JCI The Journal of Clinical Investigation

The renal tubular handling of aldosterone and its acid-labile conjugate

Murphy T. Scurry, … , Leroy Shear, Kevin G. Barry

J Clin Invest. 1968;47(2):242-248. https://doi.org/10.1172/JCI105720.

Research Article

Stop-flow studies using infusions of aldosterone-^{3}H or its ^{3}H acid-labile conjugate were done on five rhesus monkeys. The aldosterone-^{3}H urine-to-plasma (U/P) ratio decreased in the same distal urine samples as sodium. The^{3}H acid-labile conjugate U/P-to-inulin U/P ratio increased in the more proximal samples either with conjugate formed endogenously during aldosterone-^{3}H infusions or with labeled conjugate infused alone. Aldosterone reabsorption occurred at a distal site in the renal tubule, and secretion of its acid-labile conjugate occurred at a proximal site.

The Renal Tubular Handling of Aldosterone and Its Acid-Labile Conjugate

MURPHY T. SCURRY, LEROY SHEAR, and KEVIN G. BARRY

From the Department of Metabolism, Walter Reed Army Institute of Research, Washington, D. C.

ABSTRACT Stop-flow studies using infusions of aldosterone-^{3}H or its ^{3}H acid-labile conjugate were done on five rhesus monkeys. The aldosterone-^{3}H urine-to-plasma (U/P) ratio decreased in the same distal urine samples as sodium. The ^{3}H acid-labile conjugate U/P-to-inulin U/P ratio increased in the more proximal samples either with conjugate formed endogenously during aldosterone-^{3}H infusions or with labeled conjugate infused alone. Aldosterone reabsorption occurred at a distal site in the renal tubule, and secretion of its acid-labile conjugate occurred at a proximal site.

INTRODUCTION

The urinary excretion of aldosterone and its metabolites is widely used to assess aldosterone metabolism for clinical purposes. The conjugate, which yields aldosterone after standing at pH 1, is used extensively in this regard (1, 2) and is referred to as the acid-labile or 3-oxo-conjugate. It is most likely a glucuronide at the 18 position (3).

An understanding of the renal handling of aldosterone and its acid-labile conjugate is important for interpretation of clinical assay data and data concerning the metabolism of the hormone. Renal clearance studies in man (4, 5) have shown that 72–90% of filtered nonprotein-bound aldosterone is reabsorbed. The site of reabsorption in the dog is the distal tubule (6). The renal clearance of the acid-labile conjugate was found to exceed glomerular filtration rate by 2- to 13-fold, indicating tubular secretion. The kidney has been shown to be a site of formation of this conjugate (7–9). The site of conjugate formation within the kidney and the relationships between formation and secretion of acid-labile conjugate are not known.

In this investigation, stop-flow studies with tritium-labeled aldosterone and acid-labile conjugate have been done to determine the site of aldosterone reabsorption and acid-labile conjugate secretion. The rhesus monkey was chosen as the experimental animal because its excretion of the acid-labile conjugate is similar to that in man (M. T. Scurry, Unpublished observation).

METHODS

Experiments were performed on five female rhesus monkeys (*Macaca mulatta*), weighing 3.8–6.6 kg. The principles of laboratory animal care were observed as promulgated by the National Society for Medical Research. The stop-flow method of Malvin, Wilde, and Sullivan (10) was used. The animals were anesthetized with intravenous sodium thiopental. The left ureter was catheterized with polyethylene tubing through a flank incision. A catheter placed in the femoral vein was used to infuse a priming solution of creatinine (0.5 g) and (or) inulin (200–300 mg), followed by a constant infusion (4.9 ml/min) of 10% mannitol in lactated Ringer's solution with creatinine (2 g/liter) and (or) inulin (400–600 mg/liter) added. After a 65–80 min equilibration period, three urine samples (free flow) of approximately 1.5 ml each were

---

Part of these results have been reported in preliminary form (*Clin. Res.* 1966. **14**: 388).

Dr. Scurry's present address is University of Texas Medical Branch, Galveston, Tex. 77550. Dr. Shear's present address is Cleveland Metropolitan General Hospital, Cleveland, Ohio 44109. Dr. Barry's present address is Washington Hospital Center, Washington, D. C. Address requests for reprints to the Department of Metabolism, Walter Reed Army Institute of Research, Washington, D. C. 20012.

