Stop Flow Analysis of Aldosterone and Steroidal Antagonist SC-8109 on Renal Tubular Sodium Transport Kinetics.* (25581)

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Recently(1), using the technic of stop flow analysis(2,3), we demonstrated a distal site of action of aldosterone. After adrenalectomy, the distal tubule was not able to reduce sodium concentration to minimum value achieved during stop flow in normal dogs. We have since noted that, on occasion, normally low distal sodium concentrations can be attained in the adrenalectomized animal only when associated with extremely low plasma sodium concentrations. The present paper presents experiments designed to study this relationship between plasma and distal tubular sodium concentrations in normal, in adrenalectomized and in dogs treated with synthetic steroid, SC-8109, 3-(3-oxo-17β-hydroxy-19-nor-4-androsten-17α-yl) propionic acid γ-lactone (Searle), which appears to block the renal effects of aldosterone and desoxycorticosterone(4-6).

Methods. Adrenalectomy and corticoid therapy of dogs prior to stop flow procedure. All experiments were performed on male or female mongrel dogs weighing 9-20 kg. These dogs can be divided into 4 groups, according to method of preparation utilized prior to performance of stop flow procedure: *Group 1*: Bilateral adrenalectomy was performed aseptically under pentobarbital anesthesia, either in a single operation by means of midline incision, or in 2 stages, performed one week apart and utilizing flank incisions. Cortisone acetate, 100 mg intramuscularly, was given at time of operation and on the day preceding. Thereafter, dosage of cortisone was gradually lessened, final maintenance dosage varying from 10-25 mg/day. These dogs were given normal diet supplemented with 100 mM Na/day. No animals were employed until at least one week after bilateral adrenalectomy. They were given usual dosage of cortisone 2 hours before starting the stop flow procedure. *Group 2*: These dogs were prepared exactly as in Group 1, except that all cortisone was discontinued 2 days prior to stop flow analysis. *Group 3*: Normal dogs were given a normal diet with no steroid. *Group 4*: Normal dogs were given SC-8109, approximately 0.75 mg/kg intramuscularly, 2 hours before beginning stop flow and every 2 hours during stop flow procedure. *Stop flow procedure.* All animals were anesthetized with 30 mg/kg of Na pentobarbital injected intraperitoneally. Through a small flank incision, one ureter was catheterized with polyethylene tubing pushed well up into the renal pelvis and tied firmly into place. A constant intravenous infusion of creatinine, PAH, and 18% mannitol in Ringer's Solution was supplied at 10 ml/min. Duration of occlusion varied from 4-6 min. Blood samples were taken from catheter in the femoral artery at onset and on release of occlusion. No ureteral occlusions were performed until urine flow had stabilized at about 10 ml/min. Three different experimental patterns were then followed, each on separate occasion: *Pattern 1*: Occlusions were performed at 15 min. intervals, varying length of occlusion from 4-6 min. *Pattern 2*: After completion of first occlusion, plasma sodium concentration was raised rapidly by intravenous administration of 2-3 g NaCl in 50 ml water for 5 minutes. After waiting 10 additional minutes, the next occlusion was performed, and the process repeated with another salt injection. *Pattern 3*: After completion of several occlusions, 7-40 μg of aldosterone were given intravenously for 15 min. Further occlusions were then performed after waiting 2-3 hrs. Aldosterone was given only to adrenalectomized dogs. *Analytical methods* were as follows: sodium was analyzed with Beckman direct-reading flame photometer,

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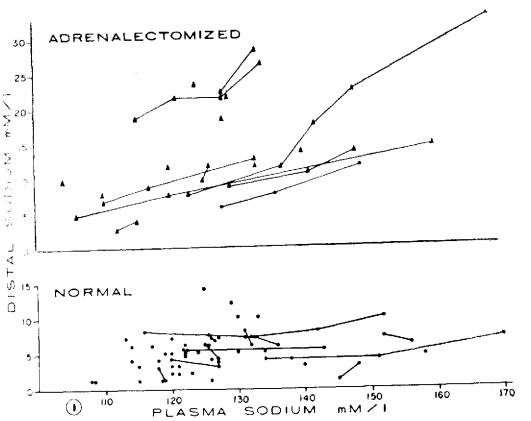
PAH according to Smith, Finkelstein, Aliminosa, Crawford, and Graber(7), creatinine by method of Bonsnes and Taussky(8).

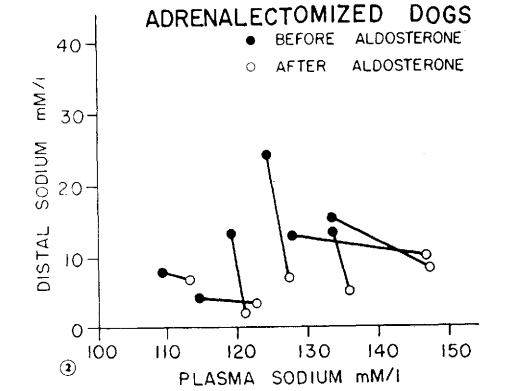
Results. Proximal tubule. It is possible, using calculations described previously(9), to determine volume of fluid reabsorbed from the proximal tubule during period of ureteral occlusion. The computed additional proximal reabsorption of sodium and water during ureteral occlusion was the same in adrenalectomized dogs both before and after administration of aldosterone.

