Absorption and Secretion of Water and Electrolytes by the Intact Colon in a Patient with Primary Aldosteronism*

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Potassium deficiency is a prominent feature of primary aldosteronism. The excessive losses of potassium in the urine have been well documented, but less well known and understood is the altered excretion of electrolytes in the faeces.

In a patient with primary aldosteronism Wrong *et al.* (1961) observed that in faecal dialysate the concentration of potassium was increased and the concentration of sodium was reduced, with a resultant decrease in the sodium : potassium ratio. These observations were confirmed in a further three patients, in one of whom the concentration of faecal electrolytes returned to normal after the removal of an adrenal adenoma (Wrong and Metcalfe-Gibson, 1965). It is likely, but by no means established, that these alterations in the ionic composition of the faeces in primary aldosteronism were brought about by the action of aldosterone on the intestinal handling of electrolytes.

Experiments in animals and several indirect studies in man have suggested that adrenal mineralocorticoids enhance the absorption of sodium and the secretion of potassium by the intestine (for review, see Shields, 1964, 1966a). Only recently, however, has it been possible to study *directly* the absorptive ability of the colon in conscious patients. In two patients with a segment of transverse colon isolated for several weeks from the rest of the gastrointestinal tract we (Shields *et al.*, 1966) have shown that potassium was secreted more rapidly by the colon when aldosterone was infused intravenously. Using the technique of colonic intubation and perfusion (Levitan *et al.*, 1962), Levitan and Ingelfinger (1965) and Levitan (1967) found that the colonic absorption of sodium was increased by aldosterone and 9α-fluorohydrocortisone (fludrocortisone).

We have developed the technique of Levitan *et al.* (1962) to allow the simultaneous measurement of the rates at which not only water but also sodium and potassium are exchanged in both directions across the colonic mucosa (Shields and Miles, 1965b ; Shields, 1966b).

In this paper we report a study of the net transport and unidirectional fluxes of sodium, potassium, and water by the intact colon of a patient with primary aldosteronism before and after the removal of a tumour of the adrenal cortex.

The Patient

At a routine examination for life assurance in 1959 the patient, who had no symptoms, was found to have an elevated blood pressure (200/100 mm. Hg). In 1963 he began to experience undue breathlessness on exertion and noticed that he passed more urine at night than during the day. The following year he suffered cramping pains in his limbs, which seemed to be rather weak. He was habitually costive but did not take purgatives ; his stool was of normal colour and consistency. He had not received digitalis or its analogues.

On clinical examination he was seen to be a healthy-looking man of 58 without any external evidence of endocrinopathy. His elevated blood pressure was confirmed. The heart was not enlarged, but in the optic fundi moderate hypertensive changes were observed. The electrocardiogram showed a marked depression of the S–T segment.

Investigations

(1) *Fluid and Electrolyte Metabolism.*—The concentration of potassium in the serum was 1.8 mEq/litre, and that of sodium 149 mEq/l. With a daily diet containing 150 mEq of potassium and 100 mEq of sodium the serum potassium rose to 3.0 mEq/l. but then fell to 2.4 mEq/l. When a similar diet, but providing less than 10 mEq of sodium in 24 hours, was given, the serum potassium rose to 4.2 mEq/l., the daily output of potassium in the urine fell from 50 to 10 mEq/24 hours, and the urinary sodium from 100 to 14 mEq/24 hours. On a diet providing 20 mEq of potassium daily, the loss of potassium in the urine remained at about 40 mEq/24 hours. The mean daily loss of potassium in faeces collected over three days was 25 mEq the normal range with the method of Crawford and Brooke (1957) is 5–10 mEq/24 hours). The mean daily faecal excretion of sodium was 3.5 mEq (normal range 1–8 mEq/24 hours). The diurnal rhythm of potassium excretion in the urine was reversed: the maximum excretion occurred at night, compared with a maximum excretion at noon in control subjects (Elmslie *et al.*, 1964). The plasma volume, determined by isotope dilution with radioiodinated human serum albumin, was 3,900 ml., the value for a healthy male patient of similar height and weight being 2,900 ml. (Nadler *et al.*, 1962). The serum calcium was 5.1 mEq/l.

(2) *Acid-base Status.*—Examination of a sample of arterial blood by the micro-Astrup technique (Astrup *et al.*, 1960) showed non-respiratory alkalosis—*p*H 7.54, P_{CO_2} 48 mm. Hg, and standard bicarbonate 38 mEq/l.

