Journal of Clinical Investigation
Vol. 45, No. 12, 1966

Micropuncture Study of the Effect of Various Diuretics on Sodium Reabsorption by the Proximal Tubules of the Dog *

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The precise sites of action of various diuretics within the nephron have been the subject of numerous investigations and considerable debate for several decades. Inhibition of sodium transport in the proximal tubule has, at one time or another, been considered to play a major role in the action of virtually every group of diuretics. However, such actions on the proximal tubules have been inferred only from indirect evidence. The technique of micropuncture allows a more direct evaluation of the effects of pharmacologic agents on sodium reabsorption by the proximal tubules.

The exploration by micropuncture technique of the effects of diuretics on various portions of the nephron is as yet very limited. Carbonic anhydrase inhibitors have been shown to impair acidification in the proximal convoluted tubules in the rat (1, 2). Recently Deetjen (3) and Malnic, Vieira, and Enokibara (4) have examined the effect of the new sulfonamide diuretic furosemide on fluid reabsorption in the proximal tubule.

For the detection of the effects of interventions on reabsorption by the tubules, a considerable advantage accrues from repeated collections from the same site in the tubules, a procedure used in recently reported studies (5). Identical convolutions of proximal tubules are sampled during each experimental phase, and changes in sodium reabsorption as measured by tubule fluid to plasma inulin ratios are compared for each tubule. Repeated collections from the same tubules do not appreciably alter tubule fluid reabsorption during continued hydropenia or continued saline infusion.

Acute saline infusion, however, markedly depressed sodium reabsorption by the proximal tubule, an effect that was independent of changes in glomerular filtration rate and occurred in the presence of high circulating levels of mineralocorticoid hormones and vasopressin. This effect of acute salt loading on reabsorption in the proximal tubule is thought to be mediated by some unknown humoral agent (5–7) and has also been shown to occur in the rat (8). The reabsorption of fluid undergoes a considerable redistribution from proximal to distal segments after saline infusion. This makes it difficult to assess the localization of the action of a diuretic from the magnitude of the diuresis produced, since the fraction of the filtered sodium that is reabsorbed in the various nephron segments may vary so extensively. It furthermore makes necessary careful attention to the state of hydration in evaluating any changes detected in reabsorption in the proximal tubule, since differences in hydration can in themselves produce marked changes in reabsorption.

In the studies to be reported here we have used the technique of collections from the same tubule segments in dogs before and after administration of diuretics. In most of the studies, the dogs were hydropenic throughout, but a few were infused with saline before the control periods, and in others sufficient isotonic saline solution was administered to replace losses due to diuresis. Although the methods were sensitive enough to detect the effects of small doses of mannitol, none of the other diuretics was found to yield significant depression of reabsorption in the proximal convoluted tubule.

* Submitted for publication May 24, 1966; accepted August 30, 1966.

Presented in part at the Eastern Section of the American Federation for Clinical Research, New York, N. Y., December 3, 1965.

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Methods

Studies were performed on mongrel dogs weighing 10 to 30 kg. The anesthesia, inulin infusion, and surgical preparation of the dogs for clearances and micropuncture were identical to those previously reported (5, 9, 10). Collections from convolutions of proximal tubules were first made during a control period, and the precise site of micropuncture was carefully marked in each case with nigrosine dye layered on adjacent tubules. A dose of a diuretic agent sufficient to produce a maximal [1] effect was then administered intravenously. This initial dose was followed by an infusion delivering the same amount of drug per hour. When the urine flow had increased to several times the control rate, samples were obtained from the same proximal tubule convolutions. Collections of fluid from convolutions of proximal tubules were made as described previously (5). The micropipette, filled to the tip with colored mineral oil, was inserted into a tubule, and a small amount of oil was injected into the lumen. The oil drop was permitted to flow distally, and tubule fluid was allowed to flow spontaneously into the pipette. After the administration of diuretics, the intratubular pressure was increased; consequently, the samples were analyzed only if the oil drop remained stationary or moved distally while in view. In this way, the possibility of retrograde collections was averted. Inulin in plasma and urine was determined by the anthrone method of Führ, Kaczmarczyk, and Krüttgen (12), and filtration rates were calculated for 15-minute periods during the micropuncture procedure. Inulin in tubule fluid was determined by a modification of the microanthrone method (13, 14) as previously described in detail (5); the method yielded reproducibility with a standard deviation of $\pm 4.7\%$. Tubule fluid to plasma (TF/P) inulin ratios were calculated with the plasma inulin interpolated to the midpoint of the sample collection when there were changes in plasma inulin concentration.

Controls. The results of control re-collection during continued hydropenia (thirsted 18 hours) or continued saline infusion have been reported (5). Additional controls obtained during continued saline infusions from seven dogs are included in this report. Dogs were again infused with 0.4 ml isotonic saline per kg per minute for 30 minutes and then 0.2 ml per kg per minute to the conclusion of the experiment.

Reduced filtration rate. The results of reduced filtration rate on proximal TF/P inulin ratios during continued saline infusion have also been reported (5). Similarly, filtration rate was reduced in seven hydropenic dogs by clamping the left renal artery with a screw clamp. Urine flow was reduced and the previously punctured tubules were resampled. The kidney after clamping was smaller and softer. The tubules appeared narrowed, and it took longer to collect a sufficient volume of tubule fluid.

