The Effect of Cyclic AMP and Dibutyryl Cyclic AMP on the Permeability Characteristics of the Renal Tubule

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A B S T R A C T   The effect of adenosine-3′,5′-cyclic monophosphate (cyclic AMP) and N^6,O^2-dibutyryl adenosine-3′,5′-cyclic monophosphate (dibutyryl cyclic AMP) on renal tubular permeability was studied by microinjection techniques in anesthetized diuretic rats. Radioactive inulin and mannitol were microinjected simultaneously into superficial proximal and distal convolutions and recovery of the isotopes was measured in the urine.

During control conditions, mannitol and inulin recovery was essentially complete. However, during infusion of cyclic AMP or dibutyryl cyclic AMP, mannitol recovery was significantly less than control after early proximal and late proximal microinjections, averaging 79 and 85%, respectively. There was no loss of mannitol from the nephron after microinjection into distal convolutions. Inulin recovery was complete after all microinjections during cyclic AMP or dibutyryl cyclic AMP infusion. Simultaneous clearances of mannitol and inulin as well as peritubular capillary microinjections studies demonstrated bidirectional fluxes of mannitol across the proximal tubular epithelium during infusion of cyclic AMP or dibutyryl cyclic AMP. Intratubular pressures were not different during control and experimental periods.

These studies demonstrate a change in the permeability characteristic of the proximal convoluted tubule during infusion of cyclic AMP and dibutyryl cyclic AMP. This change in permeability of the proximal tubule could account for the effects of cyclic AMP on proximal tubular transport processes.

INTRODUCTION

It is generally accepted that the effect of parathyroid hormone on the proximal tubule is mediated through stimulation of adenyl cyclase (1–5) which subsequently increases intracellular levels of adenosine-3′,5′-cyclic monophosphate (cyclic AMP).[1] Not only does parathyroid hormone, through cyclic AMP, decrease net reabsorption of phosphate by the kidney, but it also decreases the reabsorption of sodium, bicarbonate, and amino acids. Agus, Puschett, Senesky, and Goldberg (5) have shown that both parathormone and cyclic AMP inhibit absolute and fractional reabsorption of phosphate and sodium in the proximal tubule of the dog. The work by Scriver and associates (6–7) in vitamin D deficiency in infants as well as in the rat has demonstrated phosphaturia and aminoaciduria in association with the secondary hyperparathyroidism of vitamin D deficiency. Further, Winnberg and Bergström (8) have shown an acidification defect in untreated vitamin D deficiency rickets secondary to decreased bicarbonate reabsorption. These studies indicate that parathormone, through stimulation of cyclic AMP, affects many aspects of proximal tubular transport. Although these effects are well documented, there is very little information concerning the underlying mechanism through which cyclic AMP may inhibit net reabsorption in the proximal tubule. Theoretically, cyclic AMP could inhibit specifically these various active transport systems. However, it is equally possible that cyclic AMP could interfere with membrane permeability and allow for greater passive back flux of these substances from the peritubular capillary into the tubular lumen. To evaluate the passive permeability characteristics of the proximal tubule during infusion of cyclic AMP, we have carried out experiments utilizing the microinjection technique. These studies indicate that during infusion of cyclic AMP or dibutyryl cyclic AMP the proximal tubular epithelium remains impermeable to inulin but becomes permeable to man-

[1] *Abbreviations used in this paper:* ADH, antidiuretic hormone; C, clearance; cyclic AMP, adenosine-3′,5′-cyclic monophosphate; dibutyryl cyclic AMP, $N^6,O^{2'}$-dibutyryl adenosine-3′,5′-cyclic monophosphate.

nitol to which the proximal tubule is normally imperme-
able.

