SUBTRACTION POTENTIAL DIFFERENCE: IN-VIVO ASSAY FOR MINERALOCORTICOID ACTIVITY

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Summary Parallel fluctuations of potential difference (P.D.) across oral and rectal mucosa are probably related to the activity of autonomic nerves rather than adrenal steroids. Unlike rectal mucosa, oral mucosa does not respond to exogenous or endogenous aldosterone. Therefore subtraction of oral P.D. from rectal P.D. gives a closer indication of mineralocorticoid activity than does rectal P.D. alone. In normal subjects plasma-aldosterone correlated well with subtraction P.D. (r=0·74; P<0·001). A subtraction P.D. higher than 26 mV in subjects on a normal sodium intake indicated primary or secondary hyperaldosteronism; if the level was lower than 11 mV four hours after intramuscular injection of 0·25 mg tetracosactrin this suggested mineralocorticoid deficiency. Measurement of oral and rectal P.D. permits rapid and inexpensive diagnosis of aldosterone excess and deficiency. The method may also be used in study of the mineralocorticoid effect of other adrenal steroids: as assessed with this bioassay, the plasma 18-OH-deoxycorticosterone, which is raised in some patients with essential hypertension, lacked any in-vivo mineralocorticoid activity.

Introduction

A SIMPLE method for determining in-vivo activity of aldosterone would enable us to screen for primary hyperaldosteronism,[1] to use aldosterone antagonists more rationally in suspected secondary hyperaldosteronism, and to diagnose adrenocortical insufficiency rapidly without hormone measurements. Although electrical potential difference (P.D.) across the rectal mucosa increases consistently in response to aldosterone and other mineralocorticoids,[2] this measurement has been of limited value as a screening test for mineralocorticoid excess because in clinical practice there is no correlation between plasma or urinary aldosterone and rectal P.D.[3–6] Also we have observed consistently normal rectal P.D.s in patients with adrenocortical insufficiency and in patients treated effectively with spironolactone. In other words, mineralocorticoids are but one determinant: in rabbits rectal P.D. is known to be influenced by the autonomic nervous system.[7] One of us has observed a steroid-independent circadian variation of both rectal and oral mucosal potential difference; and this suggested that rectal potential difference could be corrected for non-steroidal influences by subtraction of the oral potential difference.[8]

Subjects and Methods

Normal Subjects

Oral and rectal potential differences and plasma-aldosterone levels were measured in 48 healthy volunteers (8 female). 11 subjects (all male) were studied after being equilibrated for one week on normal, high, and low sodium intakes. In 23 subjects measurements were made after intramuscular injection of 0·25 mg tetracosactrin ('Synacthen').

Patients

(i) The same measurements were made repeatedly on different days in 6 patients with primary and 10 patients with secondary hyperaldosteronism; all of them were studied on a normal sodium intake. 5 of the patients with primary hyperaldosteronism had adrenal adenomas which were located by the dexamethasone-modified scintiscan and later removed at operation: 1 had bilateral adrenal hyperplasia. The diagnosis of primary hyperaldosteronism was established by the finding of consistently suppressed plasma-renin activity and supine plasma-aldosterone levels above 20 ng/dl on a normal sodium intake. 10 patients had secondary hyperaldosteronism associated with cirrhosis of the liver (4), heart-failure (4), pseudo-Bartter's syndrome (1), and phæochromocytoma (1). In these patients the supine plasma-aldosterone level was above 20 ng/dl.

(ii) 9 patients with primary adrenocortical insufficiency were studied repeatedly on different days: 3 had had bilateral adrenalectomy for Cushing's disease and 5 had idiopathic Addison's disease. 1 patient had previously undergone bilateral adrenalectomy for malignant neuroblastoma and was still excreting large amounts of catecholamines from multiple metastases. Adrenocortical insufficiency was confirmed in each case by an 8 A.M. plasma-cortisol below 4 μg/dl, which did not rise after corticotropin, and by an undetectable plasma-aldosterone.

(iii) 5 patients with congestive heart-failure were assessed who had received between 100 and 200 mg spironolactone daily for at least a week before the study, but whose endocrine status was not assessed before the medication.

(iv) Studies were also done in 29 patients with untreated essential hypertension (W.H.O. stage 1 and 2).

Protocol

24-hour urinary sodium excretion was determined on the day before the study. Plasma aldosterone, 18-OH-deoxycorticosterone (18-OH-D.O.C.), and cortisol levels were measured at 10 A.M. after an overnight fast and 2 hours' supine bed rest. Rectal and oral electrical potential differences were measured 3 hours later at 1 P.M. This time was chosen since we have shown that the maximal response of rectal P.D. to aldosterone occurs 3 hours after its injection.