[1] The case of furosemide is an exception. Greater responses than those obtained in these studies have been reported to occur with the administration of 100 mg per kg (11).

The following experiments with diuretics were conducted.

Mannitol. Graded intravenous infusions of mannitol were used to assess the sensitivity of the technique for the detection of depressed fluid reabsorption by the proximal convoluted tubules. Mannitol was infused into a leg vein at various flow rates (2 to 10.7 ml per minute) and at varying concentrations (5, 10, and 20%) in eight hydropenic dogs. This resulted in the excretion of 2 to 46% of the volume of glomerular filtrate (V/GFR) during resampling of previously punctured tubules. In four hydropenic dogs the repeat collections were done in periods when V/GFR fell in the range of 10 to 46%. In four hydropenic dogs, repeat collections were made when urine flow was 2 to 9% of glomerular filtration rate.

Ethacrynic acid. Initial collections were performed in six hydropenic dogs. Ethacrynic acid [2] was then administered intravenously in doses of 1 to 5 mg per kg with an equal hourly infusion rate. In five dogs, initial collections were performed after initial saline loading as described for the saline controls. Ethacrynic acid was then administered as above, and the fluid loss resulting from the diuresis was replaced with intravenous infusion of an equal volume of isotonic saline solution. A single experiment in which the initial collections were carried out in hydropenia but in which the fluid losses were replaced with isotonic saline was combined with this latter group.

Chlormerodrin. Three dogs were loaded with 10 g ammonium chloride 18 hours before the experiment. Control collections were performed in hydropenia, and then chlormerodrin was administered intravenously as a priming dose of 2 mg Hg per kg with an equal hourly infusion rate. Collections were repeated when diuresis had increased the flow rate severalfold over control values. In three dogs initial collections were obtained during saline loading as described and then chlormerodrin was administered as above. The losses due to diuresis were replaced by the intravenous infusion of roughly equal volumes of isotonic saline solution. In one dog, the diuretic-induced fluid losses were similarly replaced after initial collections were performed in hydropenia.

Furosemide. Furosemide [3] was studied in four hydropenic dogs. In two animals furosemide was administered as an initial dose of 5 mg per kg followed by an infusion of the same dose each hour. The other two animals received five times this dose. No attempt was made in the furosemide experiments to preserve the animal's sodium balance with isotonic saline infusion.

Hydrochlorothiazide. In three dogs, initial collections were performed in hydropenia. Hydrochlorothiazide was administered at a dose of 2 mg per kg initially, followed by 2 mg per kg per hour. The fluid losses from diuresis were not replaced in these experiments.

[2] 2,3-Dichloro-4-(2 methylene-butyryl)-phenoxyacetic acid supplied by Merck, Sharp and Dohme Research Laboratories, West Point, Pa.

[3] 4-Chloro-N-(2 furylmethyl)-5 sulfonylanthranilic acid supplied by Dr. Raymond L. Moreland, Lloyd Brothers, Inc., Cincinnati, Ohio.

Acetazolamide. Initial collections were performed in six hydropenic dogs. Acetazolamide was administered as an initial dose of 10 mg per kg and infused at a rate of 15 mg per kg per hour. Collections were repeated almost immediately after initial drug administration because of the brief diuresis that generally ensued.

Results

Control. Figure 1 illustrates 56 paired collections from 13 dogs, previously reported in part (5), during continued saline infusion. Initial TF/P ratios are plotted on the abscissa. The ratio of the TF/P of the second collection to that of the first is plotted on the ordinate. An ordinate value of 1.0 indicates no change in fractional reabsorption of glomerular filtrate between collections

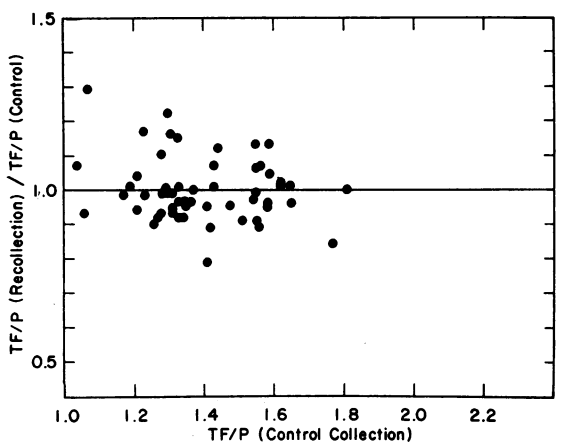

FIG. 1. EFFECT OF RE-COLLECTION ON PROXIMAL TUBULE TO PLASMA RATIO (TF/P) OF INULIN DURING CONTINUED SALINE INFUSION IN 13 DOGS.

and is indicated by the horizontal line. Values greater and less than 1.0 represent, respectively, increased and decreased fractional reabsorption by the proximal tubules up to the point of collection. The ratios $(TF/P)_2/(TF/P)_1$ average 1.00 with a standard deviation of 0.09 and a standard error of 0.012. Sixty-three pairs of collections previously reported (5) from seven dogs during continued hydropenia resulted in a mean value of 0.97 (SE $\pm$ 0.013).

Reduced filtration rate in hydropenia. Figure 2 illustrates the data from 43 paired collections during left renal artery clamping in seven hydropenic dogs. Filtration rate reductions, indicated by the symbols, averaged 50%. The mean value of $(TF/P)_2/(TF/P)_1$ was 1.00 (SE $\pm$ 0.024).

