the response of the two groups of rats cannot be attributed to inhibitors present in the albumin solution.

The growth hormone (NIH GH S7) was a gift of the Endocrinology Study Section of the National Institutes of Health. The superb technical assistance rendered by Mrs. Rosalie Snyder is gratefully acknowledged.

1. Fain, J. N., Endocrinology **82**, 825 (1968).
2. Goodman, H. M., Proc. Third Intern. Congr. Endocrinol. Mexico City (1968). in press.
3. Wieland, O., Biochem. Z. **329**, 313 (1957).
4. Goodman, H. M., Endocrinology **83**, 300 (1968).

Received Oct. 30, 1968. P.S.E.B.M., 1969, Vol. 130.

## Aldosterone Production in Chronic Renal Failure (33685)

JOHN P. HAYSLETT, JOHN E. BOYD, AND FRANKLIN H. EPSTEIN
*Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut*

Changes in salt and water balance consequent to renal failure might be expected to involve changes in the secretion of sodium-retaining hormones. When renal mass is reduced by renal disease or by ablation of renal tissue, sodium excretion per nephron increases so that normal sodium blanace is maintained. Previous studies using micropuncture techniques in rats showed that the mechanism for this adjustment is not dependent on either the compensatory increase in glomerular filtration per remaining nephron which occurs in these circumstances, or on a reduction in intrinsic reabsorptive capacity in the proximal tubules (1). It seems likely, therefore, that the early adjustments in sodium excretion in renal failure take place in the distal tubule. One possible explanation is that aldosterone secretion is diminished. Another hypothesis is that distal absorption of sodium is decreased in renal insufficiency by an augmented load of urea per nephron. This too might modify aldosterone secretion, but in the opposite direction. For example, the obligatory losses of sodium imposed by a urea diuresis might be expected to deplete uremic individuals of salt, stimulate the secretion of aldosterone, and thus secondarily modify distal reabsorption of sodium so as to maintain sodium balance. The rate of aldosterone secretion in experimental renal failure is therefore of special interest.

*Methods.* Experiments were performed upon two groups of male Sprague-Dawley rats. One group of 10 rats with an initial weight of 212 ± 10 g (mean ± SE), served as the control group. The second group of 10 rats, initial weight 199 ± 5 g, were azotemic after ablation of approximately 85% of renal tissue. Reduction of nephron mass was accomplished in two stages. In the first stage the upper and lower poles of the left kidney were removed under ether anesthesia. Four to 5 days following this procedure, the right kidney was removed. Sham operations were performed in the control animals. Both groups were then placed on a normal diet containing 109 meq of sodium/kg and 120 meq of potassium/kg and tap water *ad libitum*. Animals from both groups were pair-fed to maintain an identical intake of sodium, since the intake of food by azotemic rats was reduced.

Fourteen days after the second stage of surgery, anesthesia was induced with sodium pentobarbital, blood was obtained from the abdominal aorta, and both adrenal glands were excised, cleaned of adherent fat, weighed, and quartered. The two adrenal glands from each rat were combined and incubated in Krebs–Ringer bicarbonate buffer with added glucose, 200 mg/100 ml, in an atmosphere of 95% $O_2$ and $CO_2$ at 36–37°. After preincubation for 30 min, the media was renewed and incubation was performed for 2 hr. The aldosterone content in the bathing medium was estimated by methods previously described (2). The rate of production

TABLE I. Summary of Data in Control Rats and Rats with Azotemia.[a]

| | Control | Azotemia | $p$ value |
|---|---|---|---|
| Sodium intake (meq/day/rat) | 1.67 ± 0.05 | 1.67 ± 0.05 | NS |
| Serum sodium (meq/liter) | 144.4 ± 0.6 | 144.5 ± 0.6 | NS |
| Serum potassium (meq/liter) | 5.5 ± 0.6 | 5.1 ± 0.8 | NS |
| Blood urea nitrogen (mg/100 ml) | 25.7 ± 1.2 | 51.4 ± 2.2 | 0.001 |
| Adrenal weight (mg/rat) | 76.9 ± 3.3 | 79.9 ± 4.8 | NS |
| Aldosterone secretion (mg/100 g of adrenal/hr) | 0.96 ± 0.08 | 0.85 ± 0.05 | NS |

[a] Values represent means ± SE.

of aldosterone measured by this method *in vitro* in normal and sodium depleted rates, corresponds closely to adrenal secretion measured *in vivo* (3, 4).

Serum values for sodium and potassium were determined on an internal standard flame photometer. The blood urea nitrogen was measured on an auto-analyzer.

*Results* (Table I). The rate of production of aldosterone *in vitro* was the same in partly nephrectomized rats (blood urea nitrogen 51.4 ± 2.2 mg/100 ml) as in their pair-fed controls (blood urea nitrogen 25.7 ± 1.2 mg/100 ml). There was also no significant difference in adrenal weight between the two groups. It should be noted that there was no systematic difference in serum sodium or potassium between the two groups of animals. The values obtained for aldosterone secretion rate (0.8–0.9 g/100 g/hr) were similar to those reported by others in normal rats, measured both *in vitro* and *in vivo*.

*Discussion.* It is clear that mineralocorticoids, including aldosterone, can reduce sodium excretion in normal subjects during intense osmotic diuresis with urea (5). In addition, mineralocorticoids can modify urinary excretion of sodium when the mass of functioning nephrons is reduced (6). When hypoaldosteronism coexists with renal failure there is an excessive excretion of sodium and retention of potassium (7). When excessive salt wasting results from intrinsic renal disease, aldosterone excretion is increased (8). Thus, there is reason to think that changes in aldosterone secretion might play a role in adjustments by remaining nephrons to renal failure. Nevertheless, the actual role of aldosterone in the maintenance of sodium balance in chronic renal insufficiency is unknown.