(3) *Steroid Investigations* (Dr. J. K. Grant).—In urine, collected over four days, the mean excretion rate of 17-oxysteroids (Medical Research Council, 1963 ; James and Caie, 1964) was 12 mg./24 hours (normal range for this age and sex: 4–15 mg./24 hours), and of 17-hydroxycorticosteroids (Few, 1961) 17 mg./24 hours (normal range: 4.5–18.5 mg./24 hours). Though the urinary excretion rate of aldosterone was within the normal range (6.8 μg./24 hours (Brooks, 1960)), the rate of its secretion measured on two separate occasions, with tritiated aldosterone 3.0 μCi (1,2-^{3}H-aldosterone) was greatly increased, being 524 μg. initially and on a subsequent occasion 2.4, 3.4, and 2.6 mg./24 hours (normal 25–500 μg./24 hours). The rates of secretion of cortisol and corticosterone (with 0.5 μCi 4-^{14}C-cortisol and 1 μCi 1,2-^{3}H-corticosterone (Cope and Black, 1958) were, respectively, 26.4 and 11.3 mg./24 hours, the latter rate being above the normal range of 1.3–4 mg./24 hours.

(4) *Renal Investigations.*—The measurement of the diurnal output of urine over 72 hours showed that 1,300 ml. was excreted at night (6 p.m. to 6 a.m.), and 600 ml. during the day. The reaction of the urine, usually *p*H 7, fell to *p*H 5.8 after an acid load (ammonium chloride, 0.1 g./kg. body weight). There was a trace of albumin in the urine, microscopical examination of which did not show any abnormality. The blood urea was 50 mg./100 ml. ; serum and urine osmolalities were respectively 303 and 530 Osm/kg. water.

(5) *Radiological Investigations* (Dr. R. G. Pitman).—Intravenous pyelography, perirenal insufflation with oxygen, and aortography did not reveal any abnormality. The appearances of an *x*-ray film of the chest were within normal limits.

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Operation

The operation was performed by Professor A. P. M. Forrest. The adrenal glands were approached through the abdomen. A discrete mass, diameter 2.5 cm., was easily palpated in the right adrenal gland. Both the gland and its contained tumour were removed. The left adrenal gland was rather dark in appearance and had a number of small purplish specks scattered over its surface. A biopsy was taken from the right kidney.

Pathology

Right Adrenal Gland (Professor T. Symington).—The specimen, which weighed 9.8 g., contained a well-encapsulated yellow-orange tumour composed predominantly of cells similar morphologically to those of the zona fasciculata of the normal adrenal cortex. They were filled with lipid and showed little pleomorphism. The nuclei were small and dense. Mitoses were inconspicuous. A few islands were noted in which the cells of the tumour bore a distinct morphological resemblance to zona glomerulosa, and there were occasional foci with tumour cells similar to the compact cells of the zona reticularis. The last two types of cells composed only a few areas of the lesion. The entire tumour was divided into alveoli of varying sizes by prominent fibrovascular trabeculae derived from a well-formed capsule. The associated normal adrenal gland showed focal lipid depletion consistent with the reaction to stress. The zona glomerulosa was focally hyperplastic and projected as tongue-like areas into the substance of the normal cortex to varying depths, some almost reaching the zona reticularis. The arteries and arterioles within the tumour and in its capsule showed prominent hypertensive changes. The appearances of the growth were those of a benign tumour associated with primary aldosteronism. There was focal hyperplasia of the zona glomerulosa of the attached gland and a similar focal hyperplasia in the biopsy of the contralateral adrenal gland.

Renal Biopsy (Dr. W. Jones Williams).—The features were those of non-specific chronic pyelonephritis with benign hypertension.

Subsequent Progress

Apart from a small pulmonary infarct on the fourteenth day after operation, the postoperative course was uneventful.

The patient remained well and free of symptoms. His arterial blood pressure was still high (190/100 mm. Hg) but the serum potassium remained within normal limits (4.2 mEq/litre) without medication or potassium supplements.

Control Subjects

The control subjects were eight adult males aged 20–65 years. Four were medical students and the rest otherwise healthy patients admitted for the operative repair of inguinal hernia. Permission was obtained from the patients and from the control subjects according to the proposals laid down by the Medical Research Council (1964).

Measurement of Rates of Colonic Transport of Sodium, Potassium, and Water

In the patient the transport of sodium, potassium, and water across the colonic mucosa was studied one week before and three months after the removal of the tumour in the right adrenal cortex. The preoperative studies were performed after correction of the potassium deficiency. For six days preceding the absorption studies, however, the patient was on the usual ward diet containing 150 mEq of sodium and 75 mEq of potassium per 24 hours and was not receiving any potassium supplements or other medication.

