The functional morphology of the kidney of the Cape Fur Seal, *Arctocephalus pusillus* (Schreber)

by
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ABSTRACT

The anatomy and histology of the kidney of *A. pusillus* were studied by means of resin infusions, corrosion casts and standard histochemical procedures using quantitative measurements where possible. It was found that the kidney of *A. pusillus* is typically renculate with each renculus conforming to the general architecture of the unilobar rodent kidney. The blood vascular system comprises an intrinsic pathway, typically found in cetaceans, but described here for the first time in pinnipeds. Quantitative measurements of relative medullary thicknesses, percentage distribution of thin segments and size distribution of glomerular volumes showed that certain aspects of the histology share common features with desert forms.

Physiological studies involving chemical analyses of urine and plasma, however, revealed that although *A. pusillus* possesses efficient renal function (Plasma: Urine ratio of 7,2), this is not nearly as spectacular as that found in desert rodents. *A. pusillus* is therefore only independent of fresh water within the confines of its naturally cool and moist habitat.

1. INTRODUCTION

During recent years increasing interest has been shown in comparative studies of renal function. Many of these studies were concerned with elucidating basic physiological phenomena. Many of them, however, have also been directed towards explaining ecological problems, particularly in regard to survival of desert forms which do not have access to free water (Abdallah and Tawfik 1969, Munkacsi and Palkovits 1965, Schmidt-Nielsen and O'Dell 1961). Marine mammals, although they inhabit a mesic environment, must by necessity also be independent of fresh water and therefore their renal function has also excited considerable interest recently (Depocas, Hart and Fischer 1971, Kooyman and Drabek 1968, Pilson 1970, Tarasoff and Toews 1972).

As neither the morphology nor the functional efficiency of the kidney of *A. pusillus* has been investigated and because a large amount of material was available during routine sealing operations, it was decided to examine the functional renal morphology of this species. To this end resin infusions, corrosion casts and histo-chemical studies were made to examine both the macro and micro anatomy of the kidney. Whenever possible an attempt was made to quantify the morphological data. Functional efficiency was studied by comparing the chemical composition, including osmolality, of the urine and plasma of this species.

2. PROCEDURE

2.1. Collection and analyses of urine and plasma samples

Samples were obtained from Cape fur seals slaughtered on land during routine annual sealing opera-

tions at the Cape Cross rookery, South West Africa. Samples were collected from the yearling class during July and from adult rutting males during December.

Urine was collected by means of disposable hypodermic syringes from exposed bladders and preserved by freezing. Blood samples from eleven yearlings were similarly obtained from the azygos vein or anterior vena cava. Blood from fourteen adult males was drained into heparinised plastic containers from severed anterior caval veins. Blood samples were immediately centrifuged on a clinical centrifuge for 30 minutes at 3 500 r.p.m. and the supernatant was frozen in sealed Eppendorf plastic vials. The yearlings, individually killed by clubbing, suffered from respiratory failure but blood circulation remained functional for some time. Blood was therefore obtained under optimum conditions although some difficulty in preventing haemolysis was encountered as Pugh (1959) experienced when sampling Weddell seals. This suggests a fragility of the red blood cells. The method used to collect blood samples from the adult males, which were killed by shooting, was entirely satisfactory and is recommended.

The total plasma protein content was determined using the biuret method (Henry 1964) and a photometer (Eppendorf model 1101 M, filter Hg 578 nm). Plasma protein fractions were determined by electrophoresis on cellulose acetate membranes (Beckman model R-100 microzone electrophoresis system). Urea determinations were carried out enzymatically using the method of Richterich (1968) and the abovementioned photometer, using filter Hg 578 nm. Osmolalities were determined using an automatic high precision research osmometer (Advanced instruments, model 67 31 RAS) and chloride determinations were carried out using an automatic chloride titrator (Radiometer, CMT 10). Sodium and potassium were determined using standard flame photometric techniques (Instrumentation Laboratory, IL 343). Uric acid was determined enzymatically by the uricase method (Henry 1964) utilising a double-beam spectrophotometer, Beckman DB, at 293 nm. Finally magnesium determinations were carried out using an atomic absorption spectrophotometer (Techtron, model AA 120). Strontium was used to stabilise magnesium counts.

2.2. Kidney Morphology

Gross morphology was examined by dissection and medial sections revealed the gross internal structure. The renal blood vascular and ureteral systems were studied by the following methods. Through the use of an infusion system comprising a wide diameter burette, rubber tubing with clamps, plastic cannula and Marco resin (Tompsett 1956), the latter was infused under slight pressure via the renal artery and renal vein respectively into the two different kidneys. The ureteral system was also infused in conjunction with the arterial system to determine relative positions. Differently stained resins were used in each instance to facilitate identification of the related systems. Fresh undamaged kidneys, frozen apart in close fitting polythene bags to prevent dessication, were thawed and manipulated underwater to expel any trapped air. The vascular system was first flushed with distilled water using gravity flow. The water was substituted with 4% formaldehyde, the emergent vessels clamped and left overnight. Resin with sufficient catalyst added to ensure a fluid condition was then introduced into the relevant systems. After ligating the appropriate vessels the kidney(s) remained submerged for eight days to allow the resin to set. The tissue was then macerated with concentrated hydrochloric acid and the resulting corrosion casts washed and studied under a stereo microscope using fine tweezers for trimming. When resin was injected into the renal artery under high pressure the glomeruli trapped the staining pigment and the venous system was secondarily filled with normal coloured resin. Thus the relative positions of the arterial and venous systems could be established.

To study the terminal vascular arrangement the kidney was infused with a warm gelatin solution containing 15 ml of India ink per litre (Van der Spoel 1963) after routine flushing with distilled water. The kidney was warmed to 37° C in a waterbath prior to infusion. After ligating the vessels the intact kidney was fixed in 5% formaldehyde. Tissue blocks were removed by dissection, dehydrated in alcohol and cleared in methyl salicylate-benzol (ratio 2:1). Sectioning was performed by hand and microtome and mounted for stereoscopic and microscopic examination.

For histological study, renal tissue was fixed in 10% formaldehyde, dehydrated in alcohol and cleared in xylol, methyl benzoate colloidin and terpineol. The two latter substances prevented excessive hardening of the tissue and good results were obtained. The tissue was blocked in paraffin wax and sectioned as 3, 7 and 10μ. Complete renculi of a yearling's kidney, sufficiently small to be adequately fixed, were longitudinally sectioned. Sections were stained with Ehrlich's hematoxylin (Gurr 1956) and Eosin or Erythrosin, Azan and Azocarmine as well as with Toluidine blue (Lillie 1929) as described by Humason (1966). Mallory's phosphotungstic acid hematoxylin (Lieb 1948) did not stain well and no results could be inferred from this method.

2.3. Dimensions and thickness of kidney regions

Kidneys obtained from adult males and three pups were weighed and the dimensions recorded. The kidney size was calculated as the cube root of the product of the dimensions of the kidney (Sperber 1944). Fifty-seven renculi were isolated from these kidneys, a minimum of five per kidney. Each renculus was sectioned along its long axis. These sections generally passed through the tip of the papilla and thinnest part of the cortex. The width of the cortex and length of the medulla (pyramid) were measured with callipers and the corticomedullary ratio was calculated. The relative thickness of the layers was assessed using the formula:

$$\frac{\text{layer thickness}}{\text{kidney size}} \cdot 10 \text{ (Sperber 1944).}$$

2.4. Glomerulometrics

The volumetric analysis of glomerular size was conducted according to the method of Palkovits and Zolnai (1963). Five renculi were dissected from an adult male's kidney (405 g), fixed in 4% formaldehyde and processed in the prescribed way. Each was sectioned at 17 μ and stained with hematoxylin and eosin. Sections were projected onto an opaque glass plate at a magnification of X250. The longest diameter and the one perpendicular to it were measured in each of three hundred cross sections of glomeruli selected at random. In addition, to determine the difference in size between cortical and juxtamedullary glomeruli, the cortical and juxtamedullary zones on each of forty sections were alternatively covered. This was done by applying India ink to the cover slip under a stereo microscope (Munkácsi and Palkovits 1966). Three hundred glomeruli were measured in each zone. The logarithmic values of the volumes (volume $= \frac{\pi}{6}$ (LB) $^{3/2}$) were arranged into logarithmic classes. Table III in Palkovits and Zolnai (1963) which was compiled on the basis of an X250 magnification was used to determine the logarithmic class value from the axial measurements (mm) obtained from glomeruli in this study. The range of the measurements (12 x 10 — 41 x 32) was sufficient to allow this to be done. Furthermore, distribution curves were drawn, indicating the degree of variation in glomerular size in each zone. The logarithmic class values were plotted as the abscissa and the percentage numbers of volumes credited to each class as the ordinate.

