cells, one-quarter was associated with microsomes, and a small fraction recovered from mitochondria. This distribution pattern is similar to that reported for total lipids, and indicates that the intracellular location of DPPD may be passively determined by its lipid solubility. Alpha-tocopherol, on the other hand, was found predominantly in mitochondria.

1. Draper, H. H., Goodyear, S., Barbee, K. D., Johnson, B. C., *Brit. J. Nutr.*, 1958, v12, 89.

2. Draper, H. H., Csallany, A. S., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 739.

3. Draper, H. H., Alaupovic, P., *Fed. Proc.*, 1959, v18, 218.

4. Pudelkiewicz, W., Potter, L. M., Matterson, L. D., Singsen, E. P., *Poultry Sci.*, 1956, v35, 959.

5. Teichman, R., Dehority, B. A., *J. Dairy Sci.*, 1959, v42, 874.

6. Schneider, W. C., *J. Biol. Chem.*, 1948, v176, 259.

7. Ma, T. S., Zuazaga, G., *Ind. Eng. Chem., Anal. Ed.*, 1942, v14, 280.

8. National Alfalfa Dehydrating and Milling Co., Lamar, Colo., Technical data on preservation of carotene in dehydrated alfalfa, p22.

9. Clement, G., Clement, J., Le Breton, E., in *Biochemical Problems of Lipids*, Interscience Publishers, N. Y., 1956.

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Water Diuresis Stop Flow Analysis in the Dog.* (25972)

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When the technic of stop flow analysis was described(2,3) it appeared to offer a means to evaluate more fully the mechanism for control of urea excretion by the kidney. Our data from urea stop flow analyses performed during osmotic diuresis were difficult to interpret. Accordingly, we modified the method using water diuresis. This did not aid in interpretation of the urea data but did provide interesting findings concerning stop flow analysis. During water diuresis stop flow analyses the sodium U/P minimum appeared near the peak of the para-aminohippurate (PAH) curve and potassium and osmolal U/P maxima were distal to the sodium U/P minimum.

Method. The following procedure was found successful in producing water diuresis with rates of urine flow of 6 to 11 ml/min./kidney in the pentobarbital anesthetized dog. Alcohol was administered to the dog in drinking water or by intravenous route. The dog was then anesthetized with pentobarbital. Four hundred ml water were administered by gavage. An infusion of 0.4% NaCl and 2% glucose in water was administered by intravenous route with 10 g alcohol in each liter of infusion. The left ureter was cannulated avoiding the use of cautery. After diuresis had become brisk PAH and creatinine were added to the infusion. When rate of urine flow had become stable and greater than 6 ml/min./kidney the control clearance collections were begun.

Three 10 minute control clearance urine collections were obtained before, and 2 after, the stop flow procedure. The ureteral cannula was occluded for 8 minutes with inulin given by intravenous route 2 minutes before release of the occlusion. Upon release of the occlusion rapid serial collections were obtained from the ureteral cannula for a period of 3 minutes. Heparinized arterial blood samples were obtained at the mid-point of each control clearance urine collection and at time of occlusion, release of occlusion, and termination of the stop flow serial collections.

Osmolalities were performed with a microthermister using the Fiske osmometer. Determinations were made on urine and plasma

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samples for concentration of inulin using Schreiner's modification of the resorcinol method(4), of creatinine using Hare's method (5) without Lloyd's reagent, of PAH using Selkurt's method with ethylenediamine dihydrochloride(6), and of sodium and potassium using a flame photometer.

When osmolalities were performed the volume of the sample remaining was too small for all analytical procedures to be carried out on the same animal. Accordingly, 19 dogs were studied and selected comparisons were made. Some of the dogs were given one liter of saline the day before the study.

Water diuresis was considered to be established when the osmolal U/P ratio was well below 1.0. New filtrate was considered to be attained when inulin concentration reached 50% of the inulin peak in the serial stop flow collection samples(3).

Results. Creatinine U/P in the distal nephron collections increased as much as 5- to 7-fold above the free flow creatinine U/P, as opposed to 1.5- to 3-fold increase during osmotic diuresis stop flow. PAH concentrations in the serial stop flow urine collection samples were corrected to the creatinine concentration to construct the curve of the PAH concentration peak in the stop flow samples. This was done with the assumption that the increased creatinine concentration in the distal nephron samples was secondary to water removal.

The lowest sodium U/P ratio was found to occur near the PAH concentration peak (Fig. 1). Maximum potassium U/P occurred distal to the para-aminohippurate peak and the lowest sodium U/P (Fig. 2).

During water diuresis stop flow analysis there occurred demonstrable changes in osmolal U/P in the stop flow samples. Osmolal U/P increased in the distal nephron samples to as high as 1.25. In Fig. 3 data are presented from a dog without saline administered before the study showing the peak of the osmolal U/P to occur distal to the PAH peak and the sodium U/P minimum. The sodium U/P was lower than in the dogs that had received saline illustrated in Fig. 1 and 2. Changes in sodium U/P ratio in the serial stop flow urine collections were accordingly less pronounced.