Received for publication 18 April 1967 and in revised form 14 July 1967.

collected. The ureteral catheter was then occluded for 8 min. After release of the occlusion, multiple samples of 0.4–0.7 ml volume were collected. Arterial blood samples were drawn at the beginning and end of the occlusion period. Blood pressure was monitored by a mercury manometer connected to a femoral artery cannula.

In three animals, 15–20 μc of *d*-aldosterone-1,2-^{3}H (specific activity 122 μc/μg) [1,2] was added to the priming infusion which was followed by a constant infusion of 0.35–0.50 μc/min for 65–80 min before occlusion. Two monkeys were given infusions of the labeled acid-labile conjugate [2] instead of free aldosterone. The tritium-labeled acid-labile conjugate of aldosterone was prepared by the method of Underwood and Tait (3) from the urine of a monkey to which 750 μc *d*-aldosterone-1,2-^{3}H had been administered intravenously. A priming dose of 10–15 μc administered intravenously was followed by a constant infusion of 0.1–0.2 μc/min for 35–40 min prior to occlusion. Free aldosterone was extracted with methylene dichloride from 0.2–0.4 ml aliquots of the urine samples adjusted to 2 ml volume with water at pH 6.0. To each extract, aldosterone-4-^{14}C [3] of known amount was added for recovery, and 75–100 μg of stable aldosterone was added as a marker. The extract was purified by paper chromatography in toluene-methanol-water (2:1:1) for 12–14 hr. After acetylation, the eluate was chromatographed in cyclohexane-dioxane-methanol-water (4:4:2:1) for 13–15 hr. The final eluate was counted in a three-channel liquid scintillation spectrometer to an error of less than 5%. The acid-labile conjugate in the previously extracted urine samples was determined after hydrolysis at pH 1.0 for 24 hr at room temperature by extraction, purification, and counting in a manner identical with that used for free aldosterone. Aldosterone-4-^{14}C was added before the pH was adjusted to 1.0. Additional paper chromatography in either benzene-formamide or cyclohexane-benzene-methanol-water (4:3:4:1) did not appreciably alter tritium-to-carbon-14 ratios (Table I) and were not included in the routine purification. The mean and standard deviation of the tritium-to-carbon-14 ratios for free aldosterone and the acid-labile conjugate in five aliquots of a free-flow sample were 1.66 ± 0.04 and 1.40 ± 0.07 respectively.

1–2-ml aliquots of plasma were used for the determination of the tritium-labeled aldosterone and acid-labile conjugate. The free aldosterone was determined in a manner identical with that used for urinary aldosterone, except that the methylene dichloride extract was washed with dilute alkali and water before chromatography. In the previously extracted plasma samples, the ^{3}H acid-labile conjugate of aldosterone was determined after preliminary separation of the plasma protein with ammonium sulfate and methanol-acetone by the method adapted by Luetscher et al. (7). After hydrolysis at pH 1.0 for 24 hr, the released aldosterone was extracted, purified, and counted in a manner identical with that used for free aldosterone. Total plasma aldosterone and acid-labile conjugate are measured by these methods without determination of protein binding.

Sodium was determined by flame photometry, and creatinine was determined by the method of Folin and Wu (11) as modified for the Technicon AutoAnalyzer, (Technicon Co., Chauncy, N. Y.). Inulin was determined by the method of Roe et al. (12).

Statistical analysis was done by the method of repeated measurement analysis of the variance (volume periods × animals) (13). The data were grouped by the accumulated volume period after release of stop-flow.

RESULTS

The stop-flow patterns of aldosterone-1,2-^{3}H in three monkeys are given in Figs. 1 and 2.[4] The urine aldosterone concentration is lowest in the early samples representing the distal portion of the nephron. This fact is apparent whether the

[1] New England Nuclear Corp., Boston, Mass.

[2] Stored in ethanol at −15°C and checked by paper chromatography before each experiment.

[3] Obtained from the Endocrinology Study Section, NIH.

[4] The plasma protein binding of aldosterone and its acid-labile conjugate was not measured. Binding would have no effect upon the configuration of the curves depicted, but it would decrease the amount of steroid filtered and increase the calculated U/P ratios.