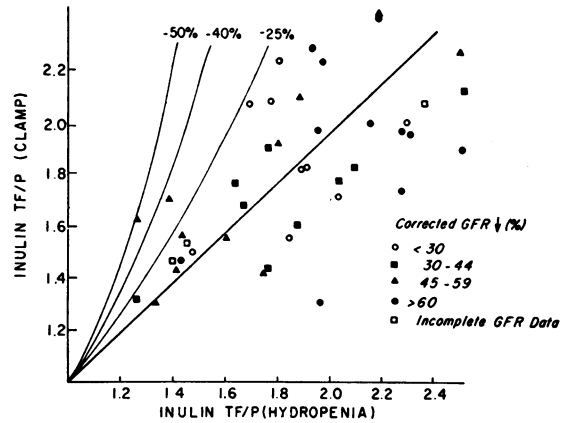

FIG. 2. EFFECT OF REDUCED FILTRATION RATE (RENAL ARTERY CLAMPING) DURING HYDROPENIA ON PROXIMAL TF/P INULIN IN SEVEN DOGS. Corrected glomerular filtration rate (GFR) decrease is per cent decrease in experimental kidney inulin clearance compared to control kidney and corrected for any difference between experimental and control kidneys during control periods. Curves represent predicted value of the ratio if absolute rate of reabsorption had remained constant when GFR was reduced by the indicated percentage.

Mannitol infusions. Graded infusion of 5 to 20% mannitol at varying rates resulted in urine flows ranging from 2 to 46% of the volume of glomerular filtrate. Figure 3 illustrates 29 paired collections from four hydropenic dogs with V/GFR ranging from 10 to 46% (mean 21%) and grouped according to the symbols indicated. Reabsorption was depressed in all. The effect of large amounts of mannitol on isotonic fluid reab-

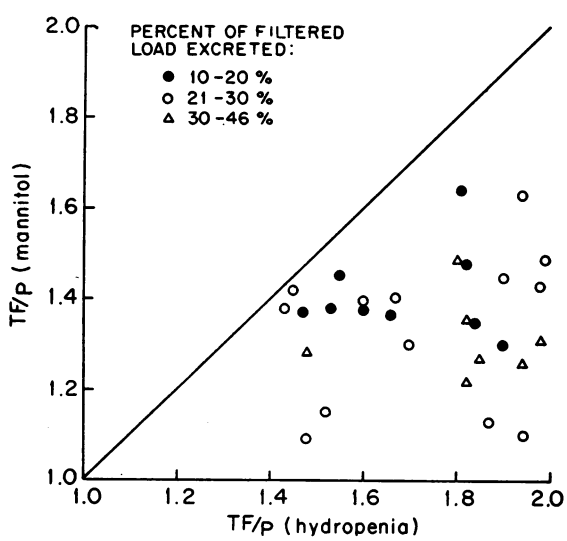

FIG. 3. EFFECT OF MASSIVE MANNITOL INFUSIONS ON PROXIMAL TF/P INULIN IN FOUR DOGS.

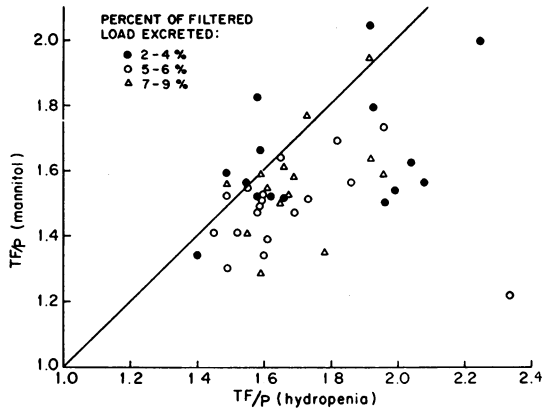

FIG. 4. EFFECT OF SMALL MANNITOL INFUSION ON PROXIMAL TF/P INULIN IN FOUR DOGS.

sorption by the proximal tubules was thus readily detected.

Lesser amounts of mannitol were administered to four hydropenic dogs, resulting in values of V/GFR of 2 to 9% (mean 5%). The results are shown in Figure 4, grouped according to the value of V/GFR. At the lowest rate of urine flow, 2 to 4% of GFR, indicated by the closed circles, no depression was detectable except in those instances with higher initial values of TF/P. The latter presumably represent collections from more distal portions of the proximal tubule where, as a result of increasing mannitol concentration, more interference with reabsorption might be expected. However, some caution is required in this interpretation since any error which tends to give too high a value in the first sample collected will also tend to give a low ratio of $(TF/P)_2/(TF/P)_1$. In the experiments with higher rates of urine flow, TF/P inulin ratios are more clearly depressed. The mean value of all 46 paired collections was 0.922 (SE $\pm$ 0.012). The difference from the results in continued hydropenia is highly significant ($p < 0.005$).

Ethacrynic acid. Six dogs received ethacrynic acid after the initial control period of hydropenia. The diuresis after ethacrynic acid administration began almost immediately and was nearly maximal about 30 minutes later. Collections were made 30 to 90 minutes after the initial dose of ethacrynic acid. Urine flow was increased by 3 to 16% (mean 7%) of the GFR during the periods in which re-collections were made. Figure 5 illustrates that the majority of the values of TF/P were increased after ethacrynic acid administration. In some instances, substantial increases in reabsorption occurred. The mean ratio $(TF/P)_2/(TF/P)_1$ for 46 sample pairs was 1.37 (SE $\pm$ 0.086), a value significantly higher than that in the hydropenic controls ($p < 0.001$).