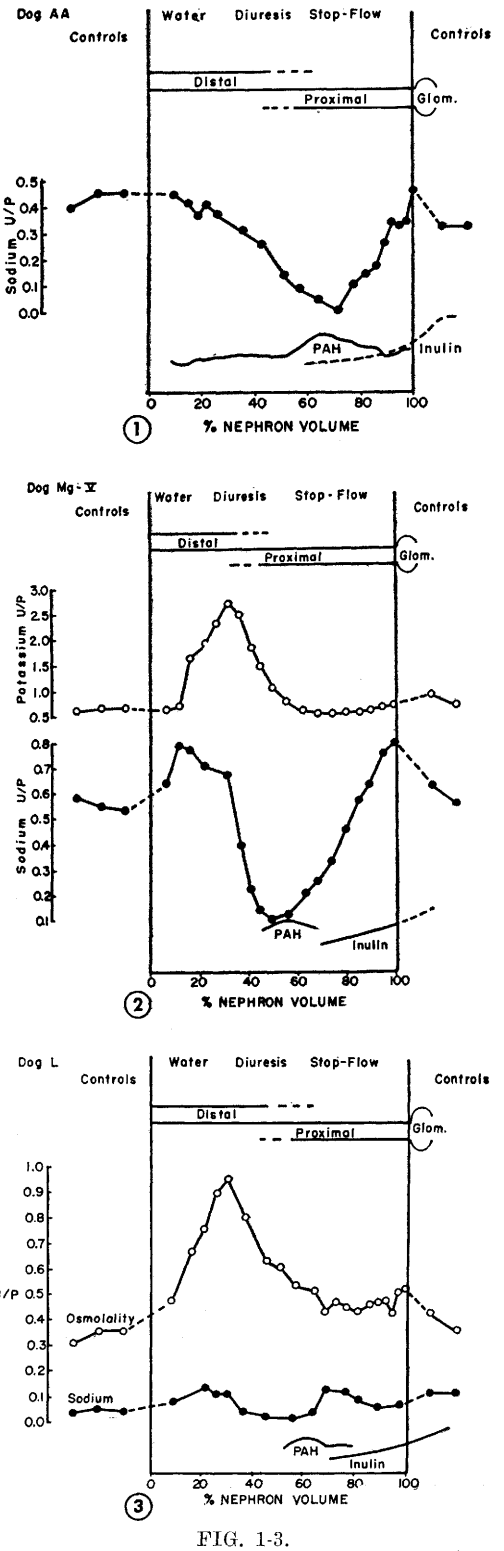

FIG. 1-3.

The nephron volume, defined as volume of serial stop flow urines obtained prior to appearance of new filtrate, was 8 to 15 ml during water diuresis stop flow as compared to 15 to 24 ml during osmotic diuresis stop flow analyses in our laboratory.

Discussion. The apparently large volume of filtrate reabsorbed during occlusion made interpretation of the data difficult. When a large volume of filtrate is reabsorbed and replacement by filtration is increased it is possible that the PAH peak might be "smeared" down the nephron to the site of the distal nephron. If this were the case PAH would not function as a marker for proximal nephron collections. In accord with the smaller nephron volume during water diuresis stop flow analyses, volumes of urine obtained prior to appearance of the PAH peak and the peak of the potassium U/P were less than found during osmotic diuresis stop flow analyses. Thus, if "smearing" occurred down the nephron it occurred in the distal as well as the proximal nephron and would not explain the presence of the sodium U/P minimum occurring near the PAH peak.

It would be conceivable that during water diuresis stop flow analysis there was a smaller mass of filtrate in the proximal nephron and sodium concentration could be decreased to a greater degree than could occur by removal of the same quantity of sodium from the larger mass of filtrate present in the proximal nephron(7) during osmotic diuresis stop flow. Against this explanation was the occurrence of the PAH peak near the mid-point of nephron volume.

A third possibility would be that active sodium reabsorption did occur in the proximal nephron and was demonstrable during water diuresis stop flow analysis but during osmotic diuresis stop flow analysis was masked by the heavy osmotic load.

The occurrence of the increase in osmolal U P in the distal nephron demonstrated the distal removal of water in absence of antidiuretic hormone.

The differences found in osmolal U/P and sodium U/P curves in osmotic diuresis and water diuresis stop flow analyses amplified the problems encountered in interpretation of stop flow analysis data.

Summary. During water diuresis stop flow analysis the nephron volume is less than during osmotic diuresis stop flow analysis, there is a demonstrable increase in osmolal U/P in the distal nephron samples, and sodium U/P minimum occurs near the PAH peak. During water diuresis stop flow analysis active proximal reabsorption of sodium is demonstrable or para-aminohippurate is not a valid marker of proximal nephron samples.

1. Murdaugh, H. V., Jr., Galloway, R. E., Hayes, E. H., *The Physiologist,* 1959, v2, 86.
2. Malvin, R. L., Sullivan, L. P., Wilde, W. S., *ibid.,* 1957, v1, 58.
3. Malvin, R. L., Wilde, W. S., Sullivan, L. P., *Am. J. Physiol.,* 1958, v194, 135.
4. Schreiner, G. E., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 117.
5. Hare, R. S., *ibid.,* 1950, v74, 148.
6. Selkurt, E. E., *Meth. Med. Res.,* 1948, v1, 191.
7. Boylan, J. W., Antkowiak, D., *The Physiologist,* 1959, v2, 15.

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Effect of Hypotension on K Excretion in the Dog.* (25973)

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Infusion of K salts following oral administration of KCl, raises the clearance of K (C_K) above the filtration clearance(1). This demonstrates tubular secretion of K and it has been assumed that this process accounts for urinary K, with all the filtered K having been absorbed(2). According to this idea, glomerular filtration would have a minimal influence

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