For the tetracosactrin test, 0·25 mg tetracosactrin was in-

jected intramuscularly at 8 A.M.; supine plasma-aldosterone was measured at 9 A.M. and potential differences at 12 noon.

Methods

Plasma-aldosterone was measured by a modification of the method of Ito et al.[9] and 18-OH-D.O.C. was measured as previously described.[10,11] Plasma-cortisol was measured by competitive protein-binding assay. Oral and rectal potential differences were measured with a specially designed battery-operated millivolt meter with an input resistance of 10^{13} ohm and digital read-out, the electrode being of silver chloride covered with a disposable plastic tip containing the NaCl junction ('Adrenosonde')*. First, 0·5 ml 0·9% saline was injected intracutaneously in the left forearm and the reference electrode was placed above the site of injection to eliminate skin potential difference.[12] The probe electrode with the disposable tip was then placed on the buccal mucosa about 3 cm from the left corner of the mouth and held there lightly while the oral P.D. was recorded. Rectal P.D. was measured by the method of Edmonds and Richards.[2] All P.D. measurements were corrected for the asymmetry of the electrodes.

Oral potential difference was not affected by dentures but varied by up to 5 mV between the left and right buccal mucosa. In order to lessen variation the reference and probe electrodes were always placed on the same side—left forearm and left buccal mucosa. Results are expressed as the (positive) polarity of the reference electrode.

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TABLE III—VALUES IN PATIENTS WITH PRIMARY ADRENOCORTICAL INSUFFICIENCY UNDER BASAL CONDITIONS AND AFTER TETRACOSACTRIN STIMULATION

—	No.	P.A.	H.C.	R.P.D.	O.P.D.	S.P.D.
Basal	16	<2·0	<4·0	23·0 ±10·4	33·6 ±11·6	−10·6 ± 8·6
Tetracosactrin	8	<2·0	<4·0	27·4 ±10·5	28·8 ± 5·6	− 1·4 ± 8·2

Results

Tables I–IV give values of plasma aldosterone, cortisol, and 18-OH-D.O.C. (in essential hypertension), the oral, rectal, and subtraction potential differences, 24-hour urinary sodium, and plasma sodium and potassium. The circadian variations of oral P.D. as measured during 25 cycles in 2 normal subjects (1 female) are shown in table v.

After injection of 0·5 mg aldosterone in a normal subject, rectal and subtraction P.D. began to rise after 60–90 minutes, reaching a maximum at 3 hours, but there was no increase in oral P.D. (fig. 1). Administration

TABLE I—VALUES IN NORMAL SUBJECTS ON NORMAL, HIGH, AND LOW SODIUM DIET AND AFTER TETRACOSACTRIN STIMULATION (MEANS ±1 S.D.)

—	No.	P.A.	H.C.	R.P.D.	O.P.D.	S.P.D.	24-Na	K	Na
Normal sodium	25	12·9 ± 4·8	11·8 ± 5·9	40·0 ± 7·3	28·5 ± 7·7	11·5 ± 8·7	209·7 ± 41·6	4·4 ±0·2	139·5 ± 1·9
High sodium	11	8·4 ± 6·7	8·5 ± 2·7	33·5 ±10·5	28·8 ± 5·2	4·7 ± 9·2	476·5 ±128·8	4·1 ±0·2	138·7 ± 1·2
Low sodium	10	106·6 ±103·1	12·7 ± 6·0	83·3 ±19·7	27·9 ± 7·5	54·5 ±16·8	13·5 ± 11·1	4·4 ±0·3	137·8 ± 1·8
Tetracosactrin	23	41·3 ± 18·5	30·7 ±10·7	58·3 ±15·4	22·3 ± 9·7	35·9 ±12·5	—	—	—

P.A.=plasma-aldosterone (ng/dl); H.C.=plasma-cortisol (μg/dl); R.P.D.=rectal potential difference (mV); O.P.D.=oral potential difference (mV); S.P.D.=subtraction potential difference (mV); 24-Na=24 h urinary sodium (mmol); K and Na, plasma potassium and sodium (mmol/l).