Figure 6 illustrates results from six dogs in which the initial collections were performed in saline diuresis in five (closed circles) and in hydropenia in one (open circles), and in which the fluid losses resulting from the diuresis were replaced by intravenous infusions of isotonic saline solution. Ethacrynic acid, with an initial dose and hourly infusion rate of 1 to 2 mg per kg, resulted in urine flows that were 7 to 24% (mean 19%) of the volume of glomerular filtrate. The ratios are now scattered about 1.0 with a mean value for 35 pairs of 1.013 (SE $\pm$ 0.017), a value not significantly different from the saline controls. Thus, no depression of proximal sodium reabsorption after ethacrynic acid was observed in hydropenia or saline infusion, and the increased reabsorption

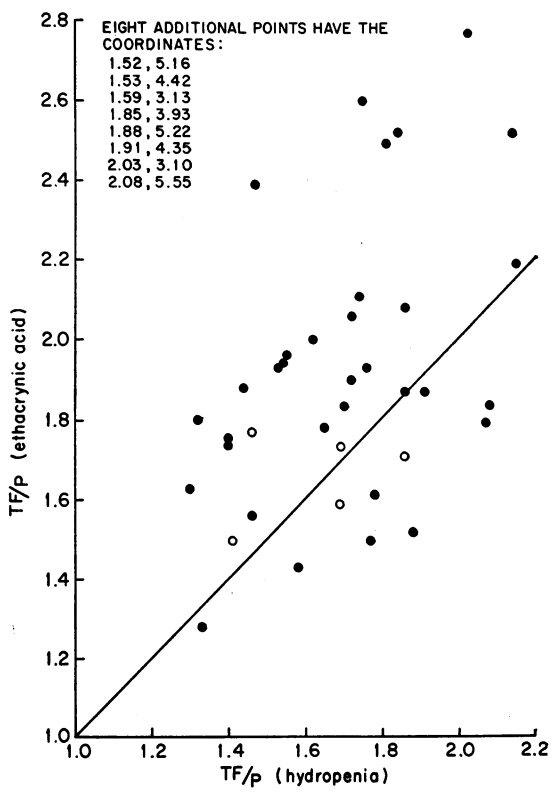

FIG. 5. EFFECT OF ETHACRYNIC ACID (ECA) ON PROXIMAL TF/P INULIN IN SIX HYDROPENIC DOGS. Solid dots, 1 mg per kg; open circles, 5 mg per kg.

seen in hydropenia was absent when fluid losses owing to the diuresis were replaced.

Chlormerodrin. Figure 7 illustrates the effects of chlormerodrin in seven dogs. The closed circles represent results from four ammonium chloride-loaded dogs with initial collections in hydropenia and repeat collections after chlormerodrin but with no replacement of the diuretic fluid losses. V/GFR was 3 to 12% (mean 7%) for the experimental kidney. Most of the values of $(TF/P)_2/(TF/P)_1$ are above unity, and the mean value for 28 sample pairs was 1.20 (SE $\pm$ 0.049), a highly significant increase compared to the hydropenic controls ($p < 0.001$).

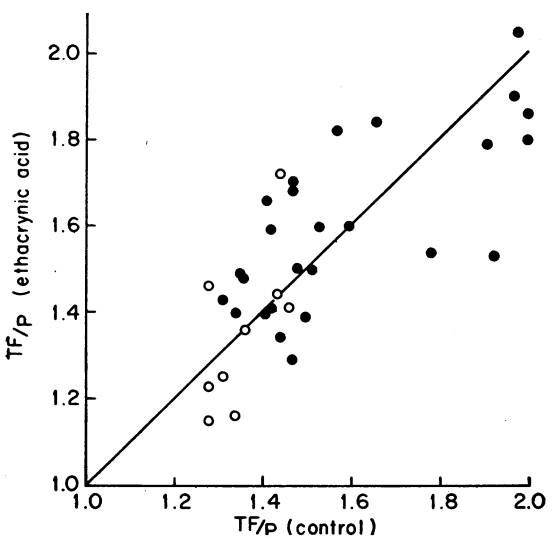

FIG. 6. EFFECT OF ECA AND SALINE REPLACEMENT ON PROXIMAL TF/P INULIN IN SIX DOGS. Solid dots, control given saline; open circles, control hydropenic.

The open circles and triangles are the results of 23 pairs of collections from three dogs in which initial collections were performed in hydropenia and saline infusion, respectively. The fluid losses due to chlormerodrin were replaced with saline infusion in each case. Fractional excretion of glomerular filtrate volume ranged from 7 to 15% for the experimental kidney. Points indicate a distribution closer to the unity line, and the mean value was 1.06 (SE $\pm$ 0.028), a smaller though still significant increase ($p < 0.05$) when compared to the results in saline controls. There is nothing to suggest that chlormerodrin depressed the reabsorption of sodium in the proximal tubule in any of these animals. The increased reabsorption observed in hydropenia was reduced when the fluid losses were replaced.