When investigation of the colon was repeated three months after the removal of the adrenocortical tumour the patient was well, was eating an unrestricted diet, and did not display any evidence of potassium deficiency. The control subjects were eating an unrestricted diet (containing plenty of salt) until 12 hours before the absorption studies.

The details of the method have already been described (Shields, 1966b). Briefly, the patient and the volunteers swallowed a long polyvinyl tube, until the tip of the tube lay in the caecum.

All subjects were fasted for 12 hours before colonic perfusion. A study was begun by washing the colonic lumen with Tyrode's solution, introduced through the oral tube and collected at the anus through a wide-bore tube which had been inserted into the rectum until its tip lay 10 cm. from the anal margin. Preliminary washing was continued until the effluent from the rectal tube was free of faeces and other solid material; usually one to two hours were required.

After an interval of two hours, test solution was perfused through the colon at 25 ml./minute by a constant infusion pump. The effluent from the rectal tube was collected in 10-minute periods. Usually 1 to 2 litres of test solution were perfused for 60 to 90 minutes. The specimens collected during the first 20 to 30 minutes of perfusion were discarded.

The test solution was freshly prepared, modified Tyrode's solution (Code and McIntire, 1956) containing ^{24}Na (2 μCi/l. solution), ^{42}K (4 μCi/l. solution), deuterium oxide (1% v/v), and, as a non-absorbed marker, poylethylene glycol of molecular weight 4,000 (1% w/v).

The activities of radioactive sodium and potassium in the test solution and in the aliquots of the effluent were determined by simultaneous counting in a Geiger–Müller liquid counter and in a thallium-activated sodium iodide scintillation counter (Veall and Vetter, 1958; Shields *et al.*, 1966). The chemical concentrations of sodium and potassium were determined by flame photometry, and the concentration of deuterium oxide in an infrared spectrometer (Perkin-Elmer 337) by a modification (Shields *et al.*, 1966) of the method of Berglund-Larsson (1956). The concentrations of polyethylene glycol were estimated by the method of Hydén (1955). The errors of the various determinations have been given in detail (Shields *et al.*, 1966).

Terminology.—Sodium, potassium, and water are simultaneously exchanged in both directions across the colonic mucosa; for each substance the difference between the rates of unidirectional movement is referred to as its net transport. The term "absorption" is restricted to net transport where more of the substance leaves the intestinal lumen and enters the body than moves in the opposite direction; by convention the rate of absorption is preceded by a plus sign. When the substance enters the intestinal lumen more rapidly than it leaves, net transport is referred to as *secretion,* whose rate is preceded by a minus sign.

Calculations.—The rates of *net* transport were calculated from the formulae proposed by Levitan *et al.* (1962). The rates of unidirectional movement of sodium, potassium, and water were calculated with the use of previously described formulae (Shields, 1966b).

Results

Rates of Intestinal Transport

Net Transport.—From the mean rates of net transport of sodium, potassium, and water calculated from 27 test periods in eight control subjects it is evident that the normal human colon absorbed sodium and water and secreted potassium under the conditions of this study. In the patient with primary aldosteronism the rates of potassium secretion into the colonic lumen were considerably increased before operation and returned to within the range of the control subjects three months after the removal of the tumour (Table I). The rates of absorption of sodium and water before and after the operation were within the range observed in control subjects.

TABLE I.—*Rates of Movement of Sodium, Potassium, and Water Across the Mucosa of Intact Colon in Patient with Primary Aldosteronism Before and After Removal of Tumour, Compared with those in Eight Control Subjects*

	Sodium (mEq./min.)			Potassium (mEq./min.)			Water (ml./min.)		
	Net	Out of Lumen	Into Lumen	Net	Out of Lumen	Into Lumen	Net	Out of Lumen	Into Lumen
Patient:									
Before operation	+0·46	0·75	0·29	−0·137	0·021	0·158	+1·7	—	—
	+0·50	0·70	0·20	−0·164	0·014	0·178	+2·2	—	—
	+0·17	1·27	1·10	−0·111	0·025	0·136	+1·1	—	—
	+0·13	0·53	0·40	−0·050	0·011	0·061	+0·1	—	—
After operation	+0·30	0·90	0·60	−0·020	0·013	0·033	+0·3	21·3	20·0
	+0·56	0·80	0·24	−0·006	0·018	0·024	+1·8	22·0	21·8
	+0·79	1·08	0·29	−0·005	0·026	0·031	+4·7	27·5	22·8
Controls (8):									
Mean	+0·39	0·68	0·31	−0·024	0·017	0·041	+1·9	21·5	19·6
Standard deviation	0·20	0·16	0·16	0·012	0·005	0·013	1·2	3·4	4·3

Unidirectional Fluxes.—In the patient before operation potassium moved into colonic lumen at a significantly greater rate than in the control subjects. After operation the rate of movement of potassium in this direction returned to within the range observed in the control subjects (Table I). The rates of unidirectional flux of sodium before and after operation, and those of water after operation, were within the range of those determined in control subjects.

