

Spirolactone-Induced Inhibition of Aldosterone Biosynthesis in Primary Aldosteronism: Morphological and Functional Studies

J. W. Conn and D. L. Hinerman

Twenty-five patients harboring aldosterone-producing adenomas were treated with spironolactone for 2-170 days immediately preoperatively. In the early period of administration of the drug (up to 27 days), plasma and urinary aldosterone decreased sharply while plasma renin activity (PRA) and serum potassium were rising. During this period of time, spironolactone bodies (SB), which form exclusively in cells actively producing aldosterone, were forming rapidly in the tumor cells but not in the inactive glomerulosa cells proper. The SB appear to be a morphological expression of a block in aldosterone biosynthesis. Since SB do not occur in normal fasciculata cells, which, like glomerulosa cells, also synthesize corticosterone, it is concluded that spironolactone inhibition of aldosterone biosynthesis occurs between corticosterone and aldosterone. Recent studies *in vitro* by others have suggested that the inhibition occurs at the corticosterone-methyl oxidase step, 1 (Ulick's nomenclature). The great diuresis of sodium and retention of po-

tassium resulting from continued administration of the drug sharply activates aldosterone stimulatory factors. Aldosterone production may return to baseline levels in several weeks but it is inappropriately low in relation to the levels of PRA and serum potassium. With the further passage of time (average 4-6 wk), aldosterone production may increase 50%-100% above baseline levels, suggesting that the block has disappeared or is receding. At this time SB are diminishing in number and by 170 days of the drug they have virtually disappeared. We have hypothesized, among other possibilities, that recovery of the ability to convert corticosterone to aldosterone occurs by virtue of a mechanism activated by sodium deficiency, independent of angiotensin, which stimulates step 1 of the corticosterone-methyl oxidase system. As the block in the final step(s) of the biosynthetic pathway recedes, the existing elevated levels of angiotensin become much more effective in stimulating the production of aldosterone.

SPIRONOLACTONE antagonizes the sodium-retaining effects of aldosterone by competing with the hormone at its cellular receptor sites (kidney, sweat glands, lacrimal glands, salivary glands, and intestine). From a physiologic point of view, its major influence is via the kidney, where it inhibits aldosterone-mediated sodium reabsorption and aldosterone-mediated potassium secretion. The sodium diuresis thus induced results in a contraction of intravascular volume and stimulation of renin release. This effect should result in increased production of aldosterone. Stimulation of the renin-angiotensin-

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aldosterone system is expected from any agent which produces a loss of sodium and water from the body. However, we have previously reported in abstract form¹ that spironolactone, in addition to being a peripheral aldosterone antagonist, has a direct inhibiting effect on aldosterone production in the cells of aldosterone-producing adenomas.

Five years after the 17-spirolactone steroids and spironolactone came into clinical use, Janigan,² studying autopsy material, described the occurrence of eosinophilic, laminated bodies within the cytoplasm of the cells of the adrenal glomerulosa zone in patients who, during life, had received these compounds for the management of various forms of edema. People with similar disorders, treated with other forms of diuretic therapy, failed to exhibit such bodies in their adrenal glands. Six years later these observations were confirmed and extended by Jenis and Hertzog³ and Davis and Medline⁴ with the aid of electron microscopy. These bodies consist of whorls of concentrically arranged cytoplasmic reticulum containing a central core of lipid material. Within the cell they are 2–20 μ in diameter and they are found exclusively in the aldosterone-producing cells (glomerulosa cells) of the adrenal cortex. Careful search by Jenis and Hertzog³ indicated that "spironolactone bodies" (SB) were not found in any other region of the adrenal gland or in any other organ of the body. All of the foregoing studies were carried out on postmortem material.

In a preliminary communication we¹ reported studies concerning the formation and regression of SB in living people. We had observed these bodies in adrenal tissue surgically removed from patients with primary aldosteronism who had received spironolactone preoperatively. We proceeded with further studies in an attempt to relate the morphological changes to concurrent physiologic changes in aldosterone production and plasma renin activity (PRA). From such data we concluded that spironolactone exerts a direct effect upon aldosterone-producing cells, inhibiting the production and/or release of aldosterone, and that the SB are a morphological reflection of a block in aldosterone biosynthesis. We also reported that in primary aldosteronism due to adenoma the SB were confined to the tumor and were absent in the glomerulosa zone of the adrenal cortex proper, indicating that they form only in cells that are actively producing aldosterone. It was observed, too, that the number of bodies formed under the influence of spironolactone was related to the duration of spironolactone administration rather than to the dose administered (150–400 mg/day). Finally, to be certain that these "bodies" were induced by spironolactone and were not the result of overactivity of aldosterone-producing cells, we reviewed adenoma sections from 83 of our cases of primary aldosteronism that had never received spironolactone. In no instance was anything resembling an SB encountered.

The present report provides new details regarding the blocking action of spironolactone upon aldosterone production in patients harboring an aldosterone-producing adenoma and modifies some of our earlier interpretations.