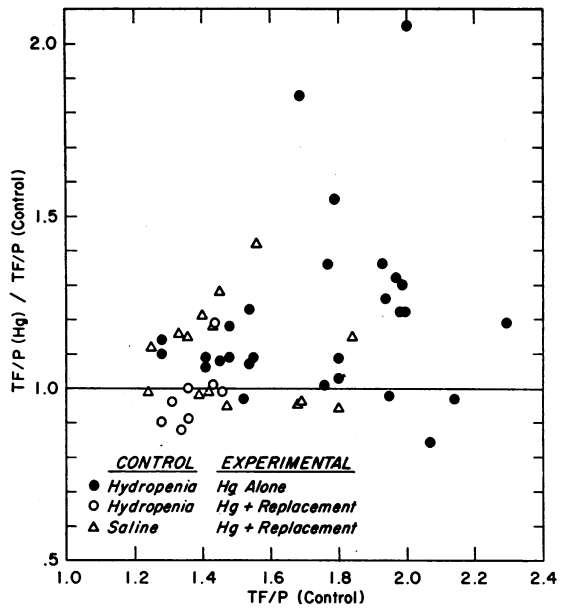

FIG. 7. EFFECT OF CHLORMERODRIN (HG) ON PROXIMAL TF/P INULIN IN SEVEN DOGS.

Furosemide. The results obtained in four hydropenic dogs given furosemide are shown in Figure 8. Diuresis was immediate in onset and nearly maximal 30 minutes after the initial dose. No replacement of the fluid losses was carried out. The closed and open circles represent, respectively, dosages of 5 and 25 mg per kg. V/GFR after furosemide ranged from 6 to 20% (mean 11%).

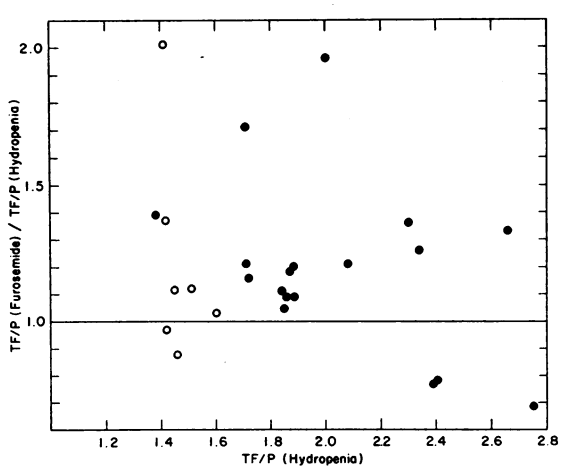

FIG. 8. EFFECT OF FUROSEMIDE ON PROXIMAL TF/P INULIN IN FOUR DOGS. Solid dots, 5 mg per kg; open circles, 25 mg per kg.

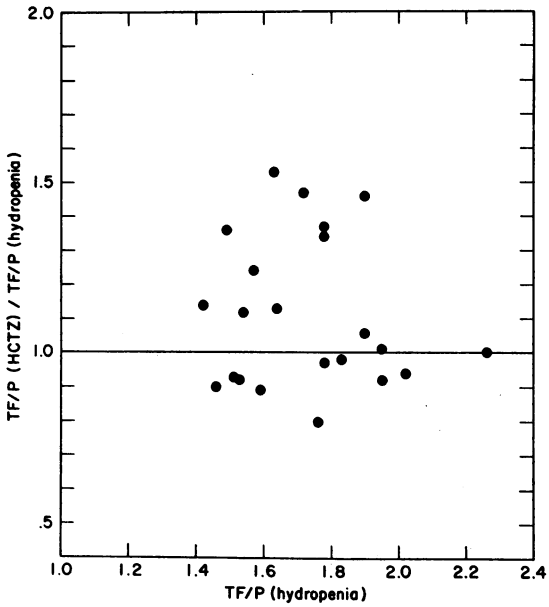

FIG. 9. EFFECT OF HYDROCHLOROTHIAZIDE (HCTZ) ON PROXIMAL TF/P INULIN IN THREE DOGS.

Most values of $(TF/P)_2/(TF/P)_1$ are higher than 1.0 with some substantial increases in reabsorption. The mean $(TF/P)_2/(TF/P)_1$ for 26 pairs was 1.20 (SE ± 0.061), a highly significant increase compared to the hydropenic controls ($p < 0.001$).

Hydrochlorothiazide. Figure 9 illustrates the results of 21 pairs of collections in three hydropenic dogs given hydrochlorothiazide. No replacement of the fluid losses was attempted, and the fractional excretion of glomerular filtrate volume increased to a modest 2 to 4% (mean 2.2%). TF/P inulin ratios increased, and the mean value of 1.13 (SE ±.046) was significantly elevated compared to hydropenic controls ($p < 0.001$).

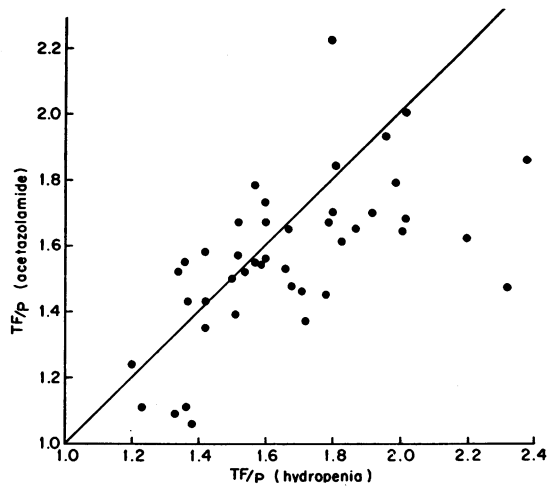

FIG. 10. EFFECT OF ACETAZOLAMIDE ON PROXIMAL TF/P INULIN IN SIX DOGS.