METHODS

Male Sprague-Dawley rats weighing 240–350 g were anes-
thetized with sodium pentobarbital 50 mg/kg body wt in-
traperitoneally and the left kidney was exposed for micro-
puncture through a midline abdominal incision which was
extended laterally along the lower margin of the left rib
cage. A metal retractor was used to immobilize the dia-
phragm from above on the left and the kidney was packed
with cotton soaked in mineral oil. Throughout all ex-
periments the animals were placed on a heated table and
body temperature was monitored continuously by means
of a rectal probe connected to a telethermometer (Yellow
Springs Instrument Co., Yellow Springs, Ohio). Body tem-
perature was maintained at 37.5±1°C. A tracheotomy was
performed and the right jugular vein was catheterized for
infusion of fluids and injections of lissamine green. A PE
50 polyethylene catheter was placed in the left carotid ar-
tery and threaded into the abdominal aorta to lie above the
level of the renal arteries. The femoral artery was catheter-
ized with a PE 50 polyethylene tubing for monitoring of
blood pressure. This tubing was connected to a Statham
pressure transducer (Model P23 DC, Statham Instru-
ments, Inc., Oxnard, Calif.) and blood pressure was re-
corded on a Grass polygraph (Grass Instrument Co.,
Quincy, Mass.). Both ureters were catheterized with PE
50 polyethylene tubing.

For the microinjection experiments, the kidney surface
was illuminated with a fiberoptic light source (American
Optical Corp., Southbridge, Mass.). The kidney was
bathed with heated mineral oil delivered by a gravity sys-
tem. Early proximal, late proximal, and distal convolu-
tions were identified by the intravenous injection of 0.05
ml of 5% lissamine green. The total amount of lissamine
green administered to any animal did not exceed 0.5 ml
and no microinjections were performed until at least 30
min had lapsed after the last injection of lissamine green.
Intratubular pressures were measured by the method of
Landis as modified by Gottschalk and Mylle (9). Random
intratubular pressure measurements were made during both
control and experimental periods. The animals were made
moderately diuretic by the infusion of 5% mannitol in
0.85% sodium chloride at a rate of 50 μl/min. Constriction
micropipettes of known volume were used for microinjec-
tion into superficial early and late proximal and distal con-
volutions. A small volume (1–5 nl) of isotonic saline
stained with nigrosine and containing trace amounts of
[³H]inulin and [¹⁴C]mannitol was injected at an average
rate of 0.26±0.06 (SD) nl/s. The injection rate was con-
trolled to avoid visible retrograde flow of the stained test
solution and dilatation of the tubular lumen. During ex-
perimental periods either cyclic AMP or dibutyryl cyclic
AMP was infused at a rate of 100 μg/min through the
catheter placed in the abdominal aorta above the level of
the renal arteries. After a 60-min equilibration period, a
convolution punctured during the control period was again
entered through the original injection site and another
microinjection performed.

[³H]Inulin and [¹⁴C]mannitol were obtained from the
New England Nuclear Corp., Boston, Mass. Cyclic AMP
and dibutyryl cyclic AMP were obtained from Calbiochem,
La Jolla, Calif. Urine from both kidneys was collected
into vials containing 10 ml of liquid scintillation fluid

(Aquasol, New England Nuclear Corp.) to which 2.5 ml
of deionized H₂O was added.

Urine collection was started at the beginning of each
microinjection and three consecutive 3-min collections were
made after injections during both control and experi-
mental conditions. Before each microinjection urine was
collected to measure residual radioactivity. Immediately
preceding a microinjection, a timed urine collection of the
same duration as each sample after microinjection was
taken. A volume of test solution equal to that which was
subsequently injected was deposited in this sample and
used as a reference standard. Significant quenching of the
tritium counts without apparent cause was observed occa-
sionally. Although there was little or no quenching of the
¹⁴C counts, these results were discarded. Radioactivity was
measured in a Beckman 3 channel liquid scintillation spec-
trometer (Beckman Instruments, Inc., Fullerton, Calif.).
Each sample was counted to a preset error of ±3%. Sam-
ples with essentially background activity were counted for
a total of 20 min. The recovery of injected radioactive
substances was calculated as follows:

$$\% \text{ recovery} = \frac{\text{isotope in urine}}{\text{isotope in injectate}} \times 100\%$$

Clearance studies were performed in an additional 10 rats
made similarly diuretic. A priming injection containing 15
μCi of [¹⁴C]mannitol and 40 μCi [³H]inulin was followed
by the intravenous infusion of 20 μCi/h of [¹⁴C]mannitol
and 60 μCi/h of [³H]inulin in 5% mannitol and 0.85%
sodium chloride at a rate of 50 μl/min. After a 30-min
equilibration period two 20-min control collections of urine
were made. Then an infusion of cyclic AMP or dibutyryl
cyclic AMP at a rate of 100 μg/min was begun in the
abdominal aorta. After a further 60-min equilibration
period, three 20-min experimental collections of urine were
performed. Blood samples were withdrawn from the fe-
moral artery at the midpoint of each clearance period.
Radioactive levels and blood and urine were determined
as described above.