TABLE II—VALUES IN PATIENTS WITH PRIMARY AND SECONDARY HYPERALDOSTERONISM

—	No.	P.A.	R.P.D.	O.P.D.	S.P.D.	24-Na	K	Na
Primary hyperaldosteronism	14	56·4 ±33·5	68·3 ±13·9	22·3 ± 9·0	46·1 ±15·1	187·3 ± 67·3	3·1 ±0·7	142·7 ± 1·6
Secondary hyperaldosteronism	10	67·2 ±28·1	64·3 ±16·6	25·7 ±12·8	38·6 ± 7·3	—	4·8 ±1·4	130·0 ± 11·5

TABLE IV—VALUES IN PATIENTS WITH ESSENTIAL HYPERTENSION WITH NORMAL AND RAISED 18-OH-D.O.C. PLASMA LEVELS

18-OH-D.O.C.	No.	P.A.	H.C.	18-OH-D.O.C.	R.P.D.	O.P.D.	S.P.D.	ΔS.P.D.	24-Na	K	Na
Normal	19	8·3 ± 4·7	7·8 ± 4·8	80·3 ± 21·2	37·6 ±11·4	25·2 ± 9·0	12·4 ±12·9	3·3 ±14·5	195·1 ± 95·6	4·2 ±0·3	138·6 ± 3·8
Raised	10	13·6 ± 6·2	10·3 ± 5·8	195·9 ± 62·7	39·0 ±11·9	29·2 ± 8·0	9·8 ± 8·1	− 5·7 ± 8·0	197·3 ± 85·4	4·2 ±0·2	139·9 ± 1·8

ΔS.P.D.=deviation of S.P.D. from the regression line of plasma-aldosterone to S.P.D. derived in normal subjects (mV).

TABLE V—CIRCADIAN VARIATION OF ORAL POTENTIAL DIFFERENCE AS MEASURED DURING 25 CYCLES IN 2 NORMAL SUBJECTS

—	8 A.M.	8.30 A.M.	9 A.M.	12 noon	4 P.M.	8 P.M.	12 midnight
Oral P.D. (mV, mean ±1 S.D.)	22·5 ± 5·3	35·0 ± 5·3	40·0 ± 4·3	34·7 ± 6·3	29·8 ± 5·7	27·0 ± 5·2	24·0 ± 5·7

of spironolactone, 400 mg intravenously, to a patient with primary hyperaldosteronism lowered rectal P.D. and subtraction P.D. but not oral P.D., within 1 hour (fig. 1). In contrast, fig. 2 shows parallel changes of rectal and oral P.D. in a bilaterally adrenalectomised patient with multiple metastases from a phæochromocytoma during a hypertensive crisis. The parallelism suggests that catecholamines can affect both rectal and oral P.D.

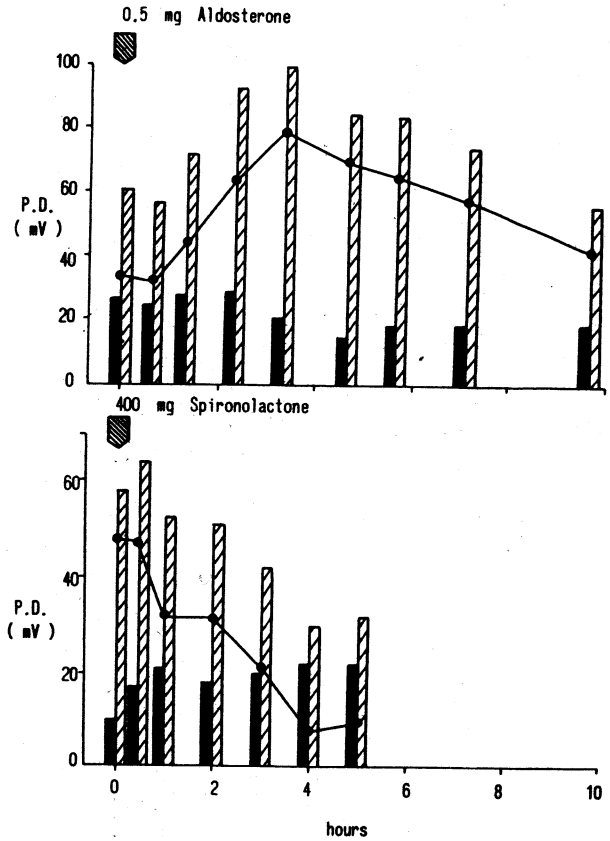

Fig. 1—Changes of oral (black bars), rectal (striped bars), and subtraction P.D.s (line) after intravenous aldosterone in normal subject (top) and after intravenous spironolactone in a patient with aldosterone-producing adrenal adenoma (bottom).