The percentage ratio of glomerular volume to cortex volume was ascertained by projecting different sections onto an opague glass plate. The latter overlaid a grid of 10 x 10 parallel lines, 1 cm apart. These lines intersected one another at 100 loci at right angles. The points of intersection which occurred on glomeruli were counted in twenty different areas. The mean value obtained expressed the percentage ratio of glomerular volume to cortex volume. When projected, a single glomerulus should not exceed the area occupied by 2 x 2 squares (Palkovits and Zolnai 1963). This was achieved by X120 magnification.

2.5. Percentage distribution of the thin segments of the loops of Henlé in the renculer medulla

The method initially employed was similar to that of Munkácsi and Palkovits (1966). A renculus fixed in 10% formalin was processed as previously described and serially sectioned at 7 μ transversely to the long axis of the renculer medulla. Sections were stained with hematoxylin and eosin. Sections were selected from twenty proportionate distances ($\pm$ 390 μ apart) from the tip of papilla to corticomedullary junction. Folowing the procedure of Palkovits and Zolnai (1963) the percentage distribution of the thin segments was calculated. The measurements were done at X1000 magnification under a microscope fitted with an ocular grid. The grid provided one hundred uniformly dispersed intersections as previously described. The grid was thus superimposed on the sections from each proportionate level and the number of intersections falling on thin segments in cross section were counted. Twenty different areas were counted from each level and the mean computed. The value obtained expressed the percentage distribution of volume of thin segments to total tissue volume in renculer medulla. A distribution curve was drawn indicating the mean percentage values at the twenty different levels. At the indicated magnification a thin segment did not exceed 2 x 2 squares, thus complying with the described prerequisite.

3. RESULTS AND DISCUSSION

3.1. Analyses of urine and plasma

The problem of water metabolism or osmotic and ionic regulation in marine mammals in general, and pinnipeds in particular, has received considerable attention in recent years. Pinnipeds do not have access to fresh water (Pilson 1970, Irving *et al.* 1935, Hiatt and Hiatt 1942) and migrations to fresh water seem unlikely (Tarasoff and Toews 1972). The question of sea water ingestion as a source of water has been repeatedly discussed. Fetcher (1942) found that ingestion of sea water resulted in selective absorption of NaCl, a process wasteful of water. Moreover, he found that dolphins showed a positive Cl^- balance in excess of 8 hours after the salt loading experiment. Smith (1936) extrapolated from the inorganic composition of urine and salts of rectal washings that *Phoca vitulina* ingested a minimum of sea water. The latter cannot utilise sea water since ingestion also induces a negative water balance (Bradley *et al.* 1954). After administration of various amounts of sea water by stomach tube, Tarasoff and Toews (1972) found that starved *P. vitulina* were unable to eliminate all the electrolytes introduced and had to draw on body water to do so, thus creating a negative water balance. Cases have been reported of deliberate sea water drinking (Rand 1959, Brown 1952) but this was related to unusual conditions. Since there exists no evidence for extra renal pathways for the excretion of salt (Pilson 1970), it can be finally concluded that a seal normally does not drink sea water and cannot benefit by it. Therefore the only water resources available are preformed water in their diet and water produced by oxidative metabolism (Depocas *et al.* 1971). Irving *et al.* (1935), Smith (1936), Fetcher (1939) and Pilson (1970) found that ample water was made available by preformed and metabolic water of ingested food at a low enough salt concentration requiring no osmoregulatory organ other than the kidney. Moreover, Pilson (1970) is of the opinion that it would be no more osmotically stressful for a seal to eat marine invertebrates (osmoconformers) than teleosts (osmoregulators). His calculations were based on the relative salt and protein contents. Fasting seals likewise derive sufficient oxidative water from their tissue to satisfy the major part of their needs (Depocas *et al.* 1971). 100 g of

fat yields 107 g of water (Harper 1967), therefore subcutaneous fat deposits (blubber) may be called upon to tide them over a period of starvation. From the above it can be inferred that the necessity to drink sea water is precluded and that sea water ingested is a hazard coincident to swallowing food (Depocas *et al.* 1971, Pilson 1970) but that water supplied by the food would be adequate to eliminate the resulting excretory products as well as the excess electrolytes (Tarasoff *et al.* 1972).

Feeding in the seal induces an increase in renal plasma flow (RPF), glomerular filtration rate (GFR) and water diuresis (Hiatt and Hiatt 1942, Ladd *et al.* 1951). According to Chew (1965) this phenomenon, diuresis being correlated with a greater RPF and GFR, is unique for the seal and rabbit. This correlation with the food intake enables the kidney of the seal to function effectively when food has supplied excess water for renal excretion (Hiatt and Hiatt 1942) and vice versa. Schmidt-Nielsen *et al.* (1959) demonstrated an increase in urea excretion (40,3 g/l) and an elevated plasma urea concentration (75 mg/100 ml – 103,2 mg/100 ml) in *P. vitulina* after feeding and found that this animal's concentrating ability for urea was rather poor, noting a maximum of 71,3 g/l, while a maximum of 74,9 g/l has been recorded for *Leptonychotes weddelli* (Kooyman and Drabek 1968). Urea also appears to be the principal osmotically active substance in urine during fasting (Smith 1936) and contributes to the high osmotic pressure of urine at low output during fasting (Tarasoff *et al.* 1972).

Keyes *et al.* (1971) found electrolyte concentrations in urine of *Callorhinus ursinus* to be comparable with those found in man. The sodium values were similar to those of non-lactating female *L. weddelli* and pups (8–151 mEq/l) and lower than those recorded for fasting lactating females (71–265 mEq/l) by Kooyman *et al.* (1968) and a wild *Zalophus californianus* male (320 mEq/l) by Pilson (1970). This suggests sea water drinking by the latter two species. However, this is not conclusive since urine composition varies throughout the day (Smith 1936). Furthermore, after the introduction of sea water by stomach tube Tarasoff *et al.* (1972) found the highest sodium and chloride values recorded for a pinniped. In this experiment *P. vitulina* exhibited the following maximum concentrations: 523 mEq Na^+/l, 508 mEq Cl^-/l, 136 mEq K^+/l and a urinary osmotic concentration of 2 050 mOsm/l. This suggests that the seal can excrete Na^+ and Cl^- at concentrations approximating that of sea water (548,3 mEq Cl^-/l, 470,2 mEq Na^+/l, 9,9 mEq K^+/l after Barnes 1954). Tarasoff *et al.* (1972) showed that body water must be utilised to eliminate all of these ions.

Blood osmotic pressures ranged from 305–336 mOsm/l in *L. weddelli* and from 335–447 mOsm/l in the marine mammals included in Fetcher's (1939) survey. Such a high osmotic pressure would reduce the kidney load slightly and Kooyman and Drabek (1968) concluded that *L. weddelli* lacked an adjustment of blood concentration to a higher level. Inspection of plasma concentration values obtained in the present investigation for *Arctocephalus pusillus* (Table 1) shows that the kidney controls the osmotically active substances of the plasma at a level similar to those found in man and other seals (Kooyman *et al.* 1968, Page *et al.* 1954). *A. pusillus*, however, apparently do not possess the ability to increase the blood concentration to reduce kidney load. Rand (1959) observed that rutting male *A. pusillus* abstained from food for ± 5 weeks during the breeding season and only resorted to limited foraging when driven to sea by the sealers. The indications were that they did not cease feeding entirely. The disturbance created by sealers was also thought to account for the deliberate drinking of large amounts of sea water since 36% of rutting bulls included in the catch contained sea water in their stomachs. Yearlings suckle for at least 8 months with little supplementary feeding and clubbing on land supplies a catch with ample milk in their stomachs (Rand 1959).