To evaluate the validity of the clearance studies, an ali-
quot of urine from each clearance period was subjected to
descending column chromatography utilizing a solvent sys-
tem of three parts n-butanol, two parts pyridine, and one
part water by volume (10). After the chromatography
procedure, the strips were analyzed for radioactivity utiliz-
ing a Beckman strip radiochromatograph scanner. The
strips were then stained with the periodate reagent (11)
to identify chemical inulin and mannitol.

Peritubular capillary microinjections were performed in
another group of five animals studied under identical con-
trol and experimental conditions. Constriction micropipettes
of 20–30 nl volume were utilized. A solution of isotonic
saline stained with lissamine green containing trace amounts
of [³H]inulin and [¹⁴C]mannitol was injected into peritubu-
lar capillaries at an average rate of 31±11 (SD) nl/min.
Urine from both kidneys was collected after microinjection
at 15-s intervals for the first 4 min, at 30-s intervals for
the next minute, and thereafter at 1-min intervals for an-
other 4 min. Radioactivity levels in the urine were deter-
mined as described above. For detection of precession only
those counts exceeding background plus 2 SD of back-
ground activity were considered significant. Recovery for
each sample was calculated as the percent of total radio-
activity recovered during the 9-min collection period fol-
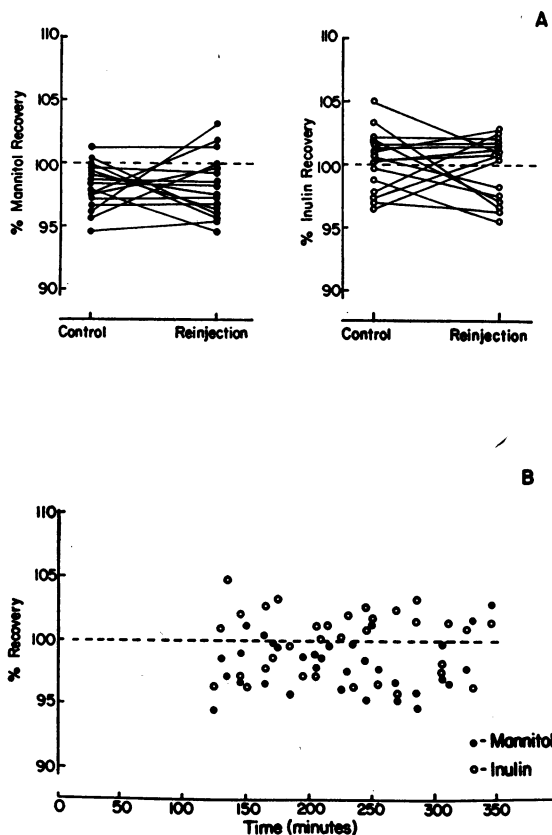lowing peritubular capillary injection.

FIGURE 1 (A) Mannitol and inulin recoveries after simultaneous early proximal microinjections and reinjections at the same puncture site. The dashed horizontal line represents 100% recovery of the injected substance. The solid lines connect reinjections pairs performed of the same puncture site. (B) Mannitol and inulin recoveries after simultaneous early proximal microinjection as a function time from the start of intravenous infusion of 5% mannitol and 0.85% NaCl at rate of 50 μl/min. The dashed horizontal line represents 100% recovery of injected substances.

Results are presented as means±1 SD. Student's *t* test was used for evaluation of statistical significance.

RESULTS

A total of 186 satisfactory microinjections were performed in 27 animals. A microinjection was not considered satisfactory if there was visible leakage at the puncture site or retrograde flow. The results presented are recoveries measured in the urine from the injected kidney. All injections were performed with a test solution which contained both inulin and mannitol.

The recovery of inulin and mannitol after early proximal microinjection and reinjections during intravenous infusion of 5% mannitol and 0.85% sodium chloride is shown in Fig. 1. Note in Fig. 1A that both inulin and mannitol recovery are essentially complete during con-

trol and reinjection periods. Inulin recovery averaged 100.2±2.6% after control injections and 99.8±2.7% after reinjections. Mannitol recovery was 97.2±2.7% after control injections and averaged 98.2±2.5% after reinjections. Neither of these was significantly different from control. In Fig. 1B, mannitol and inulin recoveries are shown as a function of time after the start of intravenous infusion of 5% mannitol and 0.85% sodium chloride at 50 μl/min. The results of these studies indicate that neither the reinjection technique or infusion of 5% mannitol and 0.85% NaCl significantly affects the recovery of inulin or mannitol after early proximal microinjection.