TABLE I

Tritium-to-Carbon-14 Ratios after Paper Chromatography

	Tritium-to-carbon-14 ratios							
	Free aldosterone				pH-1 Released aldosterone			
Methylene dichloride extract	5.63		3.60*		11.70		5.87*	
Toluene-methanol-water	2.70*	1.69	1.68		2.82*	1.63	1.62	
Benzene-formamide	2.63		1.76		2.69		1.53	
Acetylation								
Cyclohexane-dioxane-methanol-water	2.64	2.63	1.68	1.62	2.70	2.66	1.47	1.40
Cyclohexane-benzene-methanol-water	2.61		1.69		2.65		1.33	

* Sample divided after this step.

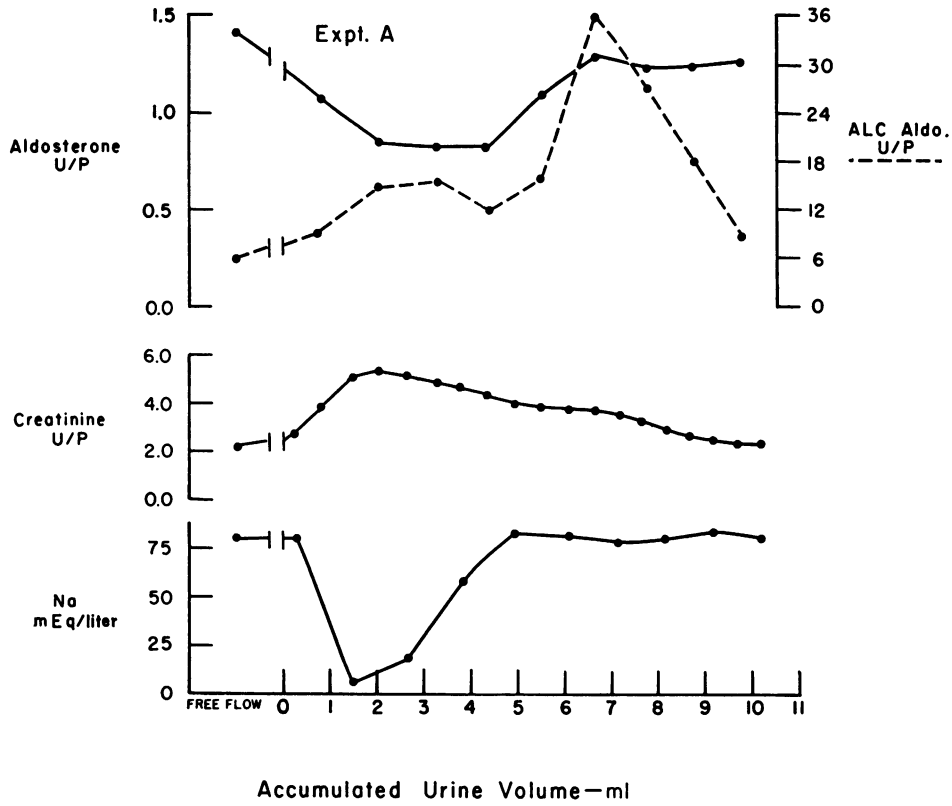

FIGURE 1 Stop-flow pattern during the infusion of aldosterone-^{3}H. Expt. A. The aldosterone and its endogenously formed acid-labile conjugate (ALC Aldo.) are expressed as urine-to-plasma ratios (U/P).

data are plotted as U/P ratio (Fig. 1) or as aldosterone U/P over inulin U/P ratio (Fig. 2) to eliminate changes in the curves because of fluid reabsorption. The aldosterone pattern is similar to that of sodium, except that the sodium concentration returned to preocclusive values before aldosterone. The aldosterone U/P ratios in the distal area are significantly different ($P < 0.01$) from the ratios in the free-flow and proximal samples.