Table 1. Plasma analyses:

AGE GROUP	MEAN	S.D.	RANGE	N
		Osmolality (mOsm/l)		
Bulls	326,6	± 9,3	306–340	14
Yearlings	308,5	± 10,8	284–320	11
		Sodium (mEq/l)		
Bulls	148	± 1,1	146–153	14
Yearlings	149	± 3,8	141–158	11
		Chloride (mEq/l)		
Bulls	108	± 1,9	106–114	14
Yearlings	102	± 1,0	97–105	11
		Potassium (mEq l)		
Bulls	5,7	± 2,2	5,0–7,0	14
Yearlings	5,2	± 0,9	4,4–7,6	11
		Magnesium (ppm)		
Bulls	35	± 4,4	30–46	10
Yearlings	35	± 5,8	29–50	10
		Urea (mg/100 ml)		
Bulls	57,9	± 11,7	38,3– 78,6	14
Yearlings	71,3	± 20,8	27,1–111,8	11

This feeding regime was reflected in the blood constituents measured in the present investigation since plasma collected from the rutting bulls exhibited a low urea content (57,9 mg/100 ml) compared with that of the yearlings (71,3 mg/100 ml). The latter is lower than that recorded for *P. vitulina* after a meal of herring (Schmidt-Nielsen *et al.* 1959) which may possibly be attributed to the lower protein content of seal milk. A single sample of *A. pusillus* milk contained 10% protein (Rand 1956) compared with 16,3–19,5% in herring (Depocas *et al.* 1971). A further observation that confirmed that at least some of the yearlings had recently been suckled was that most of them regurgitated milk as a result of the clubbing. Fifty percent of the plasma samples obtained from the pups were very lipaemic showing

a distinctive white surface layer after centrifugation. This could be the result of the high fat content of seal milk, up to 53,2% for *Pagophilus groenlandicus* (Cook and Baker 1969). This assumption is substantiated by the findings of Nelson (1970) for a single male *Mirounga augustirostris*. The latter exhibited lipaemic plasma one hour post prandially and exhibited a characteristic elevation of the plasma triglyceride levels when compared to fasting seals included in the study. Triglycerides constituted 39,08% of the neutral lipids in the plasma. This effect would no doubt also occur after the ingestion of milk with its high fat content.

The total plasma protein content obtained for *A. pusillus* in the present investigation together with the various fractions are listed in Tables 2 & 3. The total protein content is comparable, if not higher, than those encountered in *Halichoerus* pups (Harrison and Tomlinson 1967). The electrophoretogram of plasma proteins (Fig. 1) possesses five distinct peaks. The lower albumin fraction of bulls, expressed as a percentage of total protein (44,2%), compared to that in yearlings (57,5%) suggests a fasting condition when circulating plasma protein becomes depleted, causing a degree of proteinemia. The higher average plasma osmolality found in the adult category suggests that sea water may be ingested. For example, Bradley *et al.* (1954) found an increase in plasma osmotic concentration after electrolyte loading in *P. vitulina*. Chew (1965) stated that the lowest urine concentrations in *P. vitulina* are associated with the lowest urine flow as in fasting seals, whereas maximum concentrations occur at intermediate rates of urine flow after food ingestion. Moreover, Tarasoff and Toews (1972) found that fasting and therefore dehydrated harbour seals exhibit maximum reabsorption of sodium and chloride ions resulting in a low urine concentration.

Table 2. Total plasma proteins.

AGE GROUP	MEAN (g/100 ml)	S.D.	RANGE	N
Bulls	6,8	±0,4	5,8–7,4	14
Yearlings	6,1	±0,4	5,5–6,6	11

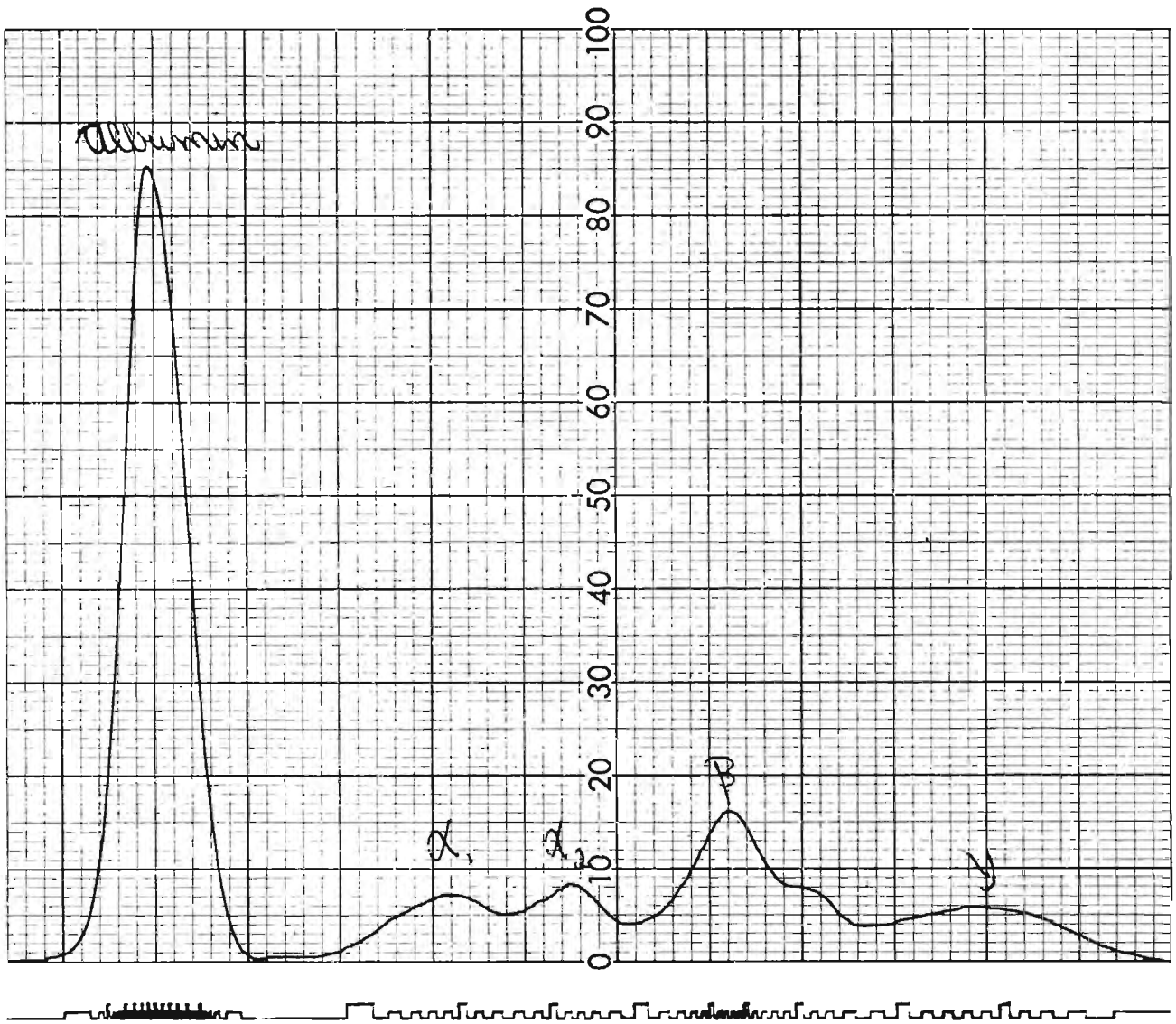


Figure 1. Typical electrophoretogram of plasma proteins.

Table 3a. Percentage of various plasma protein fractions in bulls.

	Albumin (%)	Globulins (%)			
		α_1	α_2	β	γ
MEAN	44,2	12,1	16,6	11,3	15,8
S.D.	±2,6	±1,5	±1,7	±1,6	±2,5
RANGE	38,8–49,6	8,0–14,6	13,8–21,8	8,7–15,1	12,3–20,5
N	14				

Table 3b. Percentage of various plasma protein fractions in yearlings.

	Albumin (%)	Globulins (%)			
		α_1	α_2	β	γ
MEAN	57,5	6,7	7,2	18,9	9,7
S.D.	±5,4	±1,7	±2,1	±1,0	±3,7
RANGE	48,0–68,6	3,2–8,6	3,1–10,8	17,2–20,5	4,4–18,9
N	10				

Table 4. Urine analyses.

AGE GROP	MEAN	S.D.	RANGE	N
		Osmolality (mOsm/l)		
Bulls	2115,2	±200,7	1748–2364	10
Yearlings	1987,2	±162,1	1717–2188	10
		Sodium (mEq/l)		
Bulls	125	± 88,1	48– 368	11
Yearlings	91	±258,9	10– 272	10
		Chloride (mEq/l)		
Bulls	262	±142,1	91– 567	11
Yearlings	185	± 49	105– 265	10
		Potassium (mEq/l)		
Bulls	152	± 30,3	106– 216	11
Yearlings	91	± 50,6	31– 210	10
		Magnesium (ppm)		
Bulls	226	± 91,1	104– 376	10
Yearlings	159	± 62,5	38– 234	10
		Urea (g/1 000 ml)		
Bulls	71	± 17,1	31,7–91,4	11
Yearlings	77,5	± 13,2	55,8–98,4	10
		Uric acid (mg/100 ml)		
Bulls	19,8	± 6,5	10,1–30,2	10
Yearlings	16,6	± 5,8	9,0–25,0	10

This observation was not confirmed during the present investigation in the fasting rutting male *A. pusillus* (Table 4), perhaps as a result of sea water ingestion. The maximum values obtained approach and in the case of chloride and potassium exceed those obtained by Tarasoff and Toews (1972) after forced stomach infusion of sea water in *P. vitulina*. The maximum urinary osmolality (2 364 mOsm/l) recorded for *A. pusillus* exceeds the previously recorded maximum of 2 150 mOsm/l in *P. vitulina* (Smith 1936). The higher Mg^{++} and reduced urea concentrations in the urine of the bulls when compared with the yearlings, are again suggestive of sea water drinking and fasting in the case of the former.