Acetazolamide. Administration of acetazolamide to six hydropenic dogs resulted in urine flows that were 1 to 6% (mean 4%) of GFR. Figure 10 illustrates 43 sample pairs after aceta-

TABLE I

Summary of inulin data

Experiment	Dogs	N	$\frac{(TF/P)_2}{(TF/P)_1}$* ±SE	Mean changes in GFR†
Saline control	13	56	1.00 ± 0.012	+ 5
Hydropenia control	7	63	0.97 ± 0.013	+ 5
Hydropenia clamp vs. hydropenia	7	43	1.00 ± 0.024	−50‡
Mannitol (V§/GFR >10%) vs. hydropenia	4	30	0.81 ± 0.022‖	−26
Mannitol (V/GFR <10%) vs. hydropenia	4	48	0.92 ± 0.012‖	−15
Ethacrynic acid vs. hydropenia	5	43	1.37 ± 0.086‖	−35
Ethacrynic acid+replacement vs. saline	7	43	1.01 ± 0.017	−11
Chlormerodrin vs. hydropenia	4	28	1.20 ± 0.049‖	−14
Chlormerodrin+replacement vs. saline	3	23	1.06 ± 0.028¶	+ 2
Furosemide vs. hydropenia	4	26	1.20 ± 0.061‖	−24
Hydrochlorothiazide vs. hydropenia	3	21	1.13 ± 0.046‖	0
Diamox vs. hydropenia	6	44	0.95 ± 0.017	−19

* TF/P = tubule fluid to plasma inulin ratio; second collection is divided by the first.

† GFR = glomerular filtration rate; per cent change in experimental kidney inulin clearance during re-collection periods.

‡ Per cent decrease in experimental kidney inulin clearance compared to control kidney and corrected for any difference between experimental and control kidneys during control periods.

§ V = urine flow.

‖ $p < 0.01$.

¶ $p < 0.05$.

zolamide. The mean value of $(TF/P)_2/(TF/P)_1$ (0.947 ± 0.017) was significantly below 1.0 but not significantly lower than that obtained in the control studies in hydropenic animals.

Table I summarizes the TF/P inulin values and mean per cent filtration rate changes for all the studies in this report. Significant reductions in filtration rate occurred in almost all diuretic experiments.

Discussion

The recent observation that isotonic and hypertonic saline infusions in the dog lead to depressed sodium reabsorption by the proximal tubules regardless of filtration rate changes (5) has important implications for any study of diuretics and clarifies some previous observations. It was found that although depressed reabsorption resulting from the infusion of sodium chloride solutions might permit as much as an additional 25% of the glomerular filtrate to escape reabsorption in the proximal convoluted tubule, only about 7% of the filtered sodium was excreted in the urine. Thus, depressed reabsorption in the proximal nephron must be accompanied by increased reabsorption at a more distal site to account for the actual urinary sodium excretion. Relevant to this is the observation of Goldberg, McCurdy, and Ramirez (15) that negative free water generation rises progressively with increased saline loading and does not reach a limiting value. From this, one may infer that a diuretic whose sole site of action was in the proximal convoluted tubule would result in only a modest diuresis. A considerable proportion of the isosmotic fluid rejected in the proximal tubules would be reabsorbed in more distal parts of the nephron, as when proximal reabsorption is depressed as a consequence of saline infusion.

The effectiveness of a diuretic that inhibits sodium transport in the distal segment, on the other hand, would be expected to vary with the state of hydration of the animal. It is common experience that even highly active diuretics may yield only a modest diuresis when administered to hydropenic animals. If, however, the animal is given an infusion of saline, the response is greatly enhanced, not particularly because the urine flow is higher as a consequence of the saline infusion but because the increment resulting from the diuretic is much greater. This can be explained as the elimination by the diuretic of salt transport capacity more fully utilized as a result of salt infusion. Thus, careful attention must be paid to the aspects of fluid loading infusions in any study of diuretics, and it is not safe to assume an action on the proximal tubules because the increment of excretion exceeds 20% of the filtered load.

Filtration rate changes such as may occur after diuretic administration do not of themselves alter fractional fluid reabsorption in the proximal tubules of the dog. A mean reduction in filtration rate of 45% during continued saline infusion resulted in no significant change in fractional reabsorption (5). A similar mean reduction in filtration rate during continued hydropenia, reported in this paper, revealed an identical proportionality between filtration rate and reabsorption in the proximal tubules. Such observations have also been made in the hydropenic rat (16, 17). Thus, changes in proximal fractional sodium reabsorption after the injection of diuretics cannot be explained by variations in glomerular filtration rate.