Concentration of Electrolytes in the Perfused Test Solution

When the test solution was perfused through the colon of control subjects there was a slight but significant decrease in the concentration of sodium and a small but significant increase in the concentration of potassium. In the patient before operation the concentration of potassium was increased twofold and threefold. After operation the increase in the concentration of potassium was no greater than that found in the control subjects (Table II).

TABLE II.—*Changes in Concentration of Electrolytes in Perfusion Solution in Patient with Primary Aldosteronism Before and After Removal of Tumour, Compared with Those in Eight Control Subjects*

Subject	Solution	Potassium (mEq./l.)	Sodium (mEq./l.)
Patient:			
	Infused solution	3·8	152
Before operation	Rectal perfusate	10·5	157
		11·0	147
		9·9	152
		8·0	142
	Infused solution	4·0	146
After operation	Rectal perfusate	5·5	140
		4·8	137
		4·7	140
		4·8	136
Control subjects (8)	Infused solution (mean ± S.E.)	4·2 ± 0·1	156 ± 1·2
	Rectal perfusate	5·5 ± 0·2	147 ± 2·2
	Mean difference ± S.E. of mean	1·3 ± 0·2	9 ± 2·5
	t	6·0	3·4
	P	< 0·001	< 0·01

No alteration in the concentration of sodium in the perfused solution was observed in the patient before or after operation.

Discussion

The case described in this report presented most of the clinical and biochemical features of primary aldosteronism, a diagnosis which was subsequently confirmed by the removal of a histologically typical tumour of the adrenal cortex (Neville and Symington, 1966). In the patient the striking abnormality in the colonic handling of water and electrolytes was a marked enhancement of the movement of potassium into the lumen—at a rate of four to five times that observed in control subjects. As a result the daily faecal loss of potassium was greatly increased and the concentration of potassium in an isotonic solution perfused through the colon was more than doubled. With the removal of the tumour the secretion of potassium by the colon returned to normal.

Similar changes in the colonic transport of potassium have been observed both in man and in the dog during the intravenous infusion of aldosterone (Shields *et al.*, 1966). Moreover, the intestinal action of aldosterone could be blocked in the dog by the previous administration of spironolactone (Elmslie *et al.*, 1966). Also, in dogs, the colonic secretion of potassium was increased by deoxycortone (Berger *et al.*, 1960) and during salt depletion (Clarke *et al.*, 1967). There seems good evidence, then, confirmed by the present study, that adrenal mineralocorticoids act on the colon to augment its secretion of potassium.

On the other hand, Levitan and Ingelfinger (1965) and Levitan (1967), using a technique similar to the one described in this paper, did not find any change in potassium secretion by the colon when aldosterone and fludrocortisone were given to healthy subjects. Two explanations may be advanced to explain this difference. Firstly, Levitan and Ingelfinger (1965) injected 1 mg. of aldosterone rapidly. We (Shields *et al.*, 1966) have noted that the effect of a single rapid injection of aldosterone on potassium secretion was slight and transient and that marked increase in the movement of potassium into the colonic lumen occurred only when aldosterone was given by continuous infusion, even though the total dose did not exceed 300 μg. Secondly, as Levitan (1967) pointed out, the test solution which they had used was not ideal for evaluating potassium secretion.

The absence of any change in the colonic absorption of sodium in primary aldosteronism is noteworthy and requires comment. Patients with primary aldosteronism usually become adapted to the renal sodium-retaining activity of aldosterone, a response probably related to the "escape" from sodium retention during the prolonged administration of aldosterone (August *et al.*, 1958). A similar adaptation to the action of aldosterone on the absorption of sodium by the colon may be postulated. That this explanation is not entirely satisfactory is suggested by previous reports of low concentration of sodium in faeces and faecal dialysates in primary aldosteronism (Milne *et al.*, 1957 ; Wrong and Metcalfe-Gibson, 1965).

