate the immediate diagnosis of primary or secondary hyperaldosteronism and of adrenocortical insufficiency where laboratory facilities for aldosterone determinations are not available.

In normal subjects there was complete suppression of *in vivo* mineralocorticoid activity and supine plasma aldosterone on the usual sodium intake of between 100 and 300 mmol per day, with little further suppression at higher sodium intakes. From the function between sodium intake and electrical asymmetry of sodium transporting epithelia it appears that adequate regulation of sodium balance through transepithelial transport processes may be expected only at sodium intakes lower than 50 to 80 mmol per day (3 to 4 g sodium chloride). This would give a biochemical explanation for the high incidence of hypertension, rising with age, at the usual level of sodium intake of 6–18 g salt per day. At this level of sodium intake the most important mechanism controlling sodium balance does not function, and this is probably followed by some degree of sodium retention.

In essential hypertension with low, normal and raised plasma renin activity, the relation between plasma aldosterone and *in vivo* mineralocorticoid activity is generally undisturbed: in only 4 out of 81 patients (3 with low renin hypertension) was *in vivo* mineralocorticoid activity raised above the upper limit of normal and inappropriately high for the prevailing aldosterone level. In only these few patients does the search for another mineralocorticoid excess syndrome or a possible alteration of mineralocorticoid receptor function appear to be justified.

Only in the absence of mineralocorticoids in adrenocortical insufficiency, and then even after blockade of cytoplasmatic mineralocorticoid receptors with spironolactone, do glucocorticoids raise transmucosal electrical potential difference comparably in time course to

aldosterone. It needs to be shown whether this rise of transmucosal PD is caused by an increase of transmucosal sodium transport, and whether this represents true 'illicit' mineralocorticoid action.

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