The maximum urea values in both age groups are higher than those recorded for fed *P. vitulina* (Schmidt-Nielsen *et al.* 1959) but are comparable to those of *C. ursinus* (106,8 g/l) after Keyes *at al.* (1971). This suggests a more effective urea concentrating ability in the fur seals but it is well within the carnivore range which has a maximum of 150 g urea/l (Fetcher 1939). The uric acid content is low but distinctly higher than the average value (9,1 mg/100 ml) recorded in *C. ursinus* (Keyes *et al.* 1971).

From the data obtained in the present investigation it is evident that the Cape fur seal can exist comfortably on the preformed water in its food and the water of oxidation made available during metabolism of nutrients. Moreover, the kidney does not appear to possess any unique ability to excrete salt.

In fact, it seems wasteful of water as it only concentrates urine to a level normally attained by many terrestrial mammals which are invariably dependent on free water. The maximum urinary osmotic pressure of 2 364 mOsm/l recorded for *A. pusillus* represents the highest value recorded so far for a marine mammal but it is still lower than the maximum recorded for the dog (2 425 mOsm/l) after West *et al.* (1955). Apart from the adequate water derived from food, other significant factors facilitate a positive water balance in the seal. For example, the lowering of expired air temperature, as a result of the well developed turbinate bones in seals, reduces respiratory water losses. The intermittant breathing and effective utilisation of a greater fraction of the oxygen during each breath, together with the high relative humidity of their surroundings, further reduces respiratory water loss to such an extent that a positive production of water occurs while fat is metabolised. The cold marine surroundings also minimise evaporation for temperature regulation thus contributing further to a positive water balance.

From a physiological point of view the true renculate kidney of marine mammals appears to be entirely self-sufficient under the conditions encountered and there is no necessity for extra-renal salt excretion. It does not, however, possess a distinct advantage over other kidney types found in terrestrial mammals with regard to concentrating ability or ability to excrete salt and nitrogen wastes.

3.2. Kidney morphology

3.2.1. Gross morphology

Sperber (1944) distinguished five mammalian kidney types on the basis of their shape and structure, placing the Cetacea and Pinnipedia in the category possessing renculate kidneys. These consisted of

numerous entities (renculi, reniculi or renules) organised as small kidneys. He, however, also described kidneys of several other mammals as belonging to this type.

Grahame (1953), Ommanny (1932) and Harrison and Tomlinson (1956) described these marine mammalian kidneys as multilobular or lobulated as did Slijper (1962) in the case of cattle, otters, bears and the elephant. They therefore considered each renculus as homologous with a lobule of a simple kidney. Van der Spoel (1963) did not accept this in the case of *Phocaena phocoena* and *Balaenoptera physalus* and homologized the renculi with lobi as defined by Ham (1965). Sperber (1944) also described the renculate kidney of *Phoca* as lobated and Diaconescu and Veleanu (1967) placed the aquatic mammalian kidney in a category of its own and termed it lobated, to be distinguished from cattle kidneys, termed pseudolobated. Wrobel (1963) considered the pinniped kidney to be distinct from that of the Ursidae. The fact that pinnipeds have a large number of relatively small renculi, all of which do not reach the kidney surface, as also pointed out by Guzsal (1959), and which are combined into a superficially compact organ which lacks a sinus renalis, resulted in this conclusion. This is entirely in concordance with Cave and Aumonier (1964) who regarded only the cetacean and pinniped kidney to be truly renculate.

All cetacean kidneys investigated by Cave and Aumonier (1964, 1965, 1967a, 1967b) and Ellenberg-Baum (1943) possessed a fibrous connective tissue capsule enveloping individual renculi, with varying amounts of interrenicular collagenous connective tissue. In pinnipeds this uniform relationship does not exist. Bargmann (1959) found a seal (unspecified) to have ample interrenicular connective tissue while *Phoca* (Sperber 1944; Guzsal 1959) has very little interrenicular connective tissue and *Z. californianus* completely lacks it (Wrobel 1963).

Each renculus comprises a single medullary pyramid (unipapillate) inserted in a single calyx with a circumscribing cap of cortical tissue. Renculi remain distinct but occasionally partial cortical fusion occurs between contiguous renculi as reported for *B. borealis* (Cave and Aumonier 1967b) and *Stenella attenuata* (Cave and Aumonier 1965). To date only cetaceans were found to have a sporta perimedullaris musculosa. This was defined by the latter authors as "a discontinuous, intrarenicular prolongation of the ureteric calyx consisting essentially of a basketwork of admixed collagen and muscle which surrounds the single pyramid at the corticomedullary junction".

Guzsal (1959) has reviewed earlier literature on pinnipeds (1904—1953) and states that the ureteral system lacks a pelvis, simply comprising a branched ureter terminating in single calyces each applied to

Figure 2. Surface of kidney with capsula fibrosa removed. Superficial renculi bordered by shallow depressions.

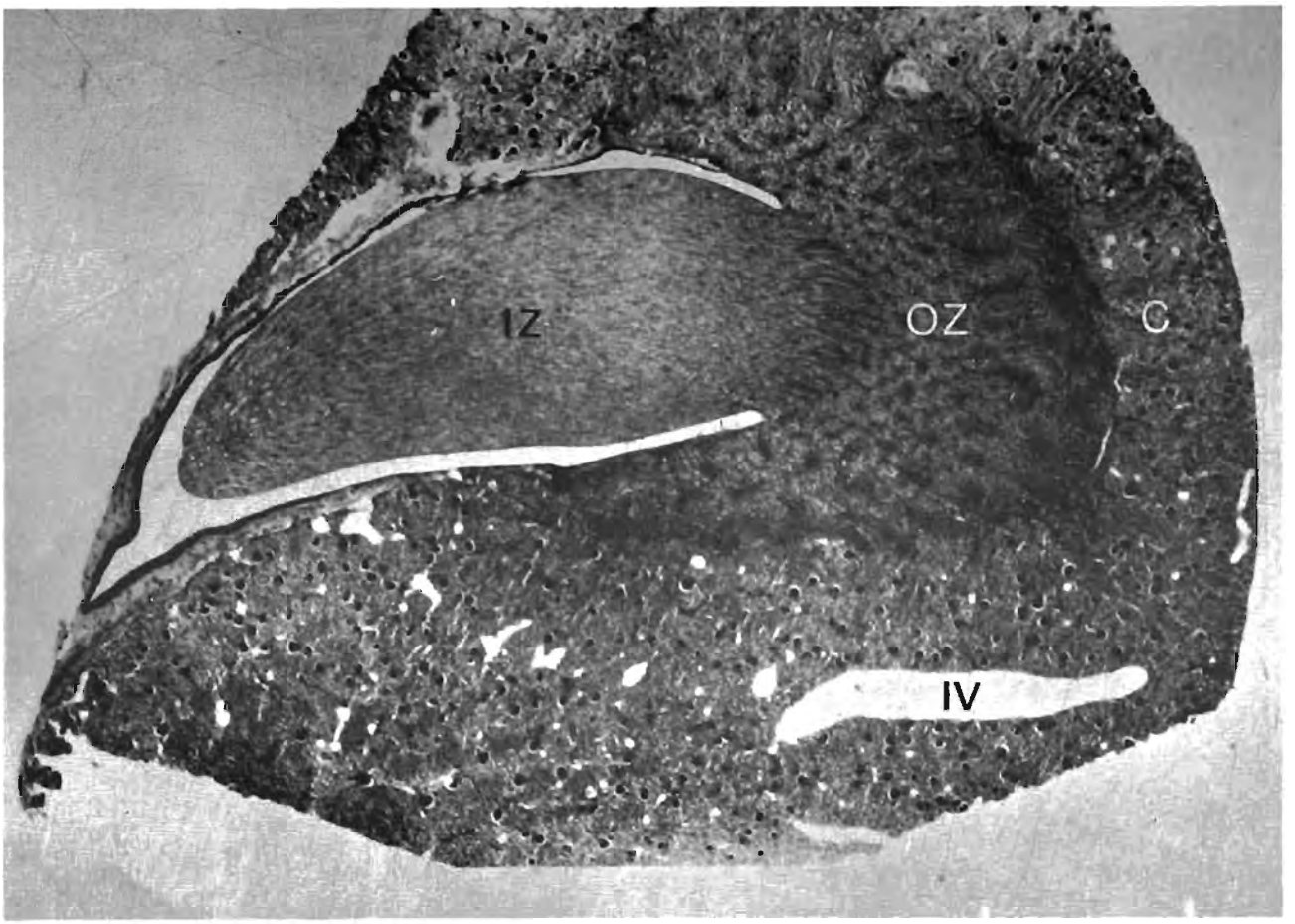


Figure 3. Medial longitudinal section through superficial renculus. C = cortex; OZ = outer zone; IZ = inner zone; IV = interrencular vein.

the papilla of a renculus. This view was supported by his own findings on *P. ladogensis* and those of Wrobel (1963) on *Z. californianus*. Harrison and Tomlinson (1956) on the other hand are of the opinion that *Halichoerus grypus* and *P. vitulina* possess a renal pelvis, while Grahame (1953, 1959) considered them to lack one. Grahame (1953), moreover, described major, intermediate and minor calyces, thus complicating matters further.