MATERIALS AND METHODS

Twenty-five patients from whom aldosterone-producing adenomas were subsequently removed have been studied. Preoperatively all exhibited the diagnostic combination of excessive excretion of aldosterone, together with subnormal levels of PRA.⁵ Twenty-three were treated with spirono-

lactone (150–400 mg/day) up to the time of operation (the last dose was given at 8:00 p.m. the evening before) for 2–170 days. Two patients received the drug for 81 and 86 days, respectively, but it was discontinued 18 and 24 days, respectively, before operation. At surgery, 24 patients were found to have a solitary adenoma and 1 patient had two adenomas in one adrenal gland.

Sections of the adrenal tissue removed at operation were stained with hemotoxylin and eosin. SB body counts were recorded by averaging the number of bodies counted in five random high-power fields at a magnification of $\times 400$. Electron microscopic study of the intracytoplasmic bodies was also carried out.

All specimens for determinations of urinary and plasma aldosterone and PRA were collected after the patient had been ingesting, for at least 3 days, a diet containing 120 mEq of sodium and approximately 70 mEq of potassium per day. Specimens for plasma aldosterone and PRA determinations were drawn at 8:00 a.m., with the patient still recumbent, after an overnight sleeping period. Aldosterone and PRA were each measured by radioimmunoassay methods previously described by laboratory.^{6,7}

RESULTS

Morphological Studies

Figure 1 is an electron micrograph of a typical SB within the cytoplasm of an adenoma cell. It demonstrates the whorling of tightly packed, concentric, agranular membranes that is characteristic of these bodies. Having observed

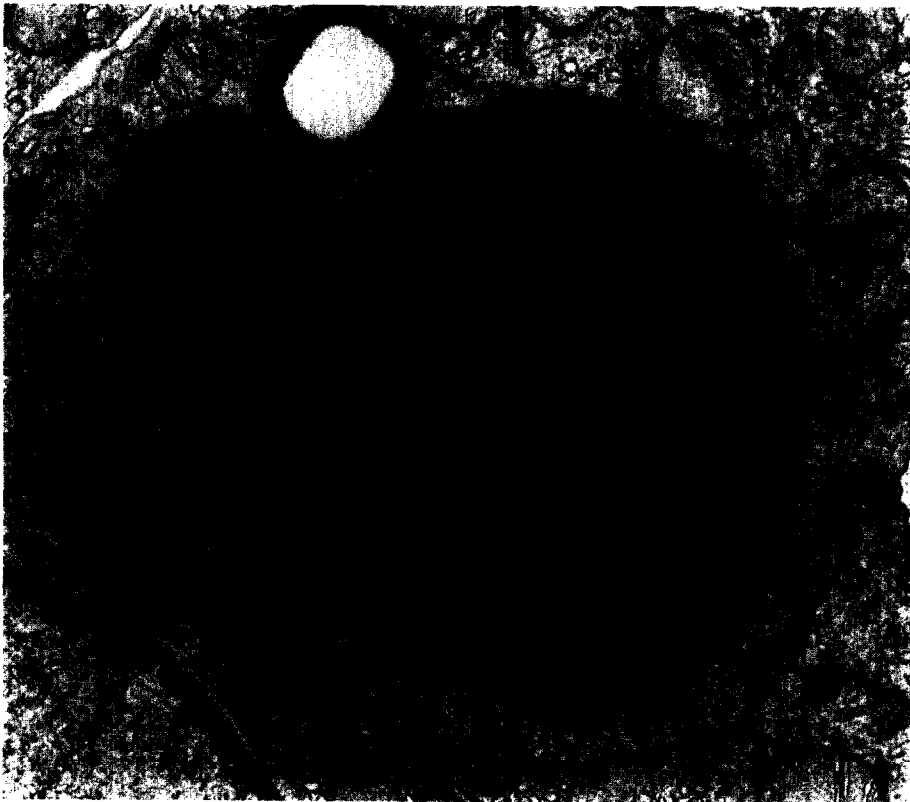


Fig. 1. Electron micrograph of a portion of an adenoma cell showing extensive whorling of cytomembranes characteristic of a spironolactone body. The cell contains abundant smooth endoplasmic reticulum and mitochondria. The broad flattened mitochondrial cristae are characteristic of glomerulosa cells. $\times 23,000$.

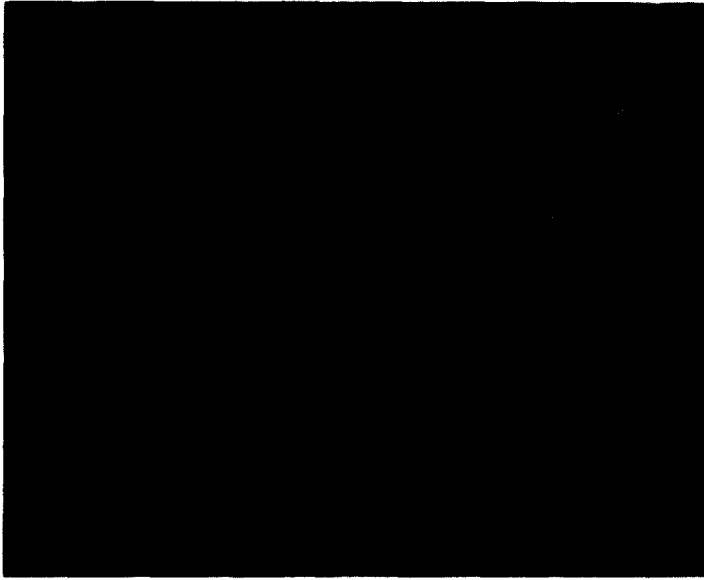


Fig. 2. Spironolactone bodies in a section from an aldosterone-producing adenoma. Pink stained, laminated, intracytoplasmic bodies are seen within a white halo. $\times 750$.