In normal subjects on varying sodium intake there was a poor but significant correlation between prevailing plasma aldosterone (P.A.) and rectal P.D. (r=0·29, $P<0·05$). The regression equation was:

Rectal P.D. (mV)=4·38 log P.A. (ng/dl)+5·58

However, the correlation between plasma-aldosterone and subtraction P.D. was highly significant (r=0·74, $P<0·001$) (fig. 3). The regression equation was:

Subtraction P.D. (mV)=36·4 log P.A. (ng/dl)−23·8

Fig. 4 shows rectal P.D. values in patients with primary and secondary hyperaldosteronism compared with those in patients with adrenocortical insufficiency or on treatment with spironolactone. There is clear overlap between the groups, and between the groups and the normal range. The two groups can be readily separated by measurement of subtraction P.D. (fig. 4). In addition, normal subjects can be readily distinguished from pa-

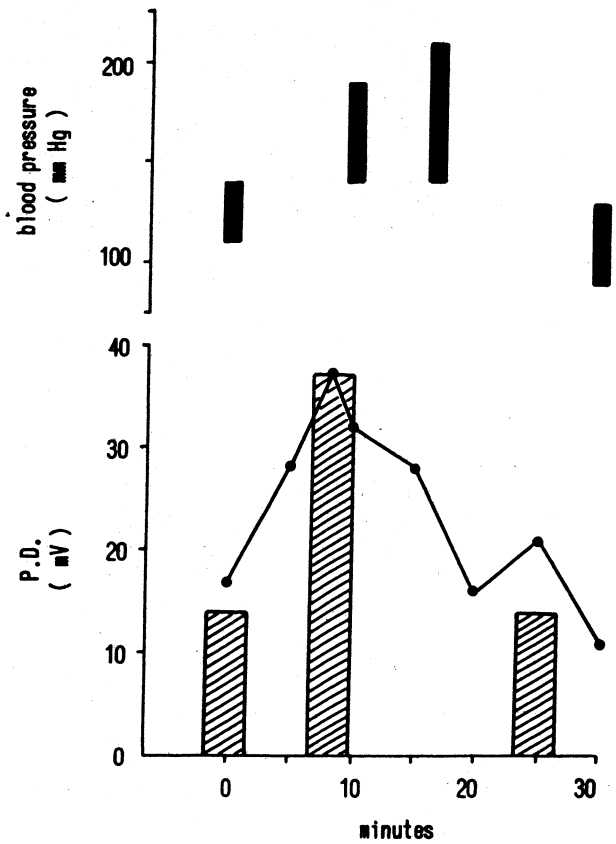

Fig. 2—Parallel changes of rectal (striped bars) and oral (line) P.D.s in a bilaterally adrenalectomised patient with metastases from a phæochromocytoma during a hypertensive crisis.

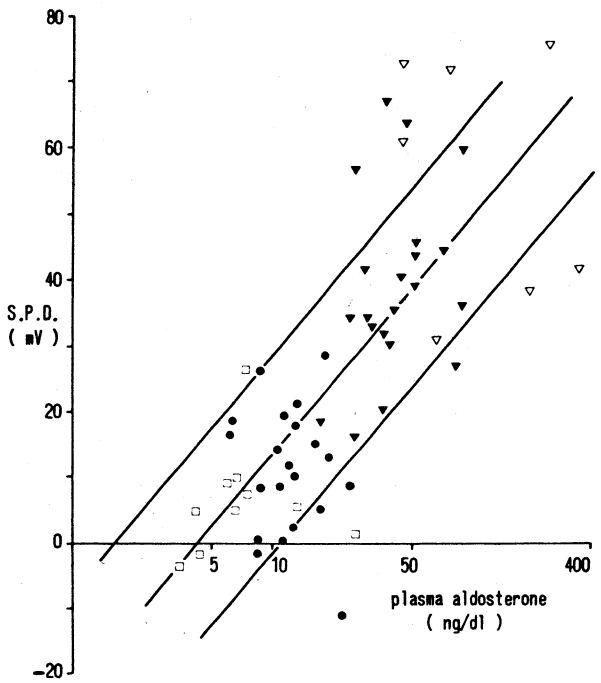

Fig. 3—Correlation between plasma-aldosterone and subtraction P.D.s in normal subjects on low (▽), normal (●), and high (□) sodium intake and corticotropin injection (▼).

tients with adrenocortical insufficiency by measurement of subtraction P.D. after tetracosactrin. In normal subjects tetracosactrin administration results in a rise in subtraction P.D. (fig. 5 top) which is not seen in adrenocortical insufficiency (fig. 5 bottom).