Distal tubule. The minimum point on the downward inflection to the left of sodium curve is thought to represent best the lowest Na concentration developed in a maximum number of low sodium distal segments during stop flow(3). This minimum distal Na concentration is plotted vertically in Fig. 1 against increasing plasma Na along horizontal axis. In the normal animal, the distal tubule is capable of lowering sodium concentration almost to zero during stop flow, as demonstrated previously(1). The minimum sodium concentration achieved appears independent of plasma sodium concentration (except perhaps at extremely high values).

The effects of adrenalectomy upon this pattern (Fig. 1) are striking. Even at very low plasma sodium concentrations, the distal concentrations achieved during ureteral occlusion are higher than normal. Moreover, there is a direct relationship between concentrations of sodium in plasma and in the distal tubule during occlusion. This indicates that adrenalectomy has reduced maximal sodium concentration gradient which can be maintained across distal tubular cells. As plasma sodium rises, the minimal sodium concentration attained during ureteral occlusion also rises. Even if duration of occlusion is prolonged from 4 to 6 minutes, the distal sodium concentration attained during stop flow is not changed, indicating that maximal distal tubular sodium reabsorption occurs within the first 4 minutes of ureteral occlusion.

There was no difference between the group of animals treated with cortisone pre-operatively and the group in which all steroid therapy had been withdrawn 2 days pre-operatively.





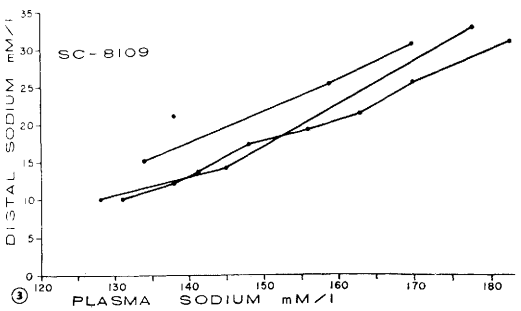


FIG. 1. Relationship between plasma Na concentration and the minimal distal urinary Na concentration developed during ureteral occlusion in normal and adrenalectomized dogs. Lines connect points derived from a single dog as plasma Na was elevated.

FIG. 2. Effect of aldosterone upon maximal distal tubular Na reabsorption during ureteral occlusion on adrenalectomized dogs. Lines connect points determined for a single dog as plasma Na was elevated.

FIG. 3. Relationship between plasma Na concentration and the minimal distal urinary Na concentration developed during ureteral occlusion in dogs treated with SC-8109. Lines connect points determined for a single dog as plasma Na was elevated.

The effects of aldosterone on adrenalectomized dogs are demonstrated in Fig. 2. Administration of aldosterone restored ability of the distal tubule to lower sodium concentration even in presence of elevated plasma sodium concentration.

Fig. 3 demonstrates the effects of SC-8109 on normal dogs. It is evident that inhibition of distal tubular sodium reabsorption produced by these large doses resembles that produced by bilateral adrenalectomy.

These studies further establish the distal tubule as site of action of aldosterone in sodium reabsorption. They also emphasize the importance of maintaining high plasma sodium whenever assays are made of possible blocking agents.

Summary. In normal dogs the distal tubule can lower Na concentration almost to zero during stop flow, the minimal concentration achieved being independent of plasma Na concentration. In adrenalectomized animals, minimal distal concentrations achieved are not as low. As plasma Na is increased, minimal distal Na concentration also increases in direct proportion, indicating that adrenalectomy reduces maximal Na concentration gradient which can be maintained across distal tubular cells. Results in adrenalectomized and in SC-8109 treated dogs are identical. Administration of cortisone to adrenalectomized dogs does not repair this defect, but aldosterone restores ability of the distal tubule to lower Na concentration, even in presence of elevated plasma Na. Aldosterone when given to adrenalectomized dogs did not alter proximal Na reabsorption, as this can be estimated by stop flow data.

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Activator of Plasminogen and Protease from Various Cell Cultures and Present in Poliomyelitis Vaccine. (25582)

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Previous reports described and characterized an activator of plasminogen produced by KB cells and both an activator of plasminogen and a protease produced by Rhesus monkey kidney (MK) cells(1-4). The present study was undertaken to determine which cell cultures produce these 2 substances and whether they were present in poliomyelitis vaccine prepared from supernatant medium from MK cell culture.

Materials and methods. Cell cultures used are described in Table I. Supernatant medium from cell cultures was obtained as previously described(3). Each supernatant medium was assayed 2 to 5 times for activator and protease using skim milk as substrate(3). Commercially prepared poliomyelitis vaccine, with preservative, without preservative and after dialysis was assayed for activator and protease.

Results. Production of activator and protease by various cell cultures. Cell cultures fell into 3 groups, depending upon production of activator, activator and protease, and detection of no activity in the supernatant medium. As seen in Table I of 21 continuous