In order to evaluate the solutes involved in regulation in the two species and to compare decerebrated with control animals, we will now look at the time periods during which the worms exhibit the greatest regulatory volume decrease (Table 3). In control P. *spiralis* there is significant reduction in total Na^+ and K^+ in the body during this period (16 and 12%, respectively). In contrast, in ablated animals there is a significant reduction in Na^+ content but no significant reduction in K^+ content. The findings for C. *arenareous* are identical such that control animals excrete Na^+ and K^+ during the regulatory period while ablated animals excrete only Na^+.

From these preliminary data, however, no information concerning the mebhanism of volume regulation can be deduced, since we have not yet determined cell volume and extracellular fluid volume in these worms. Experiments are in progress to determine these parameters. This study was supported by NIH grant AM15972-08.

URINARY RENAL PELVIC REFLUXES OF URINE

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When the urine exits from the Ducts of Bellini, it sometimes refluxes back into the renal pelvis (Schmidt-Nielsen, Fed. Proc. 36:2493, 1977). Most mammalian renal pelvises have elaborate extensions (Pfeiffer, J. Anat. (London) 102:321, 1968; Lacy and Schmidt-Nielsen, Am. J. Anat., in press a; Schmidt-Nielsen, Fed. Proc. 36:2493, 1977) which permit the urine to come into contact with the thin epithelium covering the outer medulla (Lacy and Schmidt-Nielsen, Am. J. Anat., in press b). The area of the outer medulla facing the pelvis in the hamster constitutes 50% of the total pelvic area, and is twice as large as that covering the inner medulla (Lacy and Schmidt-Nielsen, Am. J. Anat., in press a). When the urine refluxes, it reaches all of the pelvic extensions (Schmidt-Nielsen et al., Bull. MDIBL 17:96, 1977). Various hypotheses have been presented to explain the physiological role of these refluxes (Pfeiffer, J. Anat. (London) 102:321, 1968; Schmidt-Nielsen, Fed. Proc. 36:2493, 1977). No real understanding has been reached, however, due to lack of information concerning the physiological conditions under which urine refluxes into the pelvis.

The present investigation was undertaken to determine: 1) under which physiological conditions pelvic refluxes occur; 2) what effect refluxes have on the solute concentrations in the renal papilla and on the urine itself, and 3) to record the various types of refluxes on film.

A total of 90 successful experiments were carried out on 45 hamsters and 45 Munich Wistar rats. These animals were chosen because the renal papilla extends beyond the renal cortex at the hilus. The animals were anesthetized with 15 mg inactin per 100 g body weight. In the rats, the jugular vein and carotid artery, as well as both ureters, were catheterized. The left kidney was exposed through a flank incision and a small plastic shield was placed under the kidney to isolate it from respiratory movements. The urine was made visible by a continuous i.v. infusion of a lissamine green solution. The rate of infusion varied according to the experimental protocol. In the hamsters, two techniques were used: the kidney was either exposed and placed on a plastic shield in the same manner as described above for the rats, or (in the majority of experiments) the right kidney was exposed through an abdominal incision. It was left in situ and was isolated from respiratory movements by placing a plastic shield against the diaphragm. The lower part of the renal pelvis was cleared of surrounding fat. In both rats and hamsters the pelvis was left intact, and was illuminated with a fiber optic light. The reason for using the different techniques for exposing the pelvis was to determine if refluxes were caused by the experimental procedures of placing the kidney on a shield.

The studies showed that refluxes rarely occur in the normal antidiuretic state. We have seen an occasional reflux in antidiuretic hamsters or rats which occurred spontaneously, but most of the time the urine which exited from the Ducts of Bellini would move straight down into the ureter. Reflux occurred under three conditions: 1) Refluxing followed an acute injection of 0.2-0.5 ml of isosmotic saline or 0.1 ml of hyperosmotic saline or mannitol solutions. In these cases the lag time between start of injection and beginning of refluxing was 47.7 ± 6.5 seconds (n=17) in the rats and 67.3 ± 23.6 (n=8) in the hamsters. The shortest time lag was 19 seconds in the rats and 17 seconds in the hamsters. 2) Refluxing could also be induced by increasing the i.v. infusion rate from 0.5 µl/min to 20 or 50 µl/min. Refluxing then started 7-20 minutes following the increased infusion, which in turn caused an increase in the urine flow rate. In Figure 1 a typical time course for refluxing during rising urine flow is shown. It can be seen that refluxing starts following increased infusion rate. In the beginning urine does not reflux up into the pelvis with each pelvic contraction, but the reflux rate gradually increases until refluxing occurs each time the pelvis contracts.

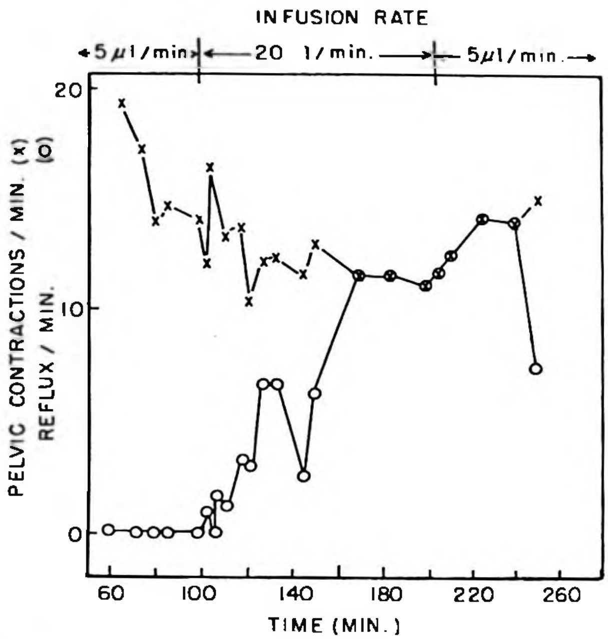

Figure 1.

