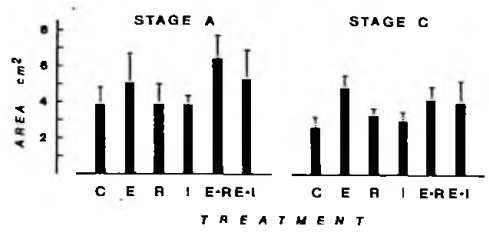

Figure 2.--Cross-sectional area of the Verschlussvorrichtung in Squalus acanthias treated with vehicles (C), 17β-estradiol (E), relaxin (R), insulin (I), estradiol plus relaxin (E+R) and estradiol plus insulin (E+I).

In stage A females, none of the hormone treatments significantly affected the maximum cross-sectional area of the Verschlussvorrichtung. Only estradiol alone was effective in stage C females in significantly increasing luminal cross-sectional area ($p < .05$). Relaxin and insulin had no effect either alone or after estradiol priming. In both stages of pregnancy, however, all groups receiving estradiol averaged larger cross-sectional areas than groups receiving vehicles or peptides. When the individual values were averaged on the basis of estradiol treatment, the means presented in Table 1 were obtained. For both stages of pregnancy the maximum cross-sectional area was significantly larger in the estradiol treated females ($p < .05$).

Table 1.--Cross-sectional area of the Verschlussvorrichtung in estradiol treated female Squalus acanthias

	Stage A		Stage C	
Group	n	area (cm^2)	n	area (cm^2)
no estradiol	13	3.86 ± 0.50	15	2.97 ± 0.28
estradiol	16	5.87 ± 0.81	17	4.36 ± 0.51

While it appears that estradiol is capable of increasing the luminal cross-sectional area of the Verschlussvorrichtung, the magnitude of the response is less than that necessary to allow passage of eggs which at ovulation measure 11 cm^2. Whether this is due to temporal and quantitative inadequacies in the estradiol treatment or to a lack of additional humoral factors is uncertain. It is clear, however, that the Verschlussvorrichtung of pregnancy must change in mass or material properties before egg transport can occur. Supported by NSF PCM 81-04144 to I.P.C.

PHYSIOLOGIC AND MORPHOLOGIC RENAL ADAPTATIONS TO A REDUCED PROTEIN INTAKE IN RATS

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Earlier findings in several mammalian species have shown that a low but adequate protein intake have the following renal effects: 1) the urea clearance is reduced relative to the glomerular filtration rate, as a result 2) the plasma urea concentration is not reduced in proportion to the reduction in the nitrogen intake, (B. Schmidt-Nielsen, Am. J. Physiol. 181:131-139, 1955; B. Schmidt-Nielsen, Physiol. Rev. 38:139-168, 1958), 3) the distribution of urea in the renal medulla differs markedly in animals on low and normal protein diets (B. Schmidt-Nielsen and R. O'Dell, Am. J. Physiol., 197:856-860, 1959; B. Truniger and B. Schmidt-Nielsen, Am. J. Physiol., 207: 971-978, 1964), 4) the recirculation index for urea (as defined by H. Valtin [Am. J. Physiol. 233:F491-F501, 1977], the fraction of filtered urea present in the distal convolutions) is significantly greater in the rat on low than in the rat on high protein diet (R.A. Danielson, B. Schmidt-Nielsen and C. Hohberger In: Urea and the Kidney, Excerpta Medica Press, pp 375-384, 1970). 5) More urea is reabsorbed from the collecting ducts by passive and active mechanisms (R. A. Danielson, B. Schmidt-Nielsen and C. Hohberger, Am. J. Physiol. 233:130-137, 1972; K.J. Ullrich, G. Rumrich and B. Schmidt-Nielsen, Pflugers Arch. 295:147-156, 1967). The present studies were undertaken to determine if renal anatomical changes may occur in the vascular bundles in the inner stripe of the outer medulla of the kidney when a rat is maintained for 30 to 40 days on a reduced protein diet. The increased

recirculation index found in the study by Danielson et al ('70) in the rat suggested an involvement of the outer medullary vascular bundles in the renal adjustments to reduced protein intake. Thus, Valtin ('77) has pointed out that there is a relationship between the type of vascular bundles an animal has and the recirculation index; i.e., the greater the incorporation of thin descending limbs of short-looped nephrons in the vascular bundles the greater the recirculation index.

Two groups of 6 week old Munich Wistar rats were placed on standardized (custom made by Siegler Brothers) reduced protein (8% protein 2.8% salt, LP) and normal protein (24% protein, NP) diets, respectively. The salt added to the LP diet insured that the excreted solute load would be equal on the two diets. After 30 to 40 days on the diet physiologic and anatomic studies were performed on the rats.