The recovery of mannitol and inulin after microinjection into early proximal convolutions during control and experimental conditions is shown in Fig. 2. During control conditions mannitol recovery averaged 97.9± 2.8%. However, during experimental periods there was a significant loss of mannitol. Average mannitol recovery was 79.0±6.8%. This is significantly less than the control ($P < 0.001$). There was also a difference between recoveries after infusion of cyclic AMP or dibutyryl cyclic AMP. During infusion of cyclic AMP mannitol recovery was 81.9±6.5%. Mannitol recovery during dibutyryl cyclic AMP infusion was 74.6±4.8%.
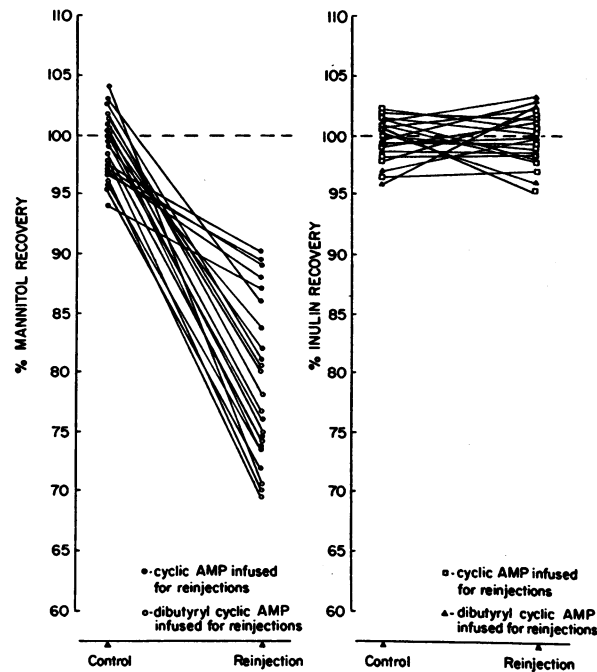

FIGURE 2 Mannitol and inulin recoveries after simultaneous early proximal microinjections during control conditions and during infusion cyclic AMP or dibutyryl cyclic AMP. The dashed horizontal line represents 100% recovery of injected substances. The solid lines connect reinjection pairs performed at the same puncture site.
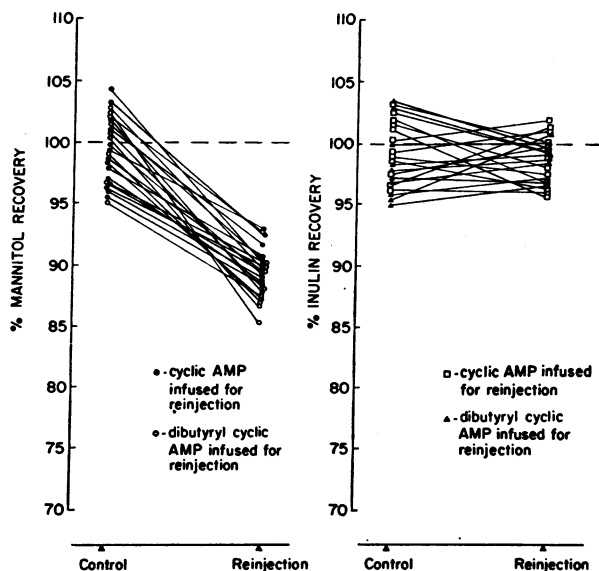
FIGURE 3 Mannitol and inulin recoveries after simultaneous late proximal microinjections during control conditions and infusion of cyclic AMP or dibutyryl cyclic AMP. The dashed horizontal lines represent 100% recovery of injected substances. The solid lines connect reinjection pairs performed at the same puncture site.