The stop-flow patterns of the labeled acid-labile conjugate, which was formed endogenously from infused aldosterone-^{3}H, are depicted in Figs. 1 and 3. In each of the three experiments, the urine-to-plasma concentration ratios for the acid-labile conjugate are highest in samples representing the proximal portion of the nephron.[5] The rise in the proximal acid-labile conjugate ratios in the three monkeys is also significant ($P < 0.01$). It can be seen in Figs. 1 and 2 that maximal values for acid-labile conjugate ratios occur at a point which is proximal to the site of aldosterone reabsorption. The smaller increase in the U/P ratio of the acid-labile conjugate seen in the more distal samples in Fig. 1 is due primarily to fluid reabsorption as demonstrated by the simultaneous increase in creatinine concentration. When acid-labile conjugate U/P ratios are compared with inulin U/P ratios to eliminate changes due to fluid reabsorption, a slight rise is still seen in the distal samples (Fig. 3). As pointed out by Malvin (14), this small distal rise is most likely due to flow of proximal tubular fluid into the distal tubule. This flow replaces fluid reabsorbed in the distal tubule during the period of stop-flow. It is also possible that a small amount of distal tubular secretion of the acid-labile conjugate contributes to the small distal rise.

The results of two stop-flow experiments utiliz-

[5] *p*-Aminohippurate was not used as a proximal tubular marker because it markedly reduced the renal clearance of acid-labile conjugate in one subject studied by Siegenthaler, Peterson, and Frimpter (4).

ing an infusion of tritium-labeled acid-labile conjugate are given in Fig. 4. Although the proximal rise depicted is not statistically significant ($P > 0.05$) on the basis of two experiments, it is apparent that the pattern is similar to that demonstrated for the acid-labile conjugate formed endogenously during aldosterone-^{3}H infusions (Fig. 3). Free aldosterone was not detected in the plasma or urine samples, indicating the absence of significant hydrolysis of the conjugate in vivo.

DISCUSSION

The present study demonstrates that aldosterone reabsorption occurs in the distal portion of the renal tubule of the monkey. Aldosterone enhances sodium reabsorption in the distal tubule, as indicated by stop-flow studies in the dog (15) and renal clearance studies in man (16, 17). Edelman, Bogoroch, and Porter (18) have demonstrated the entry of aldosterone into the mucosal cell of the toad bladder. Entry of the hormone into the tubular cell is also a likely requirement for its biologic effect. Aldosterone entry into the cell from tubular fluid is suggested by the present observations, but aldosterone entry from the plasma has not been studied. It is also not known whether the reabsorbed aldosterone is biologically active. However, if 86% of filtered aldosterone is reabsorbed (4), and if the glomerular filtrate is ⅕th of effective renal plasma flow (19), then 17% ($0.20 \times 86\% = 17\%$) of the unbound aldosterone that enters the kidney is reabsorbed, and it is probable that this amount would influence distal tubular sodium transport.

Studies in man have shown that the renal clearance of aldosterone's acid-labile conjugate is 2 to 13 times the glomerular filtration rate (4, 5). The present observations demonstrate acid-labile conjugate secretion at the level of the proximal tubule. The site of acid-labile conjugate secretion was distinctly separated from the site of aldosterone reabsorption. The organic acid secretory system