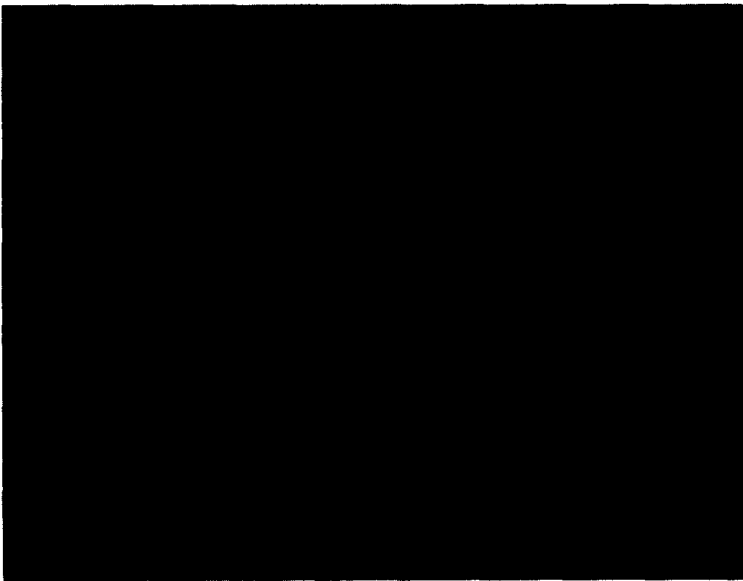


Fig. 3. Broad distribution of spironolactone bodies as seen with lower magnification. $\times 400$.

the electron micrograph, one can appreciate better the appearance of SB as seen under the light microscope.

Figure 2 is a section from an aldosterone-producing adenoma under the light microscope. The patient had received spironolactone, 400 mg/day, for 25 days. The bodies stain pink with eosin and are seen within a white halo. The coiled watch-spring-like structures are SB. No such bodies were observed in the glomerulosa cells of the nontumorous portion of the gland.

Figure 3 demonstrates a section from the same tumor at lower power and shows the broad distribution of the bodies which are easily counted.

Figure 4 shows the "life cycle" of the SB as derived from counts in the adenomas of 23 of the 25 patients with proven primary aldosteronism. The average daily dose for each patient is recorded in parentheses. The vertical axis indicates the SB count. The horizontal axis shows the duration of spironolactone administration up to the time of operation. It will be observed that the peak counts occur between 40 and 60 days, with a gradual and highly significant increase between 4 and 50 days ($r = 0.910$, $p < 0.0001$). Then there is a slow and significant decrease from 50 to 170 days ($r = 0.887$, $p < 0.005$). Note that the counts follow the regression lines in relation to time, regardless of the dosage (150–400 mg/day). Bodies are not seen until the patient has received the drug for 3–4 days. Note, too, that at 100 and 110 days there are two additional adenoma cases that were operated upon but showed no SB. In these two patients the drug had been discontinued 18 and 24 days before operation. These two values were, of course, not computed in the regression lines produced by

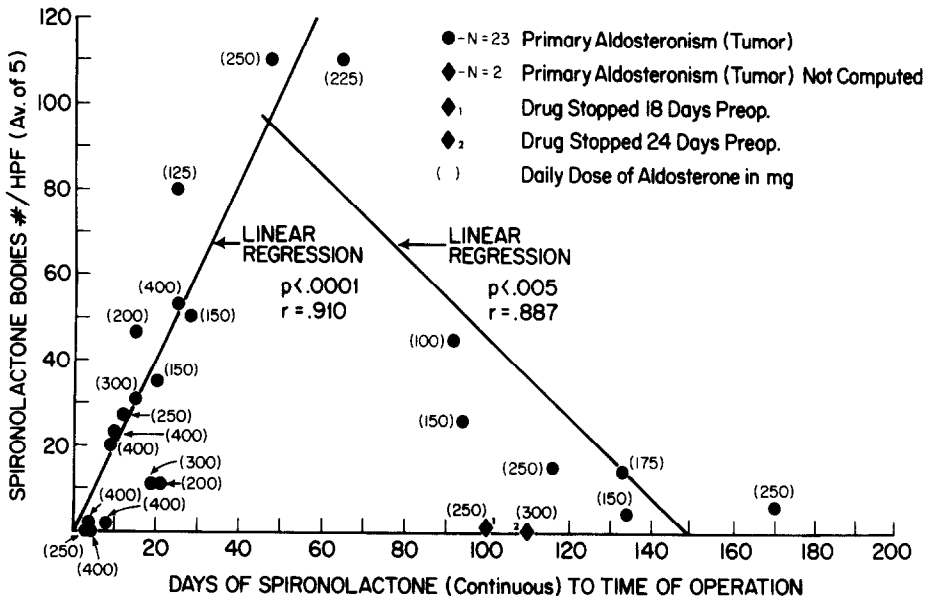


Fig. 4. "Life cycle" of spironolactone bodies based upon spironolactone body counts and the duration of therapy up to the time of operation.

the other 23 cases, but they provided information regarding the rates of formation and disappearance of SB at 100 days of administration of the drug.