However, the daily excretion of sodium in the faeces is low —from about 3 to 10 mEq/day. Consequently further reduction in the faecal loss of sodium because of increased colonic absorption will barely disturb electrolyte homoeostasis. In contrast, the enhanced colonic secretion of potassium in response to aldosterone and the subsequent loss of this ion in increasing amounts in the faeces can be of clinical importance. In health, the faecal excretion of potassium represents 20% of the total loss of potassium from the body by all routes. Though the urinary loss of potassium is greatly increased in aldosteronism and during corticosteroid therapy, the faecal excretion of potassium may also be increased and the extent of potassium deficiency may be seriously underestimated if only the electrolyte content of the urine is measured. Thus, for example, in

the present patient the daily output of potassium in the faeces represented one-half of the total sensible loss of potassium from the body.

Summary

In a patient with primary hyperaldosteronism the rates of net transport and of unidirectional fluxes of sodium, potassium, and water in the intact colon were measured before and after removal of the adrenocortical tumour, by perfusing the colon with an isotopically labelled test solution introduced into the caecum through a tube passed by mouth. The results in this patient were compared with those in eight control subjects. Before removal of the aldosterone-producing tumour the colon of the patient secreted potassium at four to five times the rate in control subjects. The unidirectional flux of potassium into the colonic lumen was greatly enhanced and the daily loss of potassium in the faeces increased. The rates of potassium transport returned to within the range observed in control subjects after the removal of the tumour.

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Preliminary Communications

Direct Evidence for Presence of Ph^1 Chromosome in Erythroid Cells

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Strong but indirect evidence (Tough *et al.*, 1963; Trujillo and Ohno, 1963; Whang *et al.*, 1963) has suggested that in chronic granulocytic leukaemia erythroid as well as myeloid precursors contain the Ph^1 chromosome. Whang *et al.* (1963), for instance, found a 90–100% incidence of the Ph^1 chromosome in bone marrow cells of 13 patients in drug-induced clinical remission known to have a moderately high proportion of dividing normoblasts. Recently, Clein and Flemans (1966) obtained more direct evidence by combining standard cytogenetic techniques with Perls's Prussian blue stain for iron. They demonstrated siderotic granules in the cytoplasm surrounding some Ph^1-positive metaphase plates derived from a patient with blastic crisis of chronic granulocytic leukaemia in whose marrow a prominent sideroblastic element was also present.

To obtain further direct evidence for the occurrence of the Ph^1 chromosome in erythroblasts, a bone marrow aspirate from a patient with chronic granulocytic leukaemia in drug-induced clinical remission was cultured with ^{59}Fe and ^{55}Fe (Suit *et al.*, 1957) and the Ph^1 chromosome was found in all erythroid metaphase plates examined.

Materials and Methods

The patient was a 56-year-old man who presented with chronic granulocytic leukaemia in January 1967. He was subsequently treated with busulphan, and was in good clinical remission at the time of this study in July 1967, when a sternal bone marrow aspirate contained 178 normoblasts per 1,000 nucleated cells as determined by May–Grünwald–Giemsa staining.

Materials.—The culture medium consisted of 12 ml. of Medium 199, an additional 0.1 ml. of Solution D-G-P for Medium 199 (Commonwealth Serum Laboratories, Melbourne); 0.01 ml. of heparin, 0.02 μg. of demecolcine, and 8 ml. of AB serum. The isotopes used were ^{59}Fe and ^{55}Fe, obtained as ferric chloride in 0.01 N HCl from the Radiochemical Centre, Amersham. Kodak AR-10 stripping film was used for the autoradiography.

Methods.—The bone marrow aspirate was added to the culture medium and the resulting cell suspension divided into 10-ml. aliquots, to which were added 200 μCi of ^{59}Fe or 200 μCi of ^{55}Fe. Cultures were incubated at 37° C. for 12 hours, which was found to be the optimal time for the uptake of isotope by actively dividing normoblasts. Both samples were then treated as follows. The cells were washed with Hanks's saline, and smears made to determine ^{59}Fe and ^{55}Fe uptake by the normoblasts. The remainder of each sample was then incubated for 10 minutes at 37° C. with 0.075M KCl hypotonic solution, after which the cells were fixed with a freshly prepared formaldehyde–acetic-acid–methanol fixative for 10 minutes. They were then resuspended in fresh fixative and chromosome preparations were made immediately by the "flaming" technique.

Autoradiograms of the smear and chromosome preparations were exposed for 21 days at 4° C., and developed for five minutes at 20° C. with Kodak D-19 developer. The smears were stained with May–Grünwald–Giemsa, and the chromo-