The relation between plasma-aldosterone, plasma-18-OH-D.O.C., and subtraction P.D. in patients with essential hypertension is shown in fig. 6. Patients with raised 18-OH-D.O.C. (above 120 pg/ml) had significantly lower mineralocorticoid activity ($P<0.05$) than those with normal 18-OH-D.O.C.

Discussion

Transmucosal potential difference is altered by several factors including histamine,[14] acetylcholine,[13] and catecholamines.[7,15] Specialised tissues such as the distal tubules of the kidney and colonic mucosa respond not only to these stimuli but also to mineralocorticoids, which raise transmucosal P.D. Therefore it is not surpris-

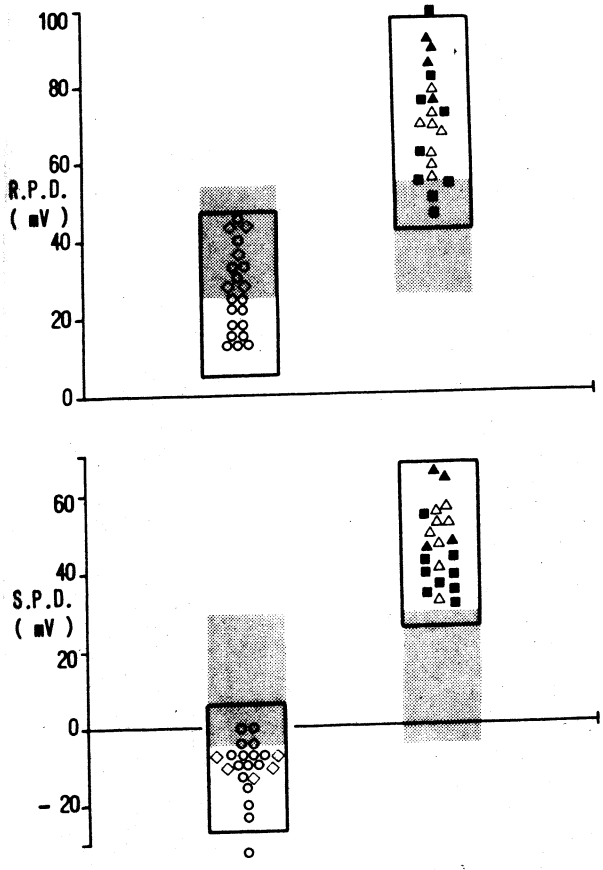

Fig. 4

Fig. 4—Rectal P.D. (top) and subtraction P.D. (bottom) in patients with adrenocortical insufficiency (○) or on treatment with spironolactone (◊) (left), compared with patients with primary hyperaldosteronism before (△) and after (▲) potassium repletion or secondary hyperaldosteronism (■) (right).

Shaded area corresponds to 95% confidence limits in normal subjects.

Fig. 5—Top: subtraction P.D. in normal subjects on normal sodium intake (left) and after corticotropin injection (right). Bottom: subtraction P.D. in patients with adrenocortical insufficiency after corticotropin stimulation (○) (right).

Subtraction P.D. in primary and secondary (■) hyperaldosteronism before (△) and after (▲) potassium repletion (left).

Fig. 6—Subtraction P.D. in patients with essential hypertension with normal (▽) and raised (▲) plasma 18-OH-D.O.C., in relation to plasma-aldosterone.

Regression line and confidence limits derived from normal subjects.