Physiologic Studies

To study the renin-aldosterone system as SB were being formed *rapidly*, we elected to observe this system in detail during the first 20 days of spironolactone administration, with removal of the adenoma immediately at the end of the short course of the drug. In less detailed studies, in nine patients with aldosterone-producing adenomas we had observed a sharp decrease in aldosterone excretion for as long as 27 days after the beginning of treatment. Those results will be presented below. The three patients chosen for detailed study received spironolactone for 10, 11, and 20 days, respectively.

Figure 5 shows the results obtained in a patient who received 400 mg daily for 10 days preceding operation. By the sixth day, aldosterone excretion had fallen to 50% of the mean baseline value. It then fluctuated at relatively low values for 4 days and again reached 50% of the mean baseline value on the day before operation. By the eighth day of spironolactone, plasma aldosterone had fallen from 18.9 and 23.6 ng/100 ml to 9.6 ng/100 ml. PRA rose slowly from very low levels into the normal range by the day before operation. However, despite this rise in PRA, plus a rise of serum potassium from 3.0 to 4.6 mEq/liter, aldosterone excretion was still 50% below baseline. Upon removal of the adenoma and of spironolactone therapy, aldosterone excretion remained in the

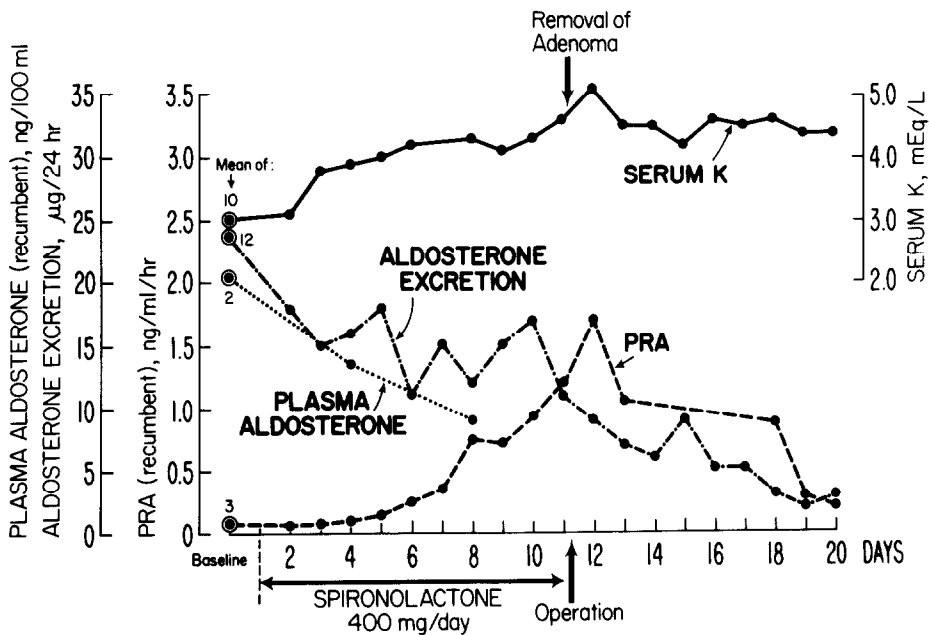


Fig. 5. Effect of spironolactone (for 10 days preoperatively) on plasma and urinary aldosterone, plasma renin activity, and serum potassium in a case of aldosterone-producing adenoma.

low normal range for several days (suggesting mild activation of the "normal" glomerulosa cells), following which the renin-aldosterone system returned to a suppressed state. Table 1 presents additional data on electrolyte excretion and weight changes in the course of this study. During spironolactone therapy there was a marked sodium diuresis and potassium retention with a rapid rise in serum potassium and a loss of 4.5 kg of body weight, but plasma and urinary aldosterone, rather than increasing, had decreased.

Figure 6 shows a similar series of events during an 11-day period of spironolactone administration (400 mg/day). Urinary and plasma aldosterone fell as PRA rose from 0.5 to 1.0 ng/ml/hr and serum potassium rose from 2.8 to 4.3 mEq/liter. Aldosterone fell from 53 to 27 ng/100 ml. Following removal of the adenoma, the normal glomerulosa cells showed no evidence of aldosterone-producing activity, even though PRA had risen from 0.5 to 2.5 ng/ml/hr and serum potassium had risen from 2.8 to 4.8 mEq/liter.