This difference is significant $(P < 0.001)$. Inulin recovery was essentially complete after both control and experimental microinjections. Inulin recovery averaged 99.4±2.7% after control injections and 99.1±1.7% after experimental microinjections. The transit time was significantly prolonged during experimental periods. The transit time for each microinjection is defined as the time from beginning an injection until the first appearance of the nigrosine-stained test solution in the distal convolution of the nephron under study. Transit time was 34±14 s after control injections and 53±25 s after experimental injections $(P < 0.001)$.

The recovery of inulin and mannitol after microinjections into late proximal convolutions is shown in Fig. 3. Mannitol recovery after late proximal injections during control periods was 98.9±2.6%. During infusion of either cyclic AMP or dibutyryl cyclic AMP mannitol recovery averaged 89.4±2.0%. This difference is highly significant $(P < 0.001)$. Inulin recovery was again essentially complete. Inulin recovery averaged 99.3±2.5% during control periods and 98.8±2.1% during experimental periods. The transit time from the start of late proximal injections until the injectate appeared in its convolution was 27±15 s during control microinjections and 32±26 during experimental injections. Although transit time was prolonged after late proximal injections this was not significant $(0.05 < P < 0.1)$. There was no difference in recoveries after experimental in-

jections between cyclic AMP and dibutyryl cyclic AMP. Mannitol recovery during infusion of cyclic AMP averaged 89.7±1.7 and during dibutyryl cyclic AMP averaged 88.8±2.4%.

In contrast to the proximal convolution microinjections, after microinjection into distal convolutions no loss of inulin or mannitol was demonstrable. Recovery after distal microinjections is shown in Fig. 4. Mannitol recovery averaged 98.6±2.1% during control conditions and during experimental periods averaged 98.7±2.6%. Inulin recovery was 100.1±2.4% during control periods and averaged 99.4±1.2% during experimental periods.

Since a difference in mannitol recovery during infusion of cyclic AMP was demonstrable, differences in the clearances between inulin and mannitol should also be present infusion of cyclic AMP. Simultaneous clearances of inulin and mannitol were performed in ten animals with two clearance periods performed during control conditions followed by three clearance periods during infusion of cycylic AMP in five animals and dibutyryl cyclic AMP in five animals. The results are shown in Table I. Since the results were similar in both kidneys, they have been pooled for purposes of analysis. There was a significant decrease in urine flow and an increase in the urine/plasma (U/P) inulin ratios during the experimental periods. There was a slight increase in mannitol clearance and a decrease in inulin clearance during the experimental periods, however, these results are not significantly different. In contrast, the ratios of mannitol clearance to inulin clearance for each clearance period were distinctly different. During control periods mannitol clearance was slightly less than inulin clear-
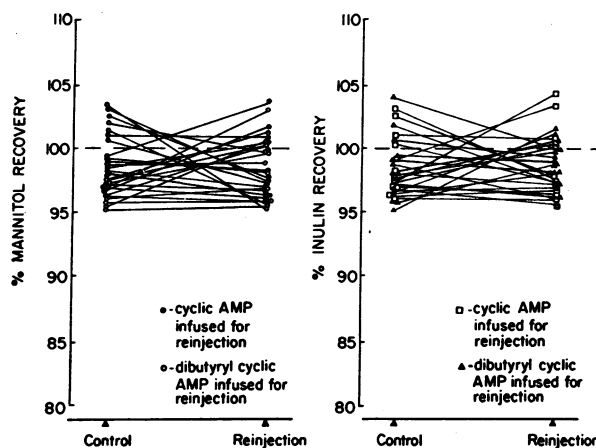

FIGURE 4 Mannitol and inulin recoveries after simultaneous distal convolution microinjections during control conditions and infusion of cyclic AMP or dibutyryl cyclic AMP. The dashed horizontal lines represent 100% recovery of injected substances. The solid lines connect reinjection pairs performed at the same puncture site.