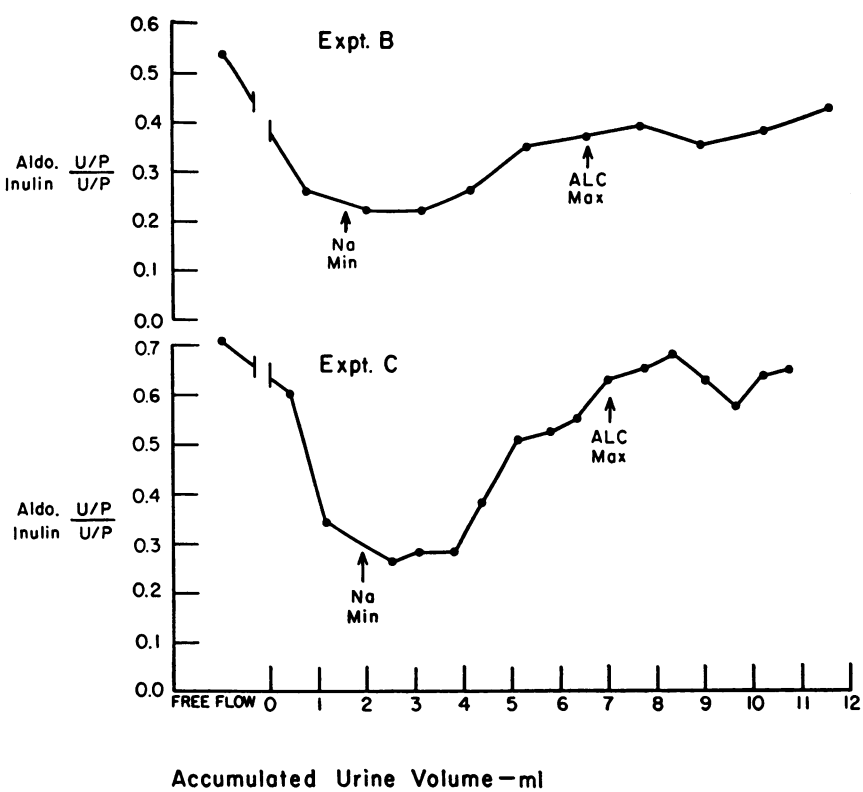

FIGURE 2 Stop-flow pattern of aldosterone-^{3}H, Expts. B and C. The aldosterone is plotted as its U/P ratio to the U/P ratio of inulin. The site of minimum sodium concentration and maximum concentration of the acid-labile conjugate are designated.

of the proximal tubule is probably utilized for acid-labile conjugate secretion, (20) since its secretion is reduced by *p*-aminohippuric acid which is known to be transported by this pathway (4). In addition, Underwood and Tait's (3) findings that this conjugate is probably a glucuronide and is more acidic than the usual steroid glucuronides are consistent with this interpretation.

Measurements of the renal extraction of aldosterone and its acid-labile conjugate in man (7–9) have shown that the kidney can convert aldosterone to its acid-labile conjugate. The comparison of the metabolism of aldosterone given orally and intravenously (8) has also revealed significant extrahepatic formation of the acid-labile conjugate. The acid-labile conjugate secreted by the proximal tubule could be primarily that conjugate which was converted from the plasma aldosterone in the tubular cell and directly secreted into the tubular fluid. The present stop-flow studies with the infusions of the ^3H acid-labile conjugate suggest that the acid-labile conjugate is transported from the plasma to the tubular fluid. These observations do not demonstrate whether or not the kidney can convert aldosterone to its acid-labile conjugate. It is probable that any conjugate formed within the proximal tubular cell would be secreted directly into the tubular fluid similar to the conjugate transported from the plasma. Cheville et al. (9) have shown that, immediately after the injection of labeled aldosterone, higher concentrations of the conjugate are found in renal-venous plasma than in renal-arterial plasma. This finding indicates that at least a portion of the acid-labile conjugate formed within the kidney enters the plasma. The cells at the distal tubular level may be the source of this conjugate entering the plasma, since aldosterone reabsorption occurs at this area without secretion of the acid-labile conjugate into the tubular fluid.