Figure 7 shows the results obtained in a patient given spironolactone for 20 days before operation. This was a mild case, as can be seen from the preoperative levels of serum potassium. Because it was mild, it telescoped in a short period of time various phenomena that usually require much more time to occur. Upon administration of spironolactone, there was a transient, initial

Table 1. Primary Aldosteronism: Preoperative Treatment With Spironolactone (400 mg/day) for 10 Days

Date	Weight (kg)	Serum Levels (mEq/liter)		Urine Levels (mEq/24 hr)		PRA, Recumbent (ng/ml/hr)	Aldo. Excret. (μ g/24 hr)	Plasma Aldo. (mg/100 ml)
		Na	K	Na	K			
Nov.								
1	76.3	144	3.2	125	70	0.14	23	18.9
2	75.5	142	3.0	159	74	0.06	21	23.6
3*	74.0	145	3.1	235	69	0.06	18	
4*	73.2	142	3.8	156	33	0.07	15	
5*	73.0	144	3.9	140	36	0.10	16	13.5
6*	72.9	145	4.0	121	42	0.14	18	
7*	72.5	144	4.2	138	48	0.24	11	
8*	71.7	—	—	144	61	0.36	15	9.6
9*	71.5	142	4.3	142	58	0.75	12	
10*	71.2	143	4.1	132	61	0.73	15	
11*	71.1	141	4.3	147	66	0.92	17	
12*	71.0	138	4.6	96	47	1.20	11	
13†	72.3	142	5.1	126	68	1.70	9	
14	70.9	137	4.5	119	87	0.96	7	
15	71.0	129	4.5	60	58	—	6	
16	70.3	138	4.2	52	54	—	9	
17	70.3	138	4.6	58	57	—	5	
18	70.1	143	4.5	63	52	—	5	
19	70.0	142	4.6	74	57	0.87	3	
20	70.9	144	4.4	113	67	0.27	2	
21	70.9	145	4.4	150	71	0.20	3	

Intake: Na, 120 mEq/24 hr; K, 70 mEq/24 hr.

*Spironolactone administered.

†Adenoma removed.

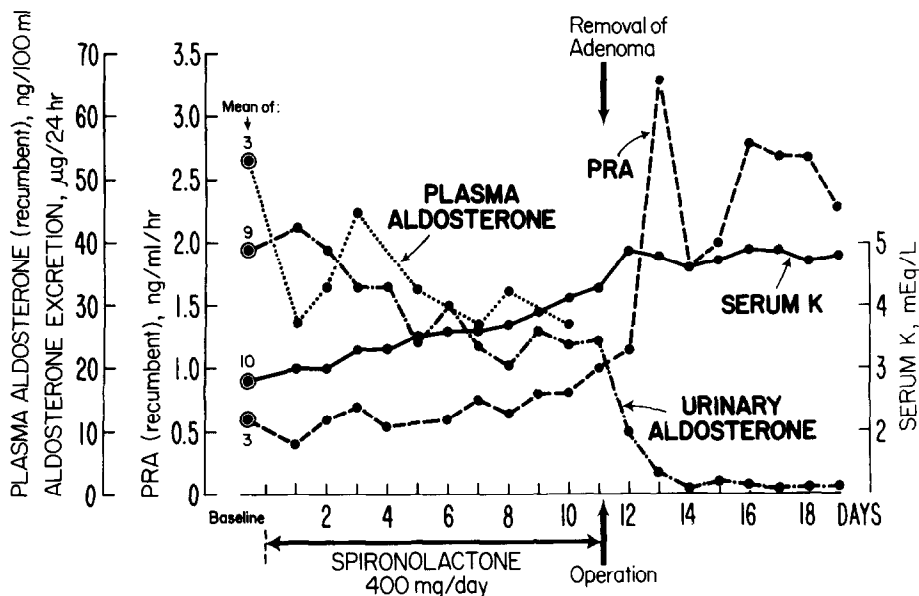


Fig. 6. Effect of spironolactone (for 11 days preoperatively) on plasma and urinary aldosterone, plasma renin activity, and serum potassium in a case of aldosterone-producing adenoma.

decrease in aldosterone excretion. By the seventh day of spironolactone administration (day 11), with a sharp rise in PRA, aldosterone excretion came back to baseline values. The obvious blocking action of spironolactone on aldosterone production seen in the other two cases is much less clear in this one. Nevertheless, the inhibiting effect on aldosterone production is still evident in that aldosterone excretion was at the prespironolactone level, while PRA had increased 12-fold (day 11).