The technique of repeated collection from identical tubule convolutions during different experimental phases of a micropuncture experiment is characterized by variability which, to some extent, sets a limit to changes in sodium reabsorption that can be detected. This variability arises from the cumulative errors of the micropuncture collections, inulin determinations, and spontaneous changes in the physiologic state of the animal. Mannitol was used to assess the sensitivity of the technique for the detection of inhibition of fluid reabsorption in the proximal tubule. When large amounts of mannitol were infused, depressed proximal fluid reabsorption was easily detected. With smaller amounts of mannitol, infused to ascertain the lower limits of inhibition that could be detected, significant changes in mean TF/P inulin could still be detected when urine flow was 2 to 9% of the glomerular filtration rate, although in the lowest part of this range (2 to 4%) the results were equivocal. In any case, relatively modest diuretic effects in the proximal tubule should be detected with the micropuncture method employed.

No evidence for a proximal site of action was found for any of the diuretics employed in this study. Such an action on the proximal tubules has been previously inferred for all the agents

tested. Convincing evidence for a major action at a more distal site has been found for ethacrynic acid, furosemide, and hydrochlorothiazide. Ethacrynic acid virtually abolishes concentrating ability and impairs the diluting capacity, indicating inhibition of sodium transport in the loop of Henle (18–20). Furosemide has effects on urinary concentration and dilution that are very similar to those of ethacrynic acid in the dog (21, 22). Deetjen (3) has reported a micropuncture study that showed an effect of furosemide on the proximal convoluted tubule of the rat when filtration rate was reduced to some 50% of normal; no inhibition was detected when filtration rate remained normal. In a similar micropuncture study with furosemide, Malnic and associates (4) found little change in proximal sodium reabsorption in the rat, but high distal TF/P chloride ratios. Hydrochlorothiazide has been shown to reduce free water formation considerably without influencing concentration in the dog renal medulla, and its effects can readily be ascribed to the cortical diluting segment (23).

Acetazolamide has clearly been shown to raise bicarbonate concentration in proximal tubule fluid in the rat (1). Acetazolamide also leads to increased free water clearance (24) suggesting an action on the proximal tubules with increased solute delivery to the distal segment. Acetazolamide combined with ethacrynic acid results in a diuresis greater than the added responses of the two agents (25). The results reported in this study show a tendency to decreased reabsorption in the proximal tubules. This depression in mean TF/P inulin did not, however, differ significantly from the changes observed in the hydropenic controls. Acetazolamide administration resulted in a modest diuretic response, and it is not unlikely that any changes might be within the limits of sensitivity of this micropuncture technique. These data together with other micropuncture and intact animal studies suggest that acetazolamide does inhibit sodium transport in the proximal convoluted tubule.

The mercurial group of diuretics have generally been considered to act in the proximal tubule. One basis of this view is the fact that in stop flow studies sodium concentration is increased and urinary/plasma (U/P) creatinine is decreased in "proximal tubule samples" by chlormerodrin (26). The results of chlormerodrin administration in situations of maximal urinary concentration and dilution have not been consistent (27). Beyer, Baer, Michaelson, and Russo (20) could superimpose no further diuresis by administering chlormerodrin to ammonium chloride-loaded dogs after ethacrynic acid infusion. Ethacrynic acid also blocked the increased secretion of potassium by chlormerodrin. These evidences suggested that the sites of action of these two drugs might be identical. The micropuncture experiments of this report indicated no depression of proximal sodium reabsorption after chlormerodrin was given to hydropenic dogs. It should be pointed out that the straight portion of the proximal tubule is not examined by such micropuncture techniques and remains a possible site of action.

The stop flow technique of Malvin, Wilde, and Sullivan (28) has been extensively used to designate the site of action of diuretics, and such studies represent the major conflict with the present findings on the effects of diuretics on proximal sodium reabsorption. Orloff and Berliner (27) have advocated cautious interpretation of stop flow analysis with respect to events occurring in the proximal segments, especially when an effect on a distal segment can be detected. Fluid originally contained in the proximal segments must flow through the distal tubule before it is collected, and changes produced by a diuretic in proximal stop flow samples may well be a reflection of a change in the extent to which they are modified as they flow through the affected distal tubule. The high sodium concentration of proximal samples after diuretics may then reflect depressed reabsorption in the proximal tubule or a failure of the concentration to be reduced during subsequent flow through the distal tubule. If no effect of a diuretic is detected in the distal samples, one can conclude a proximal site of action, provided one does not overlook significant changes in distal reabsorption reflected by a small change in distal concentrations. In addition, distal fluid sodium concentration may be reduced nearly maximally in the presence of a markedly inhibited transport system during the time of stop flow. Virtually all diuretics tested have some distal effects by stop flow analysis, and conclusions regarding proximal effects cannot be made with any confidence. Thus, high proximal fluid sodium con-

centration appeared after mercurial and thiazide administration, and an action in the proximal tubule was inferred for these two agents (26, 29, 30).[4] Subsequently, thiazides were shown to produce inhibition in the distal tubule since they block free water clearance in water diuresis (23). More recent stop flow studies have revealed definite inhibitory effects of thiazides and mercurials in the distal tubule (31–33).