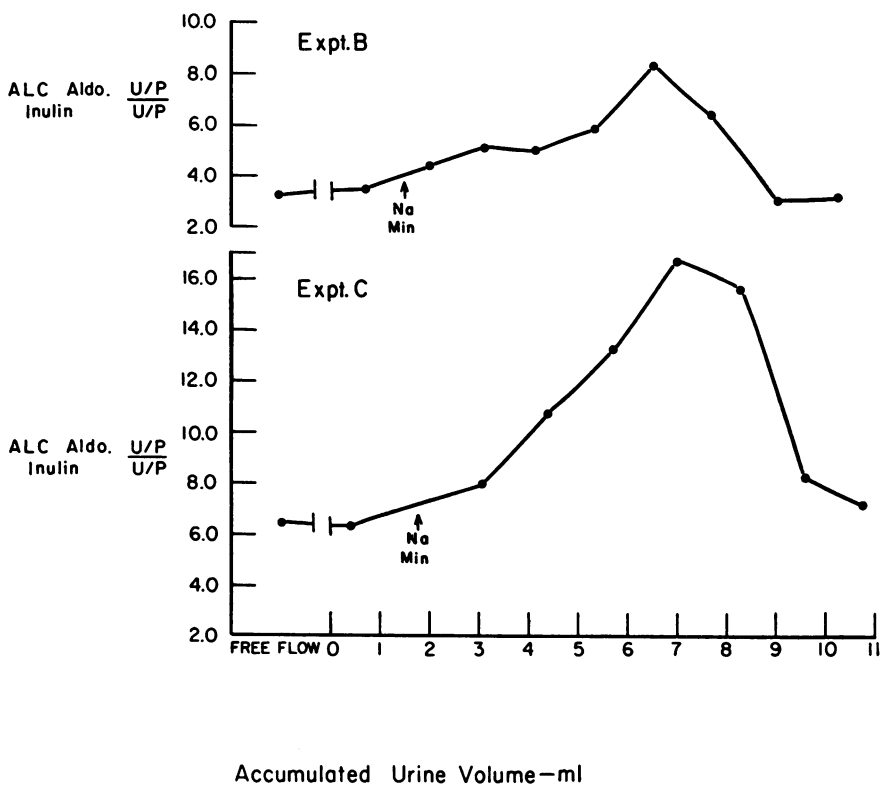

FIGURE 3 Stop-flow pattern of endogenously formed acid-labile conjugate of aldosterone. Expts. B and C. The data were obtained during the infusion of aldosterone-^3H and the curves correspond to those of Fig. 2. The site of minimum sodium concentration is designated.

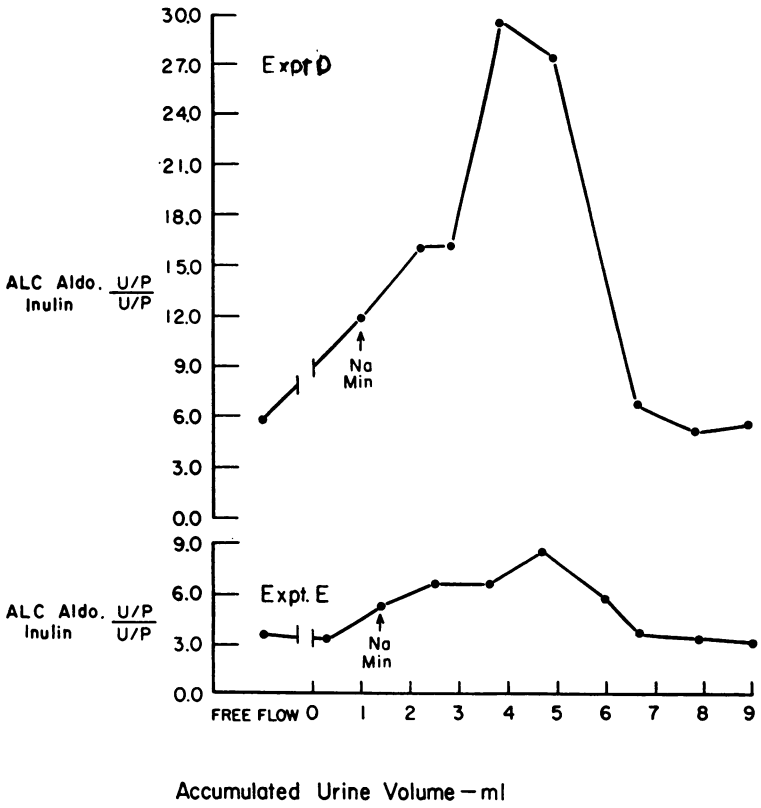

FIGURE 4 Stop-flow pattern during the infusion of the acid-labile conjugate of aldosterone-^{3}H. Expts. D and E. Displayed as in Fig. 3 Curves obtained in the absence of free aldosterone-^{3}H.

ADDENDUM

Stop-flow studies in five dogs demonstrating aldosterone reabsorption in the distal tubule and secretion of endogenously formed acid-labile conjugate in the proximal tubule have recently been reported. Deck, K. A., and W. E. Siegenthaler. 1967. Transport of aldosterone and of the acid-labile conjugate of aldosterone by tubular cells of the kidney. *Acta Endocrinol.* **55**: 648.