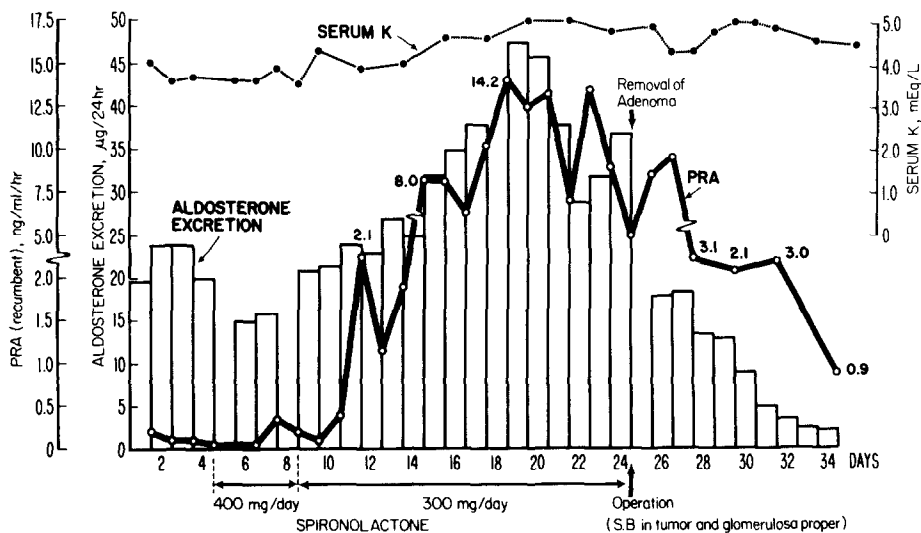


Fig. 7. Effect of spironolactone (for 20 days preoperatively) on aldosterone excretion, plasma renin activity, and serum potassium in a case of aldosterone-producing adenoma.

Table 2. Primary Aldosteronism: Effect of Spironolactone on PRA (2 hr upright, ng/ml/hr)

Case No.	Na 120 mEq/day	Na 120 mEq/day + Spironolactone 200-400 mg/day	
		10-14 days	6 wk
1	0.1	0.4	0.6
2	0.1	1.9	
3	0.1		9.6
4	0.2	0.6	9.9
5	0.4		27.0
6	0.5	0.5	0.7
7	0.5	1.2	
8	0.6	0.1	
9	0.8	6.8	
10	1.0	14.9	

Normal 7.7 ± 1.0 (SEM).

On day 15 and beyond, a *very* large increase in PRA occurred (note the break in the scale) and aldosterone excretion went to twice its baseline value. Under the latter circumstances it would be difficult to invoke a continuing spironolactone block on aldosterone biosynthesis. The same phenomenon, however, occurs in most spironolactone-treated adenoma cases, but it is usually seen after 5 or 6 wk of therapy.⁸ Table 2 indicates the great variability among patients in the time required to increase PRA by means of spironolactone therapy. In some (cases 1 and 6) it is still severely suppressed after 6 wk. In others (cases 9 and 10) it is normalized in 10-14 days, as it was in the present case (Fig. 7). Following removal of the adenoma from this patient there was evidence of transient aldosterone production by the normal glomerulosa cells for a short time, after which they again became suppressed. They appear to have assumed some function before the operation because a few SB were seen in the glomerulosa cells proper (an unusual occurrence), as well as in the adenoma cells.

Figure 8 summarizes the early changes (1-27 days) in aldosterone excretion induced by spironolactone in the 12 cases in which these data were available. The open circles represent the day before operation and the duration of spironolactone administration preoperatively. The solid circles represent aldosterone determinations done while the patients were on spironolactone *before* operation. In all 12 cases there was a decrease in aldosterone excretion of 25%-83%. The solid circle at 27 days of spironolactone therapy represents a decrease from baseline of 76%. However, by the time of operation at 170 days, aldosterone excretion was 100% above baseline. The line labeled J.M. represents the patient described in detail (Fig. 7) who telescoped the whole phenomenon into a short period of time. For comparison, the solid square averages the results obtained in 6 adenoma cases treated with spironolactone for 35 days⁸ and the solid triangle represents the mean of 27 patients with essential hypertension, treated for 70 days.⁹

DISCUSSION

From a study of 25 patients with primary aldosteronism, an attempt has been made to correlate the effects of spironolactone upon function and structure of aldosterone-secreting adenoma cells. At the beginning of administration

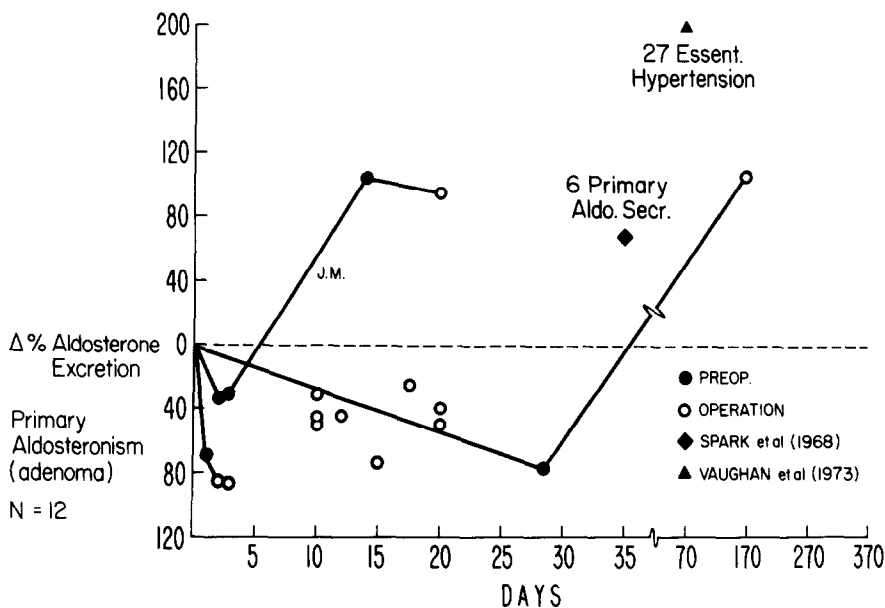


Fig. 8. Effect of spironolactone (for 2-170 days preoperatively) on aldosterone excretion in 12 patients with aldosterone-producing adenoma.

of the drug, two events occur simultaneously: (1) a sharp decrease in plasma and urinary aldosterone, and (2) the appearance in the tumor cells of the intracytoplasmic, laminated, eosinophilic bodies (spironolactone bodies).