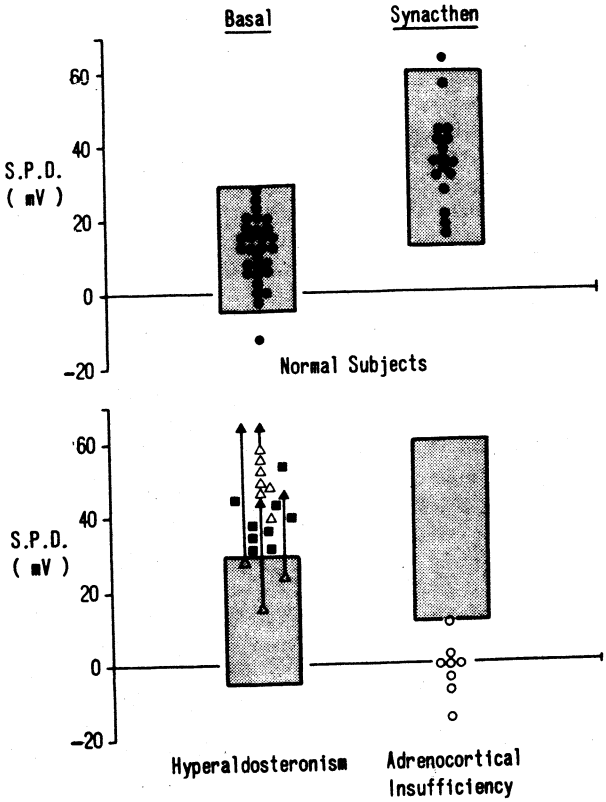

Fig. 5

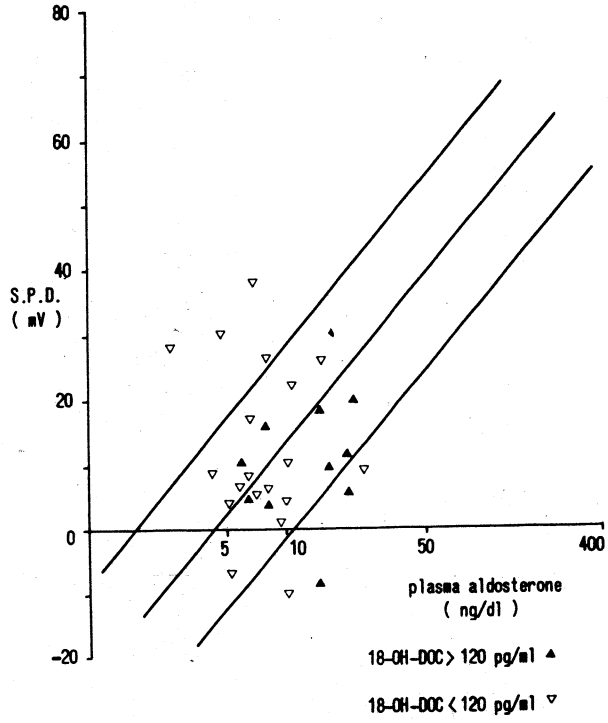

Fig. 6

ing that rectal potential difference alone is a poor index of mineralocorticoid activity. This investigation shows that, although both rectal and oral mucosa respond to the same non-steroidal influences, only rectal mucosa responds to mineralocorticoids. Therefore subtraction of oral from rectal potential difference eliminates the steroid-independent biological variation of rectal potential difference, and improves the correlation coefficient between plasma-aldosterone and transmucosal P.D. from $r=0{\cdot}29$, $P<0{\cdot}05$ to $r=0{\cdot}74$, $P<0{\cdot}001$. With rectal P.D. there is an overlap between values in aldosterone excess and those in aldosterone deficiency. Subtraction P.D. separates the two groups by 20 mV (fig. 4).

In normal subjects on various sodium intakes there was a close correlation between prevailing plasma-aldosterone and subtraction P.D. Probably this correlation could be further improved by the use of integrated plasma-aldosterone measurements, employing the exact time-course of mineralocorticoid agonist action shown in fig. 1.

As shown in fig. 5, subtraction P.D. permits diagnosis of aldosterone excess and, after the injection of corticotropin, of aldosterone deficiency in adrenocortical insufficiency. In 3 patients with hyperaldosteronism subtraction P.D.s were normal in the presence of hypokalæmia; after two days' oral potassium supplements (80 mmol per day) the subtraction P.D. rose to high levels. Therefore, when the test is used to detect mineralocorticoid excess, plasma-potassium should be measured.

This simple and inexpensive measurement may serve as an effective screening test for primary and secondary hyperaldosteronism and also for adrenocortical (aldosterone) deficiency. Furthermore, it may be useful in investigation of in-vivo mineralocorticoid effects of steroids other than aldosterone. Such an approach may prove better than the current indirect assays in animals[16,17] and isolated cell preparations[18–20] since mineralocorticoid activity can be evaluated in relation to that of the most potent known mineralocorticoid, aldosterone. An example is shown in fig. 6: 18-OH-D.O.C., which has been implicated in human essential hypertension,[21] was assessed for in-vivo mineralocorticoid action. If this compound has an important mineralocorticoid effect then subtraction P.D. in patients with raised plasma-18-OH-D.O.C. should be inappropriately high for the prevailing plasma aldosterone. As can be seen, such patients actually had lower subtraction P.D.s; hence, the steroid is unlikely to have in-vivo mineralocorticoid action. If this steroid is concerned in the genesis of essential hypertension, then it does not seem to act through sodium retention.

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