Renal function was measured in five normally hydrated animals from each group. In the anaesthetized rat the jugular vein, carotid artery, and bladder were catheterized. A priming dose of ^{3}H labelled inulin together with ^{14}C labelled urea was injected into the jugular vein and followed by a maintenance dose of these compounds. An hour later three to four urine samples were collected at approximately 30 min intervals. Blood samples were taken from the artery at the midpoint of each urine collection period. All samples were counted in a Packard Liquid Scintillation Counter. Urine collection time was corrected for catheter dead space. Urine flow rates, urine/plasma ratios and glomerular filtration rates were averaged for each animal.

Analyses of inner medullary tissues were made on 11 rats on the two diets. The animals were dehydrated for 18-24 hours and then killed in CO_2. The inner medulla was divided into three pieces as shown in Fig. 1. The

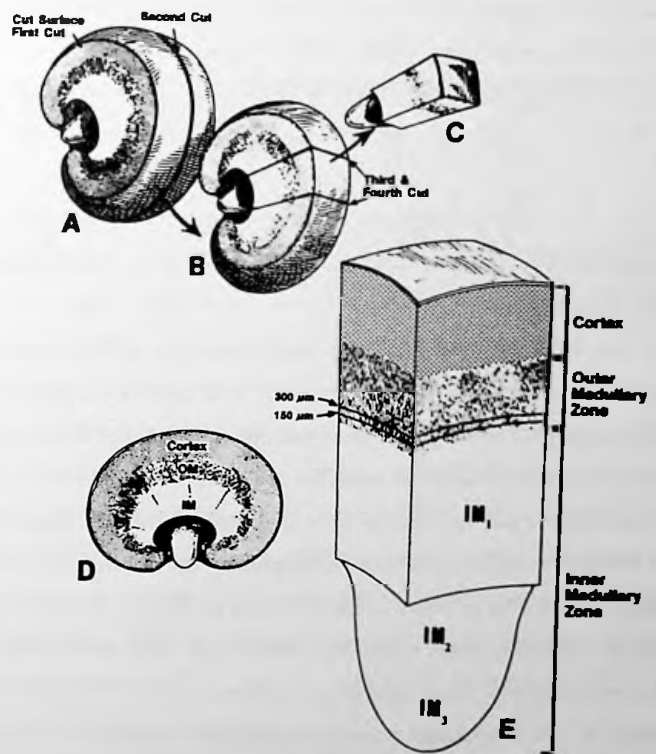

Figure 1.--Tissue sampling from the rat kidney. A. Anterior face of whole kidney is cut away (first cut) revealing intact papilla within renal sinus. B. Second cut gives the central disc with papilla. C. Third and fourth cuts give the central core within intact papilla. D. Zones of the kidney are readily identifiable (OM-outer medulla; IM-inner medulla). E. Central core showing the three layers of the inner medulla sampled (IM 1, IM 2, IM 3) and the 150 and 300 μm levels of the outer medulla used for morphometric analysis.

methods used for water and solute determinations in the tissue was as described earlier (B. Schmidt-Nielsen, B. Graves, and J. Roth, Am. J. Physiol. 244:F474-F482, 1983).

Five animals from each group were used for the structural analysis. The kidneys were fixed via retrograde abdominal aortic perfusion. Selected slices from each kidney were processed for routine microtomy in Epon plastic. Sections of these slices were examined and photographed at the light and electron microscope level. Structural parameters were quantitatively measured and compared using a computer-based morphometric analysis system. The values shown in Table 3 represent 10 measurements from each of the five animals.

The results showed a highly significant difference in urea clearance between NP and LP rats (Table 1). The facts that GFR was unaffected by the difference in diet, and that plasma urea in the dehydrated LP rats (Table 2) was

Table 1.--Renal function. Comparison between normal (NP) and reduced protein (LP) anaesthetized rats.

	NP n = 5	LP n = 5
GFR	0.957 ± 0.076	0.896 ± 0.096
Fraction of filtered urea excreted	0.253 ± 0.014	0.052 ± 0.006*
Urine urea mM	888 ±67	101 ±25*

*$p < 0.001$

Table 2.--Plasma and papilla tip concentrations in dehydrated rats

	Plasma		Papilla tip	
	NP n = 6	LP n = 5	NP n = 6	LP n = 5
Osm mOsm	316 ± 2	325 ± 3^{+}	2,218 ± 115	1,806 ± 102^{+}
Urea mM	5.9 ± 0.6	13.2 ± 1.0*	1.011 ± 57	573 ± 78*
Na mM	160 ± 2	157 ± 4	393 ± 30	417 ± 30
K mM	5.3 ± 0.5	6.0 ± 0.7	61 ± 4	78 ± 2$^{\xi}$

$^{+}P < 0.05$, $^{\xi}P < 0.01$, $^{*}P < 0.005$.

higher than in the NP rats clearly show that the change in urea clearance and urea distribution in the medulla are not secondary to a lowering of either filtration rate or plasma urea. It is concluded that urea handling within the kidney is specifically altered in LP animals, a change which does not appear to affect the handling of other solutes (see Table 2). The lower urea accumulation in the inner medulla of the LP rats might be explained by an increase in passive removal of urea from the inner medulla, and or by a decrease in the addition of urea to the inner medulla.