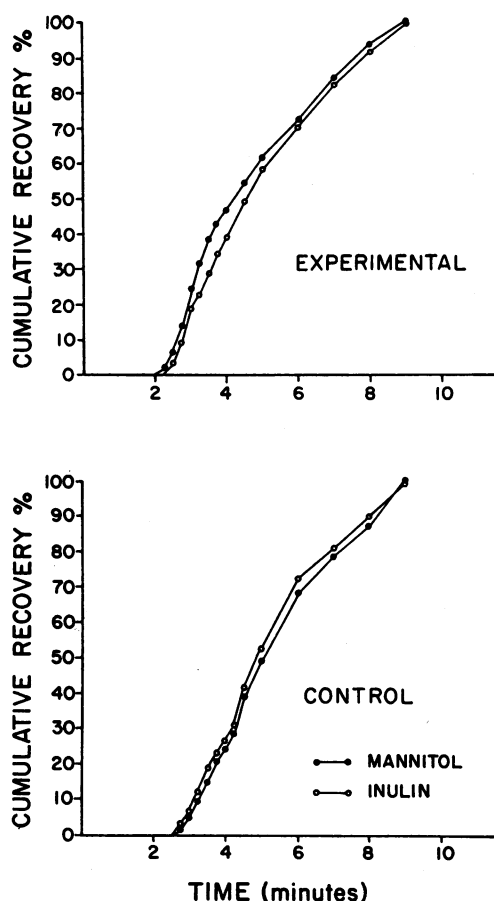*Effect of Cyclic AMP on Renal Tubular Permeability*   1253

FIGURE 5 Cumulative mannitol and inulin recoveries from the experimental kidney after peritubular capillary injection in a typical experiment. Note that mannitol recovery precedes inulin recovery during the experimental period in which dibutyryl cyclic AMP was infused.

ance. The ratio of mannitol clearance to inulin clearance for each clearance period during control conditions was

TABLE I

Effect of Cyclic AMP on Simultaneous Clearance of Mannitol and Inulin

	Control (n = 20)	Experimental (n = 30)	P
Urine flow (μl/min)	36.6±11.2	21.0±5.7	<0.001
U/P inulin	107.4±48.6	156.4±38.3	<0.001
C mannitol (ml/min per 100 g body wt)	0.95±0.33	0.98±0.30	>0.7
C inulin (ml/min per 100 g body wt)	0.99±0.31	0.89±0.29	>0.3
C mannitol / C inulin	0.97±0.04*	1.12±0.08*	<0.001

* Values represent mean±SD of ratios for each clearance period.

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0.97±0.004. During experimental periods, however, mannitol clearance exceeded inulin clearance with an average ratio 1.12±0.08. This difference was significant (P < 0.001).

To check the validity of this clearance data, an aliquot of urine from each clearance period was subjected to descending column chromatography and scanning radio-chromatography. The results showed single peaks of radioactivity which had the same mobility as [³H]inulin and [¹⁴C]mannitol from stock solutions of the radioisotopes prepared for infusion. To further identify the radioactive peaks, the strips were then stained with the periodate reagent. The radioactive peaks were found to be present in spots which stained identically with and had the same chromatographic mobility as unlabeled inulin and mannitol. The chromatography studies showed that both radioactive inulin and mannitol were excreted in the urine in an unaltered form.

The results of the microinjection studies and clearance studies suggested that there was bidirectional movement of mannitol during infusion of cyclic AMP or dibutyryl cyclic AMP. To further investigate the possibility of movement of mannitol from the peritubular capillary into the tubular lumen, peritubular capillary microinjection studies were performed in an additional five animals. The animals were made diuretic as during the microinjection and clearance studies by infusion of 5% mannitol in 0.85% saline at a rate of 50 μl/min. Microinjections into peritubular capillaries were made during control conditions and then a series of experimental microinjections into peritubular capillaries was performed during infusion of dibutyryl cyclic AMP.

During control periods inulin and mannitol were excreted in a similar fashion as is shown in the bottom panel of Fig. 5. However, during experimental periods there was precession of mannitol recovery over inulin recovery in the urine of the injected kidney. This is shown in the top panel of Fig. 5. Precession of mannitol was present in 14 of 15 microinjections during experimental periods. The percent recovery of mannitol at a point in time when 50% of the inulin from the microinjected kidney had been recovered averaged 47.0±1.2% during control periods and 58.5±3.0% during experimental periods (P < 0.001).

Random measurements of intratubular pressures in both proximal and distal convolutions were made during control and experimental periods in all animals studied. Proximal intratubular pressure during control periods averaged 13.2±2.1 mm Hg (n = 104). During experimental periods averaged proximal intratubular pressure was 12.8±2.2 mm Hg (n = 126). This difference was not significant. Distal intratubular pressures averaged 6.4±1.5 mm Hg (n = 52) during control periods and

during experimental periods averaged 6.1±1.6 mm Hg (n = 60).