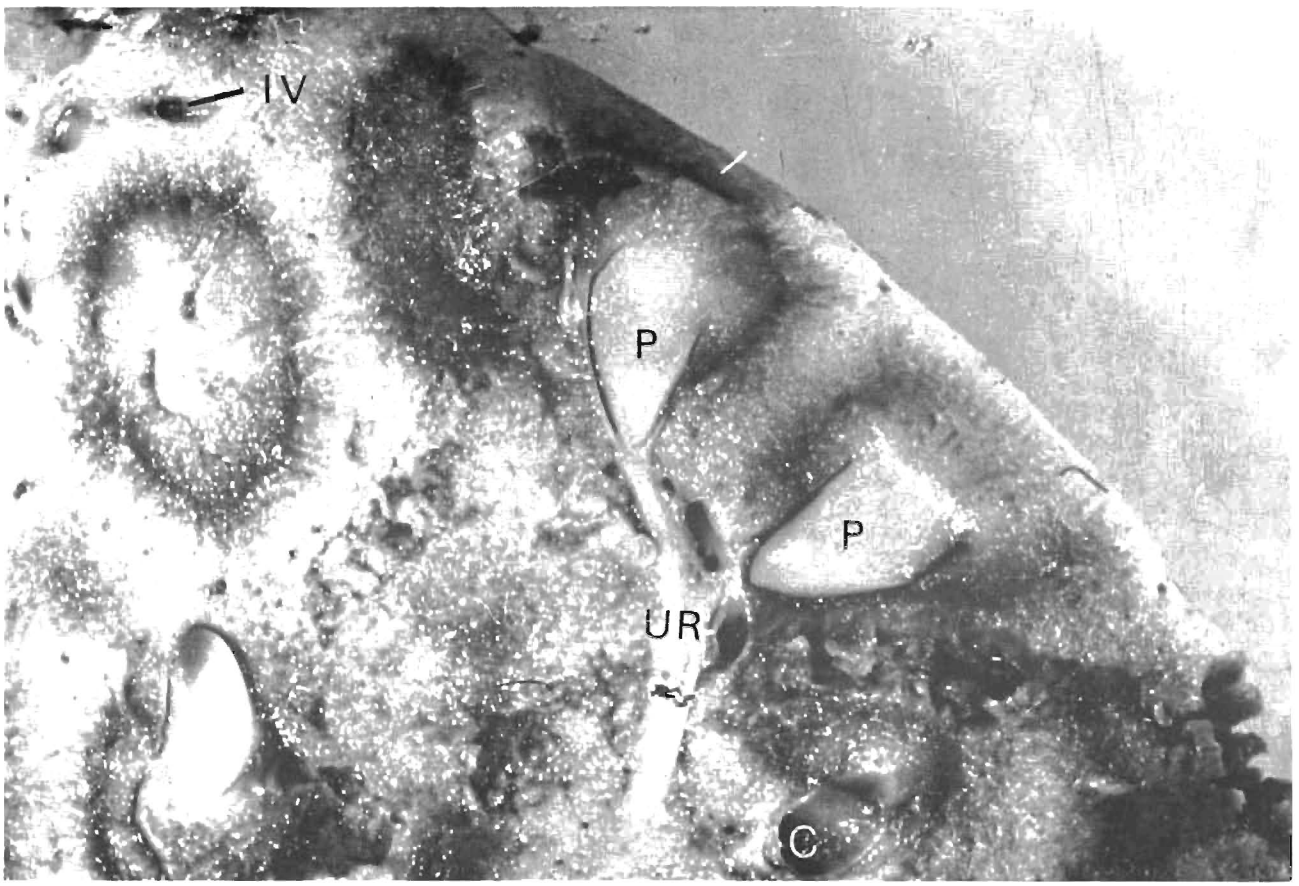
The kidney of *A. pusillus*, studied in the present investigation, is a composite organ surrounded by a tough capsula fibrosa. It comprises a large number of renculi, each of which possesses a prominent, single and undivided medullary pyramid which projects into a single calyx, as well as a circumscribing mass of cortical substances (Figs. 3 & 4). As in *Z. californianus* the borders between renculi are very indistinct. On the kidney surface the external, slightly convex aspects of the superficially situated renculi are demarcated by circumscribing shallow grooves lending the kidney its lobated appearance (Fig. 2). Their internal borders, as well as those of renculi entirely confined to the interior of the kidney, are delimitated by the interrencular veins as depicted in Figs. 3 & 4. On inspection of materials, treated with India ink injection, a narrow, yet distinct zone, lacking cortical glomeruli was detected between contiguous renculi, further substantiating the individuality of the compacted renculi. In Fig. 4, which shows part of a medial section parallel to the flattened surfaces of the kidney, an end branch of the ureter can be seen bifurcating into two radicles, each of which terminates in a cylax that receives the papilla of the renculus allocated to it. In the lower segment of Fig. 3, the conical interior of a calyx, from which the medullary papilla has been removed, can be observed. Two to four calyces (usually two) coalesce by means of short connecting ureteral ductules which unite progressively towards the hilus area into two major branches, which eventually form the single ureter. The ureter emerges from the caudal side of the hilus (Fig. 5). No indication of a pelvis was found and the ureter is thought to simply branch and terminate in the single calyces, which totalled 231 in a latex injected kidney. No fused renculi were observed and the number of calyces and renculi are thought to be the same.

3.2.2. Blood vascular system

The Cetacea and Pinnepedia appear to exhibit a fairly uniform arterial system. It usually consists of a single renal artery which starts to bifurcate before

Figure 4. Medial section, parallel to the dorso-ventral surfaces of kidney. C = calyx; IV = interrencular vein; P = papilla (inner zone); UR = ureteral radicle.

Figure 5. Resin cast of arterial and ureteral systems. AR = arteria renalis; C = calyx; U = ureter.