Refluxing usually ceased when the urine flow was no longer rising. However, when a true water diuresis was induced, refluxing continued even during falling urine flow. 3) Finally, refluxing could be induced by mechanical means. Thus, touching the pelvis epithelium with a foreign object just below the tip of the papilla induced refluxing immediately which lasted as long as the contact was maintained, even though the contact did not in any way obstruct the ureter. Refluxing could also be induced by lifting the ureter slightly. Obstruction of the ureter resulted in continuous reflux with a greatly expanded pelvis and weak pelvic conditions.

The effect of refluxing on papillary solute concentrations could be studied when only one of the two kidneys was refluxing, and the other kidney could serve as a control. Consequently, mechanical reflux was induced in the right kidney of 7 hamsters on normal diet and 6 hamsters on low protein diet. The results (Table 1) show that refluxing had no effect on tissue concentration in the animals on normal diet, but that refluxing significantly lowered the urea concentration of the inner medulla of animals maintained on a low protein diet. Osmolality, Na^+, or K^+ concentrations of the inner medulla were not affected in either group. The reason for this difference between normal and low protein animals in effect of refluxing on inner medullary solute concentrations can be found in the difference between tissue and urine urea concentration under the two conditions. In the normal protein hamsters the urine urea concentration is higher than that of the papilla (Table 2), while in the low protein hamsters, the reverse is true. Only two animals are shown in Table 2, but this phenomenon is well documented in rat (Truniger and Schmidt-Nielsen, Am. J. Physiol. 207:471, 1964) in dog (Schmidt-Nielsen and Robinson, Am. J. Physiol. 218:1363, 1970) and in sheep (Schmidt-Nielsen and O'Dell, Am. J. Physiol. 197:856, 1959). Thus, it appears that urea diffuses out of the tissue into the urine if the gradient for urea is outward when refluxing occurs, but there is no addition to the tissue if the gradient is reversed. In experiments with rats on normal diet mechanically-induced refluxing had no effect on either the urine or the renal

TABLE 1

Effect of pelvic reflux on solute concentrations in renal papilla. R/L, concentration in refluxing kidney (R) divided by concentration in control kidney (L)

		N	Osm R/L	Na^+ R/L	K^+ R/L	Urea R/L
Normal Diet	IZ2	(7)	1.09 ± .09	.99 ± .04	1.02 ± .03	1.09 ± .04
Normal Diet	IZ1	(6)	.96 ± .08	.85 ± .07	.96 ± .03	1.02 ± .07
Low Protein Diet	IZ2	(6)	1.07 ± .03	.94 ± .06	.99 ± .04	.71 ± .08*
Low Protein Diet	IZ1	(6)	.99 ± .04	.98 ± .06	.96 ± .02	.84 ± .06*

Significantly lower than unity *P < 0.05 .

IZ2 represents the renal papilla, IZ1 the upper part of the inner medulla.

TABLE 2

Solute concentrations from left kidney: in papilla (IZ2) in urine (U), and in plasma (P)

	Osm mOs	Na^+ mEq	K^+ mEq	Urea mM
Normal (n = 4)				
IZ2	1681 ± 237	286 ± 92	104 ± 4	649 ± 66
U	2102 ± 198	453 ± 28	266 ± 36	816 ± 112
U/IZ2	1.31 ± .19	1.20 ± .14	2.57 ± .36	1.19 ± .05
P (n=7)	296 ± 6	145 ± 2	7.85 ± .41	6.50 ± .53
Low protein (n = 2)				
IZ2	1498 ± 48	345 ± 42	102 ± 13	282 ± 32
U	1221 ± 117	342 ± 51	151 ± 2	186 ± 9
U/IZ2	.82 ± .10	1.02 ± .27	1.51 ± .21	.67 ± .11
P (n=6)	305 ± 4	144 ± 7	5.96 ± .41	3.78 ± .26

Urine was obtained from only four animals on normal protein diet and two animals on low protein diet. Plasma samples were obtained from a larger number of animals on two diets.

rissue concentrations. Unfortunately, no experiments were done with mechanical reflux in low protein rats.

The conclusion reached from the data obtained so far is that refluxing occurs primarily during rising urine flow, but it persists even during falling urine flow when the urine flow is very high. Urea can thus move out of the tissue into the urine during refluxing. Whether this movement takes place from inner and/or outer medulla facing the pelvic space cannot be deduced from the present data. The effect of refluxing is to reduce the urea concentration in the renal papilla. Since a substantial reduction in papillary urea concentration occurs during the change from antidiuresis to diuresis, the refluxing mechanism serves to speed up this process. This study was supported by NIH grant AM 15972-08.