Mean arterial blood pressure during control periods averaged 110±13 mm Hg (n = 112) and during experimental periods averaged 102±11 mm Hg (n = 124). This small difference was highly significant (P < 0.001). For purposes of analysis of continuous mean blood pressure recordings, the value immediately following a microinjection or the midpoint of a clearance period was chosen as the value for analysis. Transient decreases in blood pressure following administration of pentobarbital were not considered for analysis.

DISCUSSION

The results of this study demonstrate that the tubular epithelium undergoes a change in its permeability characteristics during the infusion of cyclic AMP or dibutyryl cyclic AMP. It is interesting to note that the tubules remained impermeable to inulin during all conditions studied and reaffirms previous studies which indicate that inulin is the most satisfactory marker for glomerular filtration during a variety of experimental conditions (12–16).

The segmental recovery of mannitol after experimental microinjections is shown in Table II. When mannitol recovery during infusion of either cyclic AMP or dibutyryl cyclic AMP after early proximal injections is compared to mannitol recovery after late proximal injections there is a highly significant difference. Similarly when the results of late proximal injections and distal microinjections are compared there is also a highly significant difference.

This indicates that there is a change in the permeability of the proximal convoluted tubule to mannitol during infusion of cyclic AMP. Since the entire proximal convolution is not accessible to micropuncture, the microinjections designated here as "late proximal" represent approximately 70% of the total length of the entire proximal convoluted tubule (9). The loss of mannitol after late proximal injection which has been demonstrated in this study could, therefore, be from the pars recta of the proximal convoluted tubule or from some portion of the loop of Henle. No further distinction can be made from this experimental design.

Previous work has shown that elevation of intratubular pressures can lead to increased permeability of the nephron to mannitol (16). The intratubular pressures during both control and experimental periods in this study were not different and were within the physiologic range. They are well below the range of pressures which have been shown to be associated with mannitol loss from the nephron (16). Even though transit time to the distal tubule was prolonged after experimental injections this should not have, in itself, caused loss of

mannitol. Aortic constriction which greatly prolongs transit time through the nephron, has been shown not to be associated with significant mannitol loss from the tubule (16).

The finding that mannitol clearance exceeded inulin clearance during infusion of cyclic AMP was unexpected. There was a decrease in inulin clearance during the experimental periods. Cyclic AMP has been reported to cause vasodilatation of systemic arteries, but conversely constriction of the renal artery (17). The decrease in glomerular filtration rate could be due to changes in systemic hemodynamics. However, unless there was transepithelial movement of mannitol there should have been a corresponding decrease in mannitol clearance. The results of the radiochromatography studies and staining for identification of chemical inulin and mannitol indicate that carbon-14 and tritium were excreted intact in the urine on mannitol and inulin, respectively. Therefore, these results suggest that there is net addition of mannitol to the urine. This, of course, does not contradict the results of the microinjection studies since only the unidirectional lumen to plasma flux is studied by the microinjection technique.

The clearance studies were in accord with the results of the peritubular capillary microinjections. After injection into the peritubular capillaries during infusion of dibutyryl cyclic AMP, there was precession of mannitol excretion in the urine from the experimental kidney. This indicates that there is movement of mannitol from the peritubular capillary lumen into the tubular lumen. If there were no movement of mannitol across the tubular epithelium, mannitol appearing in the final urine would have all had to be the result of glomerular filtration. Mannitol excretion should have been significantly less than inulin excretion since the microinjection studies indicate that during cyclic AMP infusion there is loss of mannitol from the proximal tubule. Therefore, all these studies indicate that during infusion of cyclic AMP or dibutyryl cyclic AMP there is a bidirectional flux of mannitol across the epithelium of

TABLE II

Segmental Recovery of Mannitol During Infusion of Cyclic AMP

Site	% Recovery	P
Early proximal (n = 23)	79.0±6.8	<0.001*
Late proximal (n = 25)	89.4±2.0	
Distal (n = 29)	98.7±2.6	<0.001‡

* Early proximal vs. late proximal.
‡ Late proximal vs. distal.

the proximal convoluted tubule and perhaps also some portion of the loop of Henle.