REFERENCES

1. Neher, R., and A. Wettstein. 1956. Physicochemical estimation of aldosterone in urine. *J. Clin. Invest.* **35**: 800.
2. Luetscher, J. A., Jr. 1956. Studies of aldosterone in relation to water and electrolyte balance in man. *Recent Progr. Hormone Res.* **12**: 175.
3. Underwood, R. H., and J. F. Tait. 1964. Purification, partial characterization and metabolism of an acid labile conjugate of aldosterone. *J. Clin. Endocrinol. Metab.* **24**: 1110.
4. Siegenthaler, W. E., R. E. Peterson, and G. W. Frimpter. 1964. A symposium organized by The Council for International Organizations of Medical Sciences established under the joint auspices of UNESCO and *WHO*. *In* Aldosterone. E. E. Baulieu and P. Robel, editors. Blackwell Scientific Publications, Ltd., Oxford. 51.
5. Gfeller, J., and W. Siegenthaler. 1965. Die renale Clearance des Aldosterons und seiner Hauptmetabolite beim Menschen. *Acta Endocrinol.* **49**: 510.
6. Deck, K. A., and W. Siegenthaler. 1965. Resorption von Aldosteron im distalen Nierentubulus. *Klin. Wochschr.* **43**: 336.
7. Luetscher, J. A., E. W. Hancock, C. A. Camargo, A. J. Dowdy, and G. W. Nokes. 1965. Conjugation of 1, 2-^{3}H-aldosterone in human liver and kidneys and renal extraction of aldosterone and labeled conjugates from blood plasma. *J. Clin. Endocr.* **25**: 628.
8. Bledsoe, T., G. W. Liddle, A. Riondel, D. P. Island, D. Bloomfield, and B. Sinclair-Smith. 1966. Comparative fates of intravenously and orally administered aldosterone: evidence for extrahepatic formation of acid-hydrolyzable conjugate in man. *J. Clin. Invest.* **45**: 264.
9. Cheville, R. A., J. A. Luetscher, E. W. Hancock, A. J. Dowdy, and G. W. Nokes. 1966. Distribution, conjugation, and excretion of labeled aldosterone in congestive heart failure and in controls with normal circulation: development and testing of a model with an analog computer. *J. Clin. Invest.* **45**: 1302.
10. Malvin, R. L., W. S. Wilde, and L. P. Sullivan. 1958. Localization of nephron transport by stop-flow analysis. *Am. J. Physiol.* **194**: 135.
11. Folin, O., and H. Wu. 1919. A system of blood analysis. *J. Biol. Chem.* **38**: 81.

12. Roe, J. H., J. H. Epstein, and N. P. Goldstein. 1949. A photometric method for the determination of inulin in plasma and urine. *J. Biol. Chem.* **178**: 839.
13. Winer, B. J. 1962. Statistical Principles in Experimental Design. McGraw-Hill Book Company, New York. 1st edition. 105.
14. Malvin, R. L. 1960. The stop-flow method and its use in studying renal function. *Progr. Cardiovascular Diseases.* **3**: 432.
15. Vander, A. J., R. L. Malvin, W. S. Wilde, J. Lapides, L. P. Sullivan, and V. M. McMurray. 1958. Effects of adrenalectomy and aldosterone on proximal and distal tubular sodium reabsorption. *Proc. Soc. Exptl. Biol. Med.* **99**: 323.
16. Yunis, S. L., D. D. Bercovitch, R. M. Stein, M. F. Levitt, and M. H. Goldstein. 1964. Renal tubular effects of hydrocortisone and aldosterone in normal hydropenic man: comments on sites of action. *J. Clin. Invest.* **43**: 1668.
17. Sonnenblick, E. H., P. J. Cannon, and J. H. Laragh. 1961. The nature of the action of intravenous aldosterone: evidence for a role of the hormone in urinary dilution. *J. Clin. Invest.* **40**: 903.
18. Edelman, I. S., R. Bogoroch, and G. A. Porter. 1963. On the mechanism of action of aldosterone on sodium transport: the role of protein synthesis. *Proc. Natl. Acad. Sci. U. S.* **50**: 1169.
19. Smith, H. W. 1951. The Kidney: Structure and Function in Health and Disease. Oxford University Press, Inc., New York. 44.
20. Weiner, I. M., and G. H. Mudge. 1964. Renal tubular mechanism for excretion of organic acids and bases. *Am. J. Med.* **36**: 743.