IV
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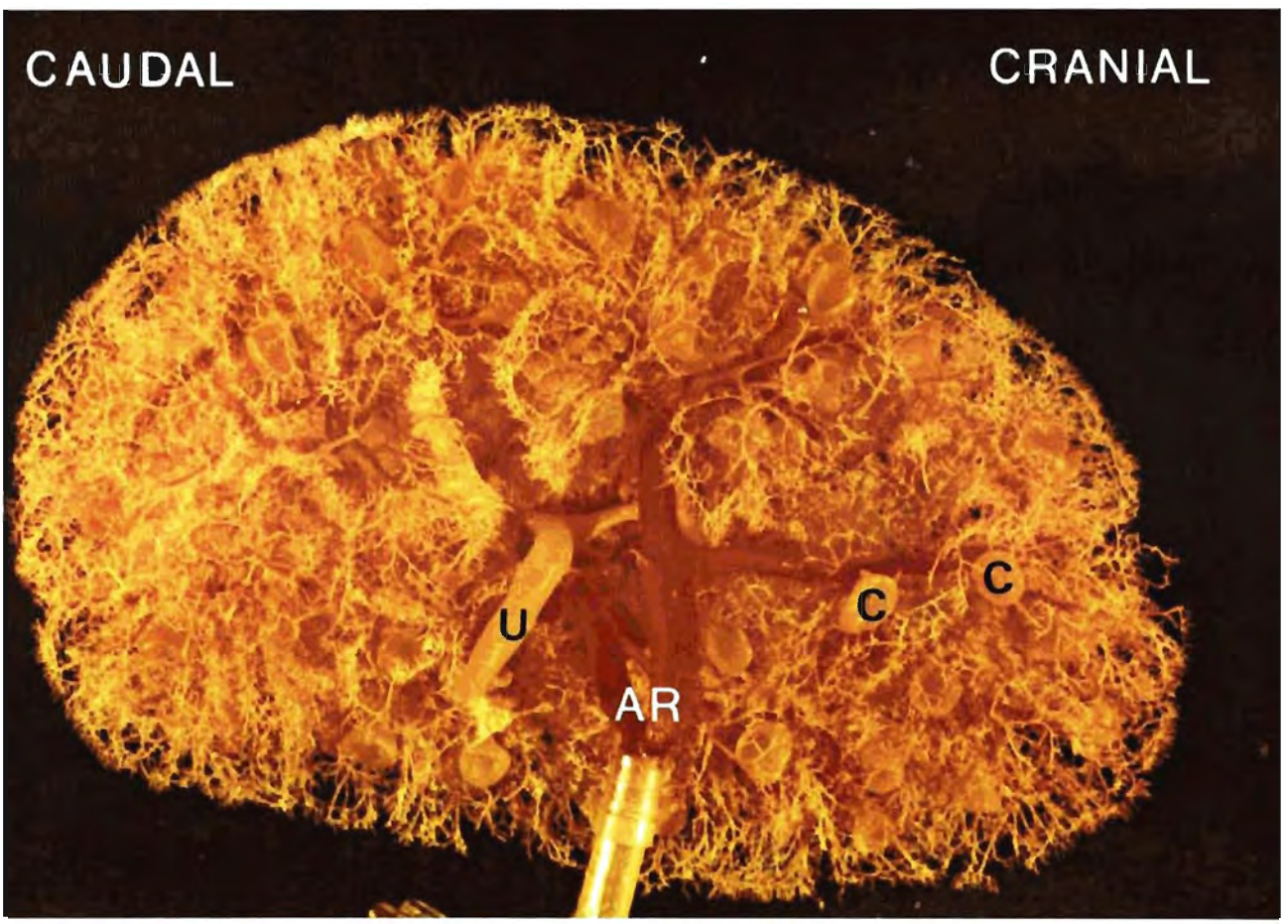
CAUDAL
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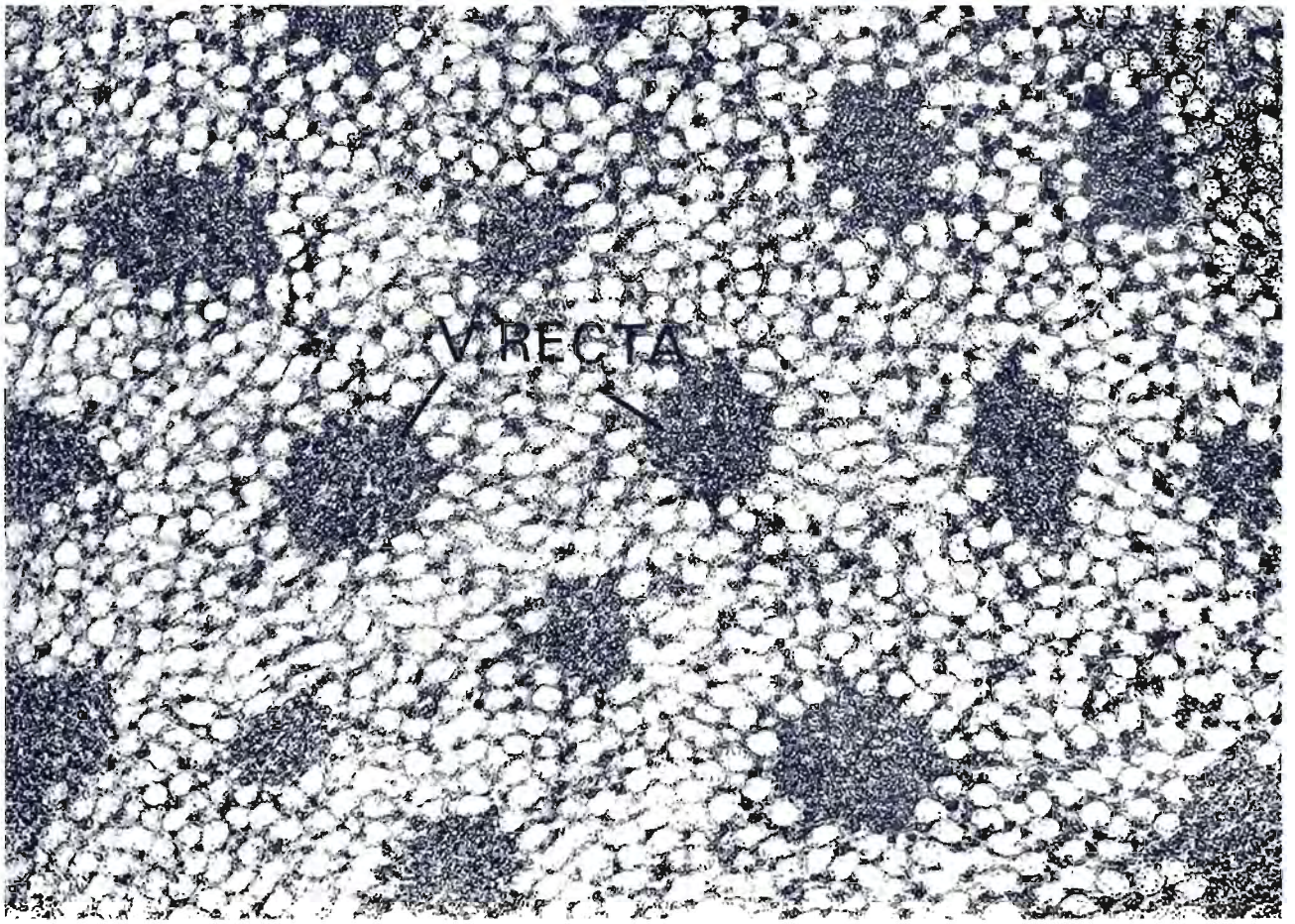

V. RECTA


AR
C
C
IA

Figure 6. Transverse section through medulla showing parallel vasa recta bundles.

Figure 7. Resin cast of arterial and ureteral system. AR = arterial rosette; C = calyx; IA = inter arterial area.

entering the kidney substance and eventually breaks up into similar renicular arterial systems. Each renculus thus possesses an exclusive arterial supply. The venous return in contrast shows considerable variation.

According to Cave and Aumonier (1963, 1967a) venous blood drains from the cetacean renculus via two pathways i.e. by means of renicular veins from the corticomedullary junction upon the calyx wall (intrinsic pathway) and/or by means of perirenicular (interrenicular, extrarenicular) veins which terminate in the renicular veins or partly as direct radicles of the renal vein (extrinsic pathway). The pathway for venous return from the renculus is also a dual one (Cave and Aumonier 1964; Harrison and Tomlinson 1956; Van der Spoel 1963). Venous blood can therefore drain in an intrarenal direction via the renal vein and in an extrarenal direction by means of interrenicular veins to a perirenal plexus (stellate plexus of plexus venosus sinus perirenalis), which is evident on the surface of the kidney. From there the blood is then secondarily conveyed to the renal vein emerging at the mesial slit (hilus) or directly into the v. cava posterior.

In the pinnepeds investigated by Van der Spoel (1963), Guzsal (1959), Harrison and Tomlinson (1956) and Blessing (1969), venous blood is drained by the extrinsic pathway, i.e. via the interrenicular veins, in an extrarenal direction to the perirenal (stellate) plexus and from there secondarily via three vv. perirenales (term instituted by Van der Spoel 1963) to the v. cava posterior. The perirenal plexus also has connections with veins of the abdominal wall, the extradural intravertebral vein and the v. azygos, a situation which is not manifested in cetaceans. A. v. renalis was thus shown to be lacking in pinnipeds. *Z. californianus*, however, lacks the perirenal plexus and the interrenicular veins merge into larger tributaries of a v. renalis proper (Wrobel 1963). The available evidence therefore indicated either an extrarenal or intrarenal pathway of venous return in pinnipeds. They are, however, not found together in the same kidney as in the cetaceans.