The most striking change observed in this study was the significantly increased reabsorption in the proximal tubule after the administration of diuretics in hydropenia. This increase in reabsorption was rather substantial for a number of re-collections. The mean increase in TF/P inulin was 35% for ethacrynic acid, 20% for furosemide, 20% for chlormerodrin, and 9% for hydrochlorothiazide. It should be pointed out again that equal but opposite changes in TF/P inulin do not represent equal changes in sodium and water reabsorption, since TF/P ratios are reciprocal functions. Large percentage increases in TF/P represent much smaller increases in sodium reabsorption. For example, the 35% increase in TF/P after ethacrynic acid compared to hydropenia represents approximately 15% increase in sodium reabsorption. This increased proximal reabsorption after ethacrynic acid was absent in experiments in which the fluid output resulting from the diuresis was accurately replaced with isotonic saline infusions, and the same was found in experiments with chlormerodrin. These results suggest that acute salt depletion due to diuretics leads to enhanced proximal sodium reabsorption. Generally, the enhancement of reabsorption in the proximal tubule varied inversely with the over-all effectiveness as a diuretic of the agent employed; e.g., ethacrynic acid, the most potent agent in this study, raised proximal reabsorption the most, and hydrochlorothiazide, which gave the least diuresis, caused the smallest increase in proximal sodium reabsorption. It is common that the effects of diuretics rapidly diminish in hydropenic man or animals. Presumably, as the diuresis depletes extracellular fluid volume, fractional reabsorption is enhanced proximally, and less fluid is delivered to distal segments where the diuretics play their inhibitory roles.

The increase in proximal reabsorption after diuresis and the resulting acute salt depletion is the opposite response to that previously found with infusion of saline solutions. It appears that proximal tubule fractional reabsorption can vary widely depending on the state of hydration. Consequently, the fluid volume delivered to more distal portions of the nephron will vary considerably. Obligatory reabsorption in the proximal tubule may apply principally to the constant fractional reabsorption in the proximal tubule observed with changing filtration rates in both the rat (16, 17) and the dog. The setting of fractional reabsorption, on the other hand, may then be determined by salt excess or deficit resulting in opposite stimuli to the postulated secretion of an unidentified humoral agent that regulates sodium reabsorption by the proximal tubules.

A number of recent studies indicate that the reabsorptive activity of the proximal tubule cells is not the sole determinant of the rate of reabsorption. Under any particular condition of hydration, for example, changes in glomerular filtration rate are accompanied by proportional changes in reabsorption. The exact mechanism underlying the latter change in reabsorption is not fully established, but it has been suggested that it may be related to changes in the diameter of the tubules (34, 35). It therefore remains possible

[4] The lower U/P creatinine ratios in "proximal" stop flow samples have also been interpreted as an indication of inhibited reabsorption in the proximal tubule. However, during mannitol diuresis, the usual condition for stop flow studies, the U/P creatinine is very highly dependent upon the plasma mannitol concentration, since *1*) mannitol is reabsorbed only very slightly, and *2*) the urine under these conditions is always close to isotonic. The U/P creatinine and U/P mannitol must be very similar and highly dependent upon the plasma mannitol concentration. To the extent that plasma mannitol concentration is constant from one stop flow to the next the U/P creatinine (and mannitol) can vary only inversely with the concentration of other urinary solutes. Since sodium chloride constitutes most of the latter, at constant plasma mannitol, variation in U/P creatinine is largely a reflection of varying sodium concentration and does not provide information which is independent of that provided by the sodium concentration. However, in most stop flow studies the plasma mannitol concentrations have not been controlled or measured so that the U/P creatinine is difficult to interpret. In any case, the same caution is required in interpreting changes in proximal samples as in the case of sodium concentration. These must flow through the distal tubule before they are collected, and effects can be assigned to the proximal tubule only if distal effects can be positively excluded.

that depression of reabsorptive activity by a drug might be counterbalanced by some dilation of the tubules so that the reabsorption of fluid in the proximal tubule might remain unchanged, as suggested by Rector, Brunner, Sellman, and Seldin (36). Such a combination of effects might explain and reconcile the apparent conflict between the results obtained with the Gertz split drop technique in animals given diuretics (36) with the results of collection in free flow as reported in this paper and by Rector and co-workers (36). It might also offer an explanation for the excretion of fractions of the glomerular filtrate far larger than can be explained by distal effects alone in animals given large doses (100 mg per kg) of furosemide (11). However, it seems worthy of emphasis that whatever direct effects of drug and compensating local adjustments there may be, the results of the present study indicate that in the dog, with the large doses of diuretics used, any such direct effects in the proximal tubule make no appreciable contribution to the diuresis produced.

Summary

The effect of various diuretics on fluid reabsorption in the proximal tubule of the dog was investigated by micropuncture technique using repeated collections from identical tubule segments during control and experimental phases.

Mannitol infusion produced consistent reductions in reabsorption in the proximal tubule when urine flow exceeded 4% of the rate of glomerular filtration.

After the administration to hydropenic dogs of ethacrynic acid or chlormerodrin there was a significant increase in the fraction of the glomerular filtrate reabsorbed in the proximal tubules. This increase in reabsorption was markedly reduced or abolished when the fluid lost as a result of the diuresis was replaced with isotonic saline solution.

Furosemide and hydrochlorothiazide in hydropenic animals also produced significant increases in proximal reabsorption.

There was a small but significant decrease in reabsorption after the administration of acetazolamide to hydropenic animals, but the change was not significantly greater than observed in repeated punctures in control hydropenic dogs.

This study indicates that depressed proximal reabsorption does not contribute significantly to the diuresis produced in dogs by ethacrynic acid, chlormerodrin, furosemide, and hydrochlorothiazide. The increased reabsorption observed appears to be a consequence of acute depletion of extracellular volume, a response opposite to that produced by infusion of saline.

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