Patients with aldosterone-producing adenomas are ideal subjects in which to demonstrate the blocking action of spironolactone on aldosterone biosynthesis because, at the beginning of administration of the drug, the major known stimuli for aldosterone production (angiotensin^{II}, serum potassium, and reduced serum sodium) are suppressed. The normal glomerulosa cells are producing little, if any, aldosterone. The adenoma is secreting aldosterone autonomously except, perhaps, for the circadian variation induced in some tumors by the normal fluctuations of ACTH release.^{10,11} Under these conditions one observes the spironolactone blockade of aldosterone production in the adenoma itself. This is the beginning of phase I of a two-phase overall reaction to spironolactone in patients with primary aldosteronism.

Phase I: Inhibition of Aldosterone Biosynthesis

Phase I is usually demonstrable for 4-6 wk, but, in unusual cases, it may be observed for only 1 wk or, occasionally, for several months. In the average case, aldosterone production diminishes significantly as SB are being formed rapidly in the tumor cells. During this time SB are not forming in the glomerulosa cells proper. Since the latter cells are not producing aldosterone, we have concluded that (1) SB are formed only in cells that are actively producing aldosterone, and (2) the SB are a morphologic reflection of a block in aldosterone biosynthesis within aldosterone-producing cells.

In addition, these morphologic observations allow us to place the site of the block beyond the formation of corticosterone in one or both of the final steps

of the aldosterone biosynthetic pathway. SB form only in aldosterone-producing cells. They do not occur in normal cells of the fasciculata zone. It has been well established that the biosynthesis of corticosterone proceeds similarly in both the glomerulosa cells and the fasciculata cells. If the SB are regarded as a morphological expression of a block in aldosterone biosynthesis, this inhibitory effect is exerted within the glomerulosa cell, where the final two steps of aldosterone biosynthesis occur. Until recently these two steps had been considered to consist of 18-hydroxylation of corticosterone to form the intermediary compound, 18-hydroxycorticosterone, followed by dehydrogenation of that compound to produce aldosterone. Ulick¹² has recently suggested a new concept involving "corticosterone-methyl oxidase" in a two-step reaction in which 18-hydroxycorticosterone is formed as a side reaction in step I and aldosterone is produced in a second methyl oxidase reaction in step II. In further discussion we employ this new nomenclature.

Morphologically, our studies appear to localize the inhibiting effect of spironolactone to one or both of the final two steps of aldosterone biosynthesis. We have no studies of precursor compounds with which to identify more precisely the site of the inhibitory effect. However, since our initial report,¹ others have observed that spironolactone inhibits aldosterone production in man and in animals.¹³⁻¹⁷ The *in vitro* studies of Cheng et al.¹⁵ suggest that step I of the corticosterone-methyl oxidase reaction is inhibited by spironolactone. That group studied mitochondria from an aldosterone-producing adenoma, and also from glomerulosa cells of beef adrenal glands. In both instances, spironolactone inhibited the synthesis of both 18-hydroxycorticosterone and aldosterone.

Phase II: "Escape" From the Inhibitory Effect of Spironolactone on Aldosterone Biosynthesis

Upon continued administration of spironolactone for about 1 mo (in the average case of primary aldosteronism), aldosterone production rises to baseline values and frequently considerably above. Formation of SB ceases and their numbers gradually diminish to almost zero by 170 days. It is to be recalled that in phase I aldosterone production was very resistant to stimulation by the elevations of PRA and serum potassium induced by the renal effects of spironolactone. It is of interest, in this connection, that Kremer et al.,¹⁸ studying a group of patients with aldosterone-producing tumors before and after 4 wk of spironolactone therapy, noted great rises in renin, but only erratic increases or none in aldosterone production. In those studies, the early decreases in aldosterone production, which we have reported above, were not observed because the second determinations were carried out too late. The fact, however, that aldosterone production, after 4 wk of spironolactone, was not increased or was only erratically higher than the prespironolactone values, while renin was considerably elevated, suggests that spironolactone was still impeding aldosterone biosynthesis.

Occasionally one encounters a case of aldosterone-producing adenoma in which it is clear that spironolactone has inhibited aldosterone production for unusually long periods of time. The case reported by Mantero et al.¹⁹ exhibited a decrease in urinary aldosterone from 48-53 $\mu\text{g}/\text{day}$ to 11-20 $\mu\text{g}/\text{day}$ after