In the present investigation the terminology used by Wrobel (1963) for *Z. californianus* will be employed. Synonymous terminology encountered in the literature will be placed in brackets. In *A. pusillus* the single a. renalis bifurcates before entering the kidney at the hilus into a major cranial and a minor caudal branch (Fig. 5). Both bifurcate again and the major branch supplies three quarters of the kidney extending from the cranial to the caudal extremity, while the minor branch and its bifurcations are confined

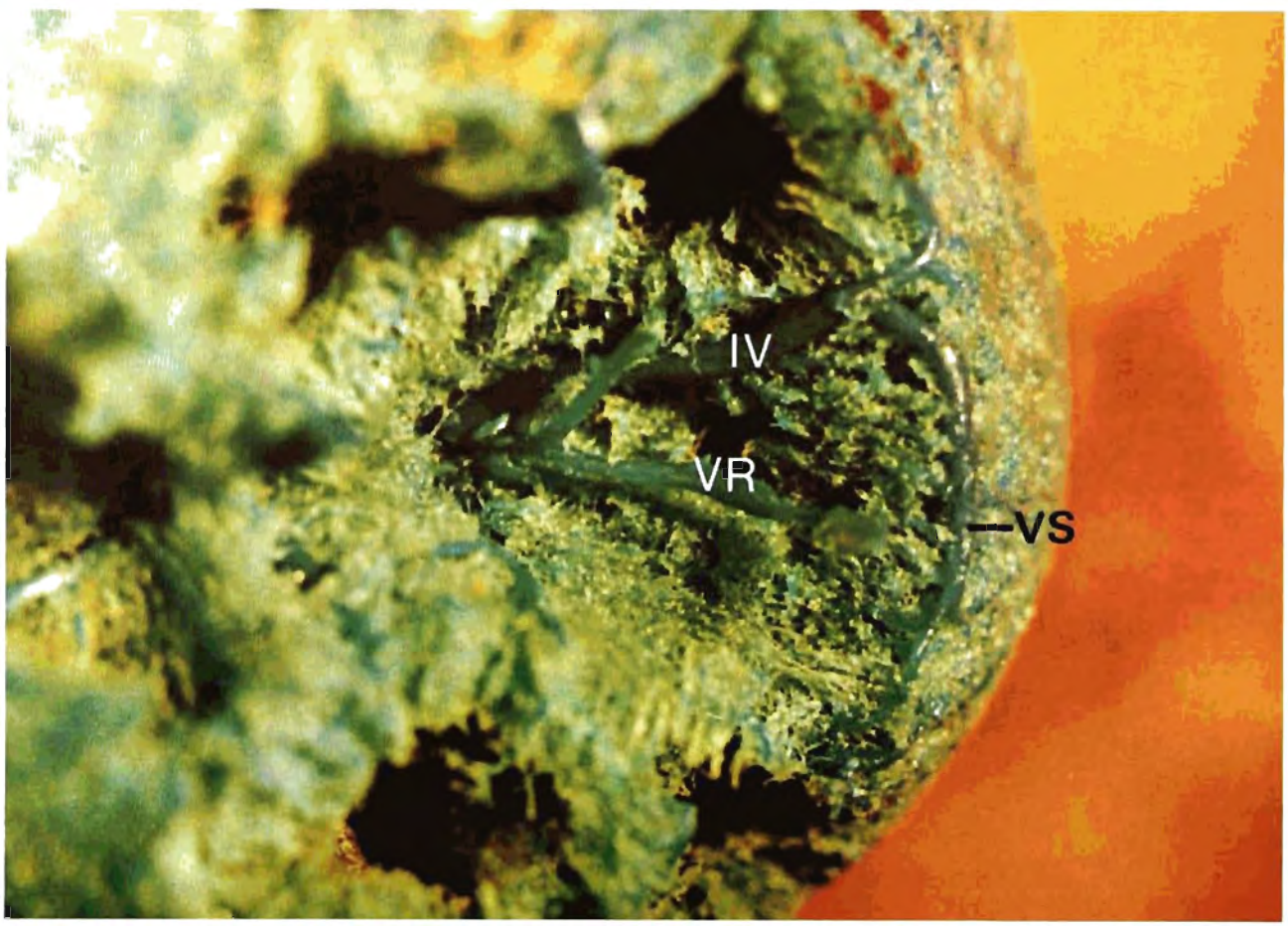


Figure 8. Resin cast of venous system. IV = interrenicular vein; VR = vena renicularis; VS = vena stellatae.

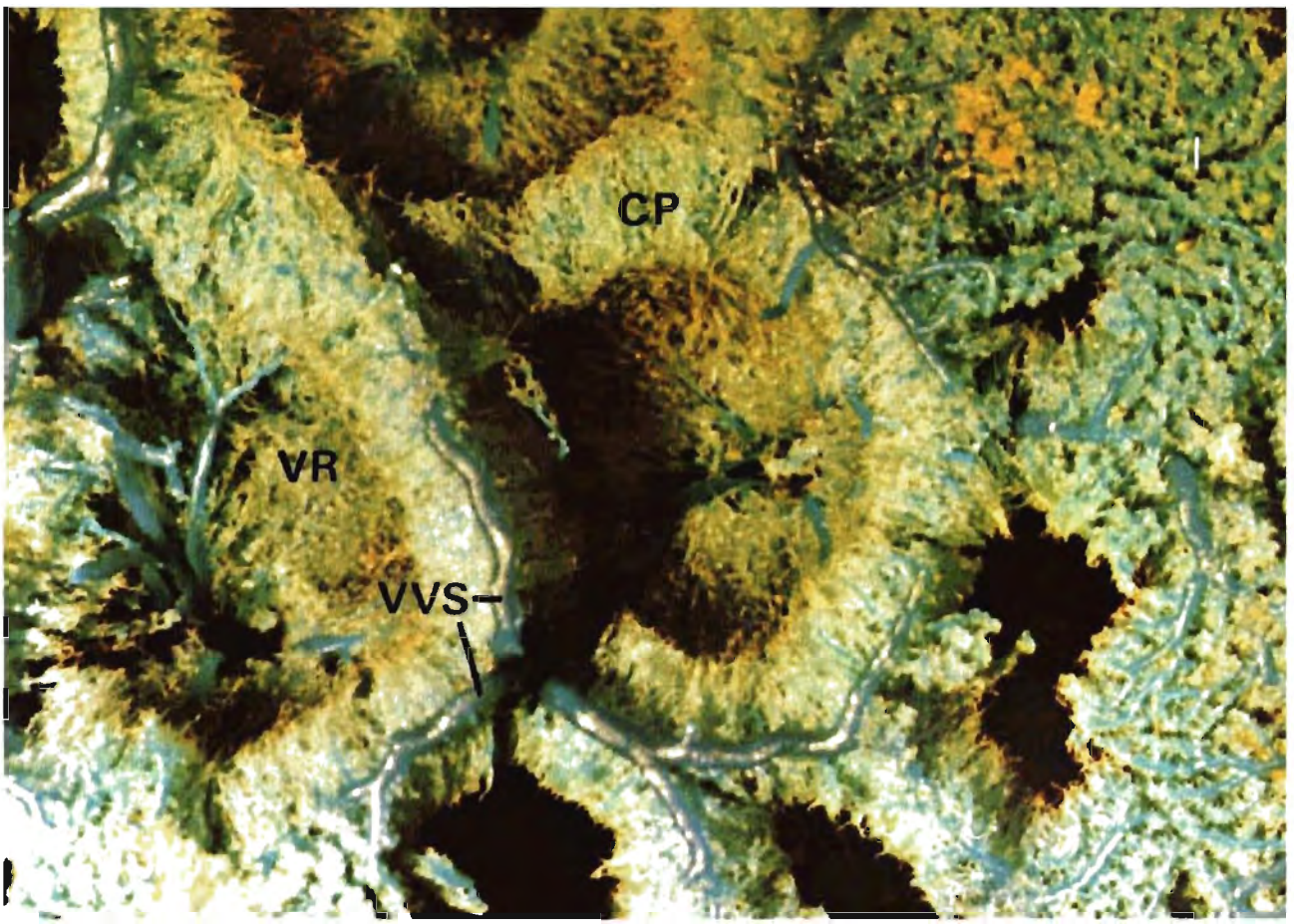


Figure 9. Resin cast of venous system. CP = cortical plexus of two neighbouring renculi; VR = vena rencularis; VVS = venae stellatae.

to the medial quarter. This pattern differs from that found in *P. ladogensis* (Guzsal 1959), nor do the branches of the a. renalis run parallel with those of the ureter as in *P. ladogensis*.