The model of renal insufficiency chosen in the present experiments was identical with that previously studied by micropuncture techniques. In the remnant kidney after 5/6 nephrectomy, sodium excretion per nephron is increased sixfold greater than control values, in a way that cannot be accounted for by a rise in glomerular filtration or a change in reabsorption by the proximal tubule (1). By exclusion, changes in distal tubular handling of sodium must be responsible. In addition, changes in distal tubular handling of potassium are implied by the maintenance of normal serum potassium and potassium balance after partial nephrectomy. If chronic alterations in aldosterone secretion played a role in these adjustments of remaining nephrons, they should have been detected in the present experiments. The fact that freshly excised adrenal glands from chronically uremic rats produce aldosterone at the same rate as normal pair-fed controls indicates that changes in aldosterone production are not primarily responsible for homeostatic adjustments in tubular reabsorption by remaining nephrons under these conditions. The ability of the adrenals to participate in regulating mineral excretion in renal insufficiency is not of course, excluded, but attention should, we believe, be focused on extra-adrenal mechanisms that might modify distal tubular behavior after partial nephrectomy in a way that subserves the needs of the body.

*Summary.* When renal mass is reduced, sodium excretion per remaining nephron increases so that normal sodium balance is maintained. Since this adjustment might be

expected to involve changes in the secretion of sodium-retaining hormones, the rate of production of aldosterone was estimated in rats with azotemia and in control rats. The rate of production of aldosterone, *in vitro*, was 0.85 ± 0.05 mg/100 g of adrenal/hour in rats with azotemia and 0.96 ± 0.08 mg/100 g of adrenal/hr in their paired controls. The similar rate of production in both groups indicates that changes in aldosterone secretion are not primarily responsible for the adjustments in sodium excretion in renal failure.

This work was supported by research grants from the U. S. Public Health Service (TIAM5015, HE00834, AM05954 and AM35402) and the American Heart Association.

1. Hayslett, J. P., Kashgarian, M., and Epstein, F. H., J. Clin. Invest. **47**, 46a (1968).

2. Kliman, B. and Peterson, R. E., J. Biol. Chem. **235**, 1639 (1960).

3. Marieb, N. J. and Mulrow, P. J., Endocrinol. **76**, 657 (1965).

4. Csanky, M. F. D., Van der Wal, B., and De Wied, D., J. Endocrinol. **41**, 179 (1968).

5. Platts, M. M., Clin. Sci. **30**, 453 (1966).

6. Gonick, H. C., Coburn, J. W., Rugini, M. E., Maxwell, M. H., and Kleeman, C. R., J. Lab. Clin. Med. **64**, 269 (1964).

7. Gerstein, A. R., Kleeman, C. R., Gold, E. M., Franklin, S. S., Maxwell, M. H., Gonick, H. C., Feffer, M. L., and Steinman, T. I., Nephron **5**, 90 (1968).

8. Walker, W. G., Jost, L. J., Kowarski, A., and Dunn, M. J., Johns Hopkins Med. J. **122**, 45 (1968).

Received Nov. 4, 1968. P.S.E.B.M., 1969, Vol. 130.

## Renal Tubular Secretion and Biosynthesis of Organic Acids*† (33686)

P. K. KNOEFEL AND K. C. HUANG
(With Technical Assistance of Leslie Talbott and Peter Kotcher)
*The Department of Pharmacology, University of Louisville School of Medicine, Louisville, Kentucky 40202*

The transtubular transport of organic acids in mammalian kidney involves at least a two-step process (1,2); the first step is from interstitial fluid to cell and the second step is from cell to lumen. Evidence of carrier-mediation in the first step of the process is readily obtained; however, evidence of method of transport in the second step is not readily available for study (2).

In the present investigation we took advantage of the well-established occurrence of a biosynthesis process of certain organic acids with the renal tubular cells (3, 4) to study the second step of transport process, as well as the interrelationship of these two processes, biosynthesis and tubular transport.

*Experimental Methods.* Mongrel dogs of both sexes were used. Using the *in vitro* kidney slice technique, the dog was ether anesthetized and one kidney was removed from the animal and packed in frozen saline. A circular core 15 mm in diameter was cut from the kidney in sagittal section and chilled for 5 min. It was then placed on a Riggs-Stadie slicer which was modified with a screw device for advancing the tissue and sections cut by hand from papilla to cortex. Each slice, averaging 0.3–0.6 mm in thickness, was alternately placed in Erlenmeyer flasks, each of which contained 5 ml of phosphate Ringer solution with either 0.075 m*M* *m*-aminohippuric acid (MAH) or 1.25 m*M* *m*-aminobenzoic acid (MAB) and 10 m*M* glycine. The flasks were gassed with $O_2$–$CO_2$ (95–5%) and incubated at 25°. One hr later the slices were removed, blotted, and homogenized with 5% trichloroacetic acid. The medium was centrifuged and diluted with trichloroacetic acid. Details of the procedure and preparation of Ringer

* Aided in part by grants from the American Heart Association, USPHS (AM-02217-11) and NSF (GB-4774).

† A preliminary communication was presented to the 23rd Intern. Congr. Physiol. Sci. Tokyo, 1965.