4 wk of spironolactone therapy; in one of our cases (Fig. 8), aldosterone excretion was similarly decreased by 76% after 27 days of therapy. Sundsfjord et al.¹⁶ have reported a case in which high values for plasma and urinary aldosterone fell to normal values and remained there for 4 mo under continuous therapy with spironolactone, even though PRA and serum potassium had long been normal or above normal. Nevertheless, most adenoma cases respond to long-term spironolactone therapy with a brisk increase of aldosterone production, which may be 50%–100% higher than the prespironolactone level of aldosterone production (Fig. 8). Spark et al.⁸ studied six patients with aldosterone-producing adenomas after they had received 400 mg/day of spironolactone for 35 days and found that the daily excretion of aldosterone was 40%–50% higher than the prespironolactone level. PRA levels were, of course, already elevated, but a 10-min infusion of angiotensin^{II} gave evidence of extreme sensitivity of the tumor to acute elevations of angiotensin^{II}. The adrenal venous effluent from the tumor side more than doubled its aldosterone concentration after 10 min of subpressor doses of angiotensin^{II}, while there was essentially no response from the contralateral side. Of great interest in those studies is the finding that 18-hydroxycorticosterone concentration on the tumor side more than tripled by the end of the infusion. It thus appears that by 5 or 6 wk of continuous administration of large doses of spironolactone, the tumor cells “escape” from their former state of drug-induced inhibition of aldosterone biosynthesis, and when this occurs both aldosterone and 18-hydroxycorticosterone are being produced in excessive amounts.

We are interested in the possible mechanisms by which a block in the conversion of corticosterone to aldosterone seems to disappear after 4–6 wk of administration of spironolactone. In the average patient with an aldosterone-producing adenoma, by this time, a large amount of sodium has been lost from the body by virtue of the peripheral effects of the drug. Blood pressure has returned to normal and serum electrolyte values have come into the normal range. There is, in fact, a sufficient sodium deficit such that renin values are quite high. The easiest explanation for the escape phase is that when plasma angiotensin^{II} concentration rises to elevated levels for a sufficiently long period of time, the adenoma cells finally respond to it. Furthermore, there is clear evidence that sodium deficit increases sensitivity to angiotensin^{II} of aldosterone-producing cells.²⁰ However, the major effect of angiotensin^{II} on aldosterone biosynthesis occurs in an early step of the biosynthetic pathway²¹ and it would not be expected to alleviate a block in the final step(s). There is no convincing evidence that angiotensin^{II} can increase the *in vivo* rate of conversion of corticosterone to aldosterone.²² However, it has been demonstrated that this conversion is clearly enhanced by sodium privation.^{23–28} Thus, a second, and somewhat more attractive postulate can be proposed, namely, that the state of sodium deficiency activates a mechanism which increases conversion of corticosterone to aldosterone; and that when this has been accomplished, the elevated levels of angiotensin^{II} become much more effective in stimulating aldosterone production. Indeed, this is probably the explanation for the increasing effectiveness of angiotensin^{II} as a stimulus for aldosterone production with increasing degrees of sodium deficit in normal man.²⁰

It should be recalled that patients with primary aldosteronism not treated with spironolactone do not augment aldosterone production upon infusion of angiotensin^{II}.²⁹ Thus, in the presence of sodium deficiency, both the early (angiotensin^{II}) and late steps of aldosterone biosynthesis would be stimulated to increased activity. Teleologically, this would be important in maximizing aldosterone production for conservation of body sodium. The implication is that sodium deficit, independent of its hyperreninemic effect, activates a mechanism which increases the activity of the corticosterone–methyl oxidase system. Similar conclusions have been suggested in the past^{27,30,31} as the result of studies of a different nature than those reported here.

In the case of neoplastic aldosterone-producing cells, the following points are relevant. These cells are producing aldosterone in large amounts in the presence of hypokalemia, hypernatremia, hyporeninemia, and normal amounts of ACTH. Presumably, the "sodium-deficit factor" is also suppressed since, in primary aldosteronism, there is an overabundance of sodium in the body. Thus, aldosterone biosynthetic enzymatic activity is proceeding in high gear despite suppression of all known stimulative factors. In fact, it has been reported by two groups^{32,33} that 18-hydroxylase activity (corticosterone–methyl oxidase activity, step I) is considerably increased in aldosterone-producing adenoma tissue. Partial or complete interference with the activity of this enzyme by spironolactone would appear to account for the early inhibition of aldosterone biosynthesis in tumor cases. After sufficient sodium deficit has occurred, induction of increased activity of the same enzyme by the "sodium-deficit factor" in the presence of hyperreninemia may account for recovery in phase II. In the rare instances in which phase I persists 4–6 mo,¹⁶ this enzyme system within the tumor cells may have been much more severely injured.

A third possibility to explain recovery of aldosterone production by the tumor cells is that intracellular potassium may have risen substantially as the result of potassium-retaining effects of the drug. However, in the tumor cells this possibility seems unlikely since before the administration of spironolactone these same cells were producing an abundance of aldosterone in the presence of severe hypokalemia and a presumed deficit of intracellular potassium.

In conclusion, spironolactone exerts an inhibiting influence on aldosterone biosynthesis in aldosterone-producing adenoma cells. The development of SB in these cells, and not in fasciculata cells, appears to place the site of inhibition after the formation of corticosterone and most likely at step I of the corticosterone–methyl oxidase reaction of Ulick.¹² With time and continued administration of spironolactone, the inhibitory effect upon aldosterone production by the adenoma lessens or disappears. SB also diminish or disappear. Several possibilities have been considered to explain the recovery phase of this two-phase phenomenon.

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