Structural changes in the outer medulla, which accompanied the physiologic changes, were highly specific (Table 3). Thus, the thin descending limbs of short loops of Henle in the LP rats had significantly smaller lumen and smaller epithelial wall area than in the NP rats. The long descending limbs of the long loops of Henle, located in the interbundle region of the outer medulla, had significantly thinner epithelial walls in the LP rats compared to the NP rats. The decrease in lumen of the short loops associated with the vascular bundles may facilitate counter-current exchange of urea with the ascending vasa recta, thus increasing the removal of urea from the medulla and account for the increased recirculation index found in LP rats (Danielson et al., '70). The decrease in the wall thickness of the long descending loops of Henle may enhance uptake of urea leaving the collecting ducts. However,

Table 3.--Morphometric analyses of anatomic changes in the inner stripe of the outer medulla. M ± SEM

	NP rats n = 50 μm^2	LP rats n = 50 μm^2	Significance of difference
Area per vascular bundle	22,800 ± 1,400	23,700 ± 1,000	NS
Thin limb of short loops			
Lumen area	166.8 ± 5.0	144.2 ± 6.3	P<0.01
Epithelium area	34.0 ∓ 1.8	29.2 ∓ 1.6	P<0.05
Thin limb of long loops			
Lumen area	167.5 ± 6.5	178.8 ± 7.8	NS
Epithelium area	74.2 ∓ 4.7	46.7 ∓ 2.8	P<0.001

until more is known about the permeability properties of these nephron segments in LP and NP rats this remains speculation , only. Further interpretation of these results must await additional data. Supported by NIH grant No. RO1 AM 15972.

THE ROLE OF THE ADENYLATE CYCLASE - CYCLIC AMP SYSTEM IN BILE SECRETION BY THE ISOLATED SKATE LIVER

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The role of hormones and the adenylate cyclase system in the regulation of biliary secretion is controversial. However, in some mammalian vertebrates, addition of dibutyryl cyclic AMP is associated with a stimulation in bile production (Graf J. Am. J. Physiol. 242:G233-G246, 1983). In this preliminary study, we have examined the effects of forskolin, a diterpene which directly activates the catalytic unit of adenylate cyclase (Seamon, K.B., Daly, J.W.: J. of Cyclic Nucleotide Research 1:201-224, 1981) and 2-chloro adenosine on bile flow in the isolated perfused skate liver. These are specific modifiers of adenylate cyclase activity in a variety of tissues and should therefore be useful in examining if this regulatory enzyme plays a role in bile formation.

Materials and Methods

Livers from the small skate (Raja erinacea) were isolated and perfused with cold oxygenated Elasmobranch Ringers, as previously described (Reed, J.J. et al., Am. J. Physiol. 242:6319-6328, 1982). In each experiment, the gallbladder was opened, the cystic duct cannulated with polyethylene tubing (PE-160) and bile collected in segments of polyethylene tubing (PE-90). Volume per unit length of tubing was calculated and, at 10 or 15 minute intervals, marks were made on the collection tube to record the rate of bile production. Bile flow was monitored for 1 to 2 hours until a stable baseline rate of secretion was established. In twelve studies, forskolin (5 μM or 0.05 μM) or dibutyryl cAMP (10 μM) was added to the system. After 1-2 hours, the perfusate was then exchanged with fresh Elasmobranch Ringers, to remove these agents and bile flow was monitored for an additional 1-2 hours.

In a second set of 5 experiments, the Ringer's solution was perfused in a single pass through the liver. After a baseline secretion rate had been established, the liver was alternately perfused (for approximately half-hour periods) with 1 μM 2-chloro adenosine, 1 μM 2-chloro adenosine plus 10 μM theophylline, or Elasmobranch Ringers alone while bile flow was continuously recorded.

Results and Discussion

Treatment with 5 μM forskolin inhibited bile secretion in all experiments (43 ± 22% of control; n = 5), and subsequent perfusion with forskolin-free ringers led to a significant recovery (80 ± 37% of control). Figure 1 illustrates a representative experiment. Reducing the concentration of forskolin to 0.05 μM abolished its inhibitory effect. Only one of five experiments showed significant depression of bile secretion from its control value, while in two ex-