The results of these studies provide, of course, no direct information concerning the structural alterations which may lead to changes in permeability or to the route of movement of mannitol across the proximal tubular epithelium. There is a considerable body of evidence that indicates the presence of low resistance intercellular shunts which involves the tight junction and lateral intercellular spaces (17–19). Boulpaep and Seely have shown an increased permeability of the necturus nephron to raffinose during conditions of volume expansion (20). Studies by Bulger, Lorentz, Colindres, and Gottschalk have shown changes in the tight junction of the proximal tubule of the rat associated with increased intratubular pressure due to elevation of ureteral pressure or partial renal venous constriction (21). Associated with this there has been expansion of the lateral intercellular spaces. Therefore it would seem reasonable to propose that the increased permeability to mannitol induced by cyclic AMP is caused by changes in the permeability characteristics of the tight junction which permits movement through the tight junction and lateral intercellular spaces.

The finding that mannitol clearance exceeded inulin clearance during infusion of cyclic AMP or dibutyryl cyclic AMP indicates that there is net addition of mannitol to the urine. Thus, the peritubular capillary to lumen unidirectional flux must exceed the lumen to peritubular capillary unidirectional flux. Further, one would have to assume that with water reabsorption in the proximal tubule mannitol is moving against a concentration gradient which would favor movement out of the tubular lumen. Since there is no known active transport process for mannitol, another explanation for this observed phenomena is necessary. Ussing and Johansen have demonstrated the phenomenon of anomalous solvent drag in the toad skin in which sucrose and urea were transported in a direction opposite to net water movement (22). During sodium and water reabsorption from the proximal tubule, net water movement is from the lumen into the peritubular capillary. According to the standing osmotic gradient theory of transport as proposed by Diamond (23), water movement is through the luminal border of the transporting epithelium into the cell and then into the lateral intercellular space. This pathway would remain impermeable to mannitol. However, under the influence of cyclic AMP the tight junction would become permeable for water to back diffuse into the tubular lumen. Thus, net addition of mannitol to the tubular lumen would be accomplished by solvent drag through the now permeable tight junction which would exceed movement of

mannitol out of the tubular lumen along its concentration gradient.

This alteration of the passive permeability characteristics of the proximal tubule during infusion of cyclic AMP or dibutyryl cyclic AMP would allow one to explain the decreased net transport of such varied substances as bicarbonate, phosphate, sodium, and amino acids which have been directly observed (5) or inferred (6–8) with states of secondary hyperparathyroidism. Agus et al. (5) have shown that the concentration of phosphate in the lumen of the proximal tubule of dogs is less than that in an ultrafiltrate of plasma after active proximal tubular reabsorption. With a change in the permeability of the tight junction, there would be movement of phosphate down a chemical concentration gradient from the peritubular capillary into the tubular lumen. There would likewise be the possibility of solvent drag if the proposed back diffusion of water does indeed occur from the lateral intercellular space through the tight junction into the tubular lumen. Since the most recent electrophysiological studies of the rat proximal convoluted tubule indicate a small potential difference across the tubular epithelium with the lumen positive, along most of its length, this movement would be down an electrical gradient (24, 25). This same potential difference would tend to inhibit back diffusion of sodium; however, one could assume that solvent drag inward would overcome the effects of the electrical gradient favoring outward movement. It is still possible that cyclic AMP could, in addition, inhibit specific active transport processes taking place in the proximal convoluted tubule. However, this change in the passive permeability characteristics of the proximal tubule allows one to advance a single hypothesis which would explain multiple effects.

Cyclic AMP and its dibutyryl derivative do not readily cross cell membranes and very high blood concentrations must be attained to produce physiological concentrations within the cell (26). To consider these results a physiological effect one would have to assume that none of the permeability changes were due to alterations in systemic or renal hemodynamics and that the resultant intercellular levels were similar to those obtained after stimulation with parathyroid hormone. These questions cannot be answered from this study.

The finding that there was no change in the solute permeability of the distal nephron was interesting when one considers that current evidence suggests that the action of antidiuretic hormone (ADH) to increase water permeability of the collecting duct is mediated through cyclic AMP (27, 28). Certainly the decrease in urine flow and increase in U/P inulin ratios in the clearance experiments during the experimental period

indicate there was an enhanced ADH effect present. Therefore, these results would be in accord with current theories concerning the activation of adenyl cyclase in the collecting duct by ADH and subsequent increases in water permeability.

ACKNOWLEDGMENTS

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