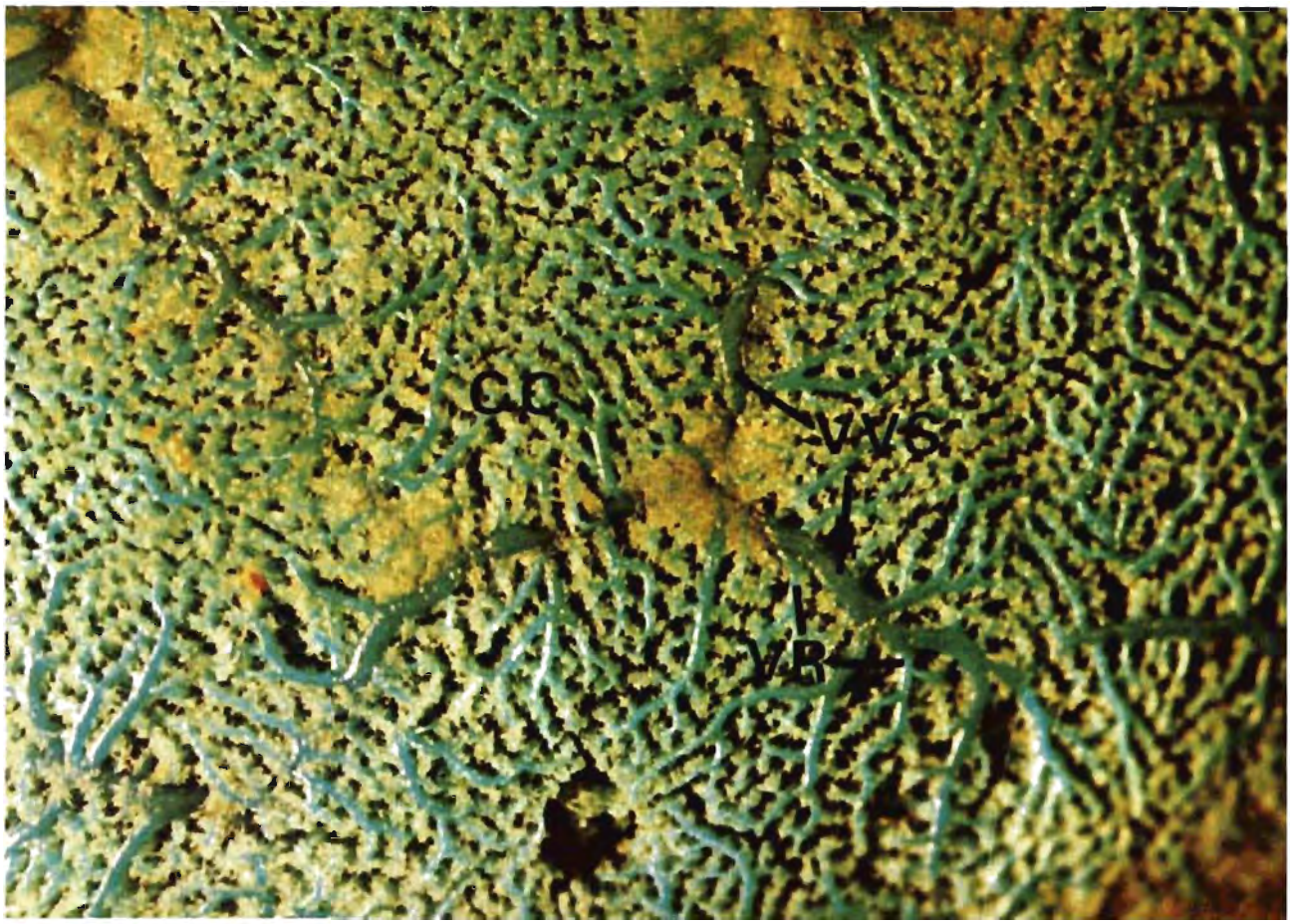
The terminal branches of the renal artery pierce the rencular substance near the calyx base as the rencular arteries (aa. interlobares) and extend along the corticomedullary boundary. The aa. subcorticales (aa. arcutae) arise from the rencular arteries and send branches, the aa. corticales radiatae (aa. interlobulares, radial arteries) radially into the cortical substance. Vasa afferentia originating in the aa. corticales radiatae conduct the blood to the glomeruli from where the vasa efferentia break up in an extensive cortical plexus. The vasa efferentia also contribute to the medullary blood supply (vasa recta) by means of the arteriolae rectae spuriae which descend in parallel bundles (Fig. 6) into the medulla.

There exists a distinct zonation of the vasa recta correlated with nephron architecture resulting in a medullary plexus in the outer zone of the medulla and mainly straight capillaries in the inner zone. The observations indicate minimal presence of Ludwig's arteriolae. No arteriolae rectae verae were detected, but intensive examination may reveal their presence, since both types are present in small numbers in *Z. californianus* (Wrobel 1963). Because they originate in the afferent system, they would provide an extraglomerular pathway for blood circulating through the renculus. It can be concluded that each renculus possesses an independent blood supply in the form of an arterial rosette, representing a complete vascular unit since no connections are present between adjacent rosettes. The arterial rosettes, each with its accompanying ureteral calyx, are seperated from one another by an area devoid of any arterial vessels (Fig. 7).

Blood drainage from the renculus is accomplished by interrencular veins which occur between renculi (Figs. 3 & 8) as well as by vv. renculares (vv. interlobares) which follow the course of the aa. renculares in the corticomedullary junction. The vv. renculares leave the renculus on the calyx wall to merge with the approximating interrencular veins (Figs. 8 & 9) to form a radicle of the v. renalis. The presence of vv. renculares in *A. pusillus* has hitherto not been described for pinnipeds but is present in the cetaceans studied by Cave and Aumonier (1967a) and Van der Spoel (1963). The interrencular veins and the subcapsular venulae stellatae, i.e. the extension of the interrencular veins which run in the

Figure 10. Superficial portion of the venous cast. CC = cortex corticis; VR = venous roots; VVS = venae stellatae.

Figure 11. Proximal part of calyx showing CT = connective tissue; L = lumen; TE = transitional epithelium.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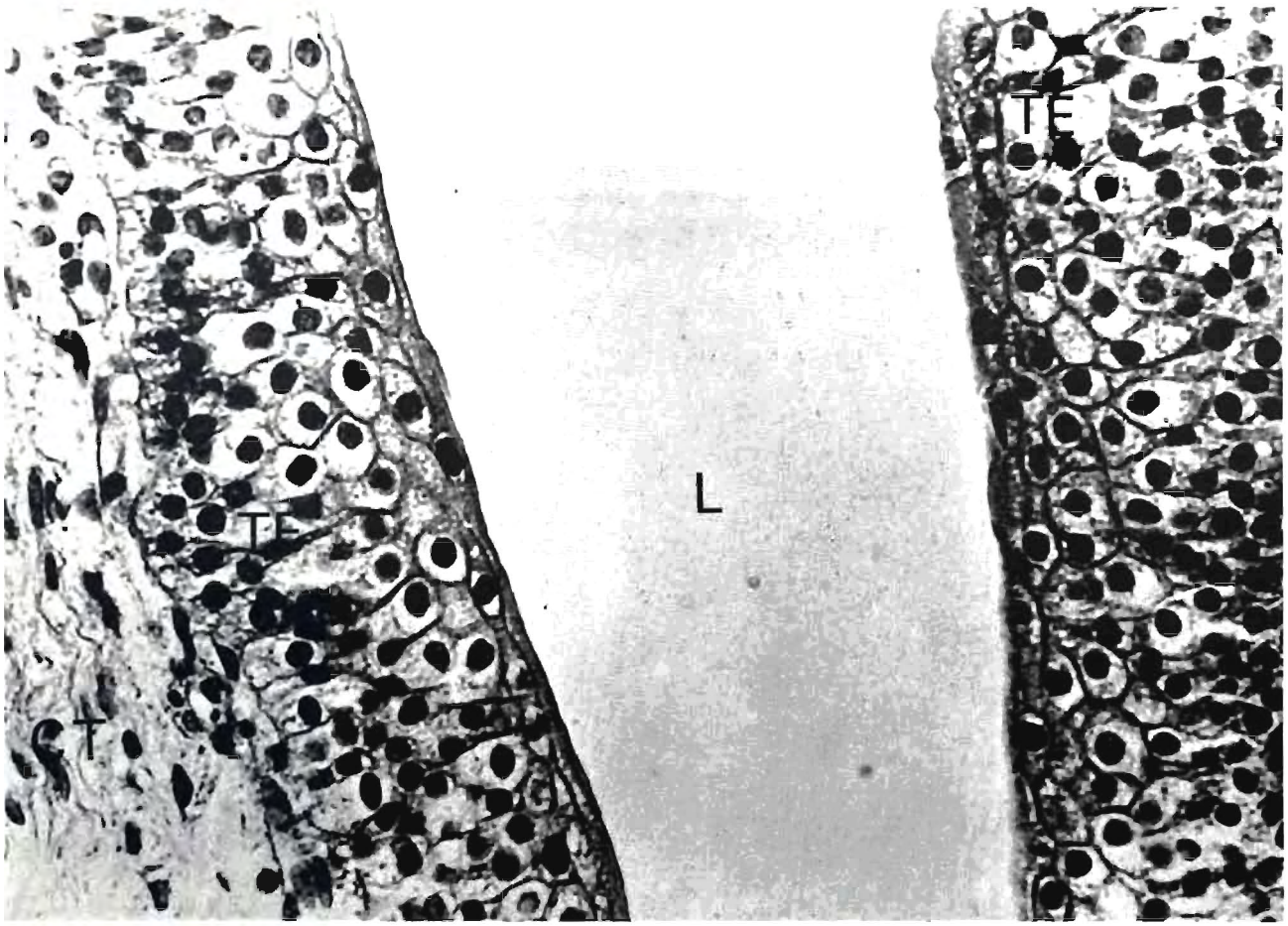
VV/S
V
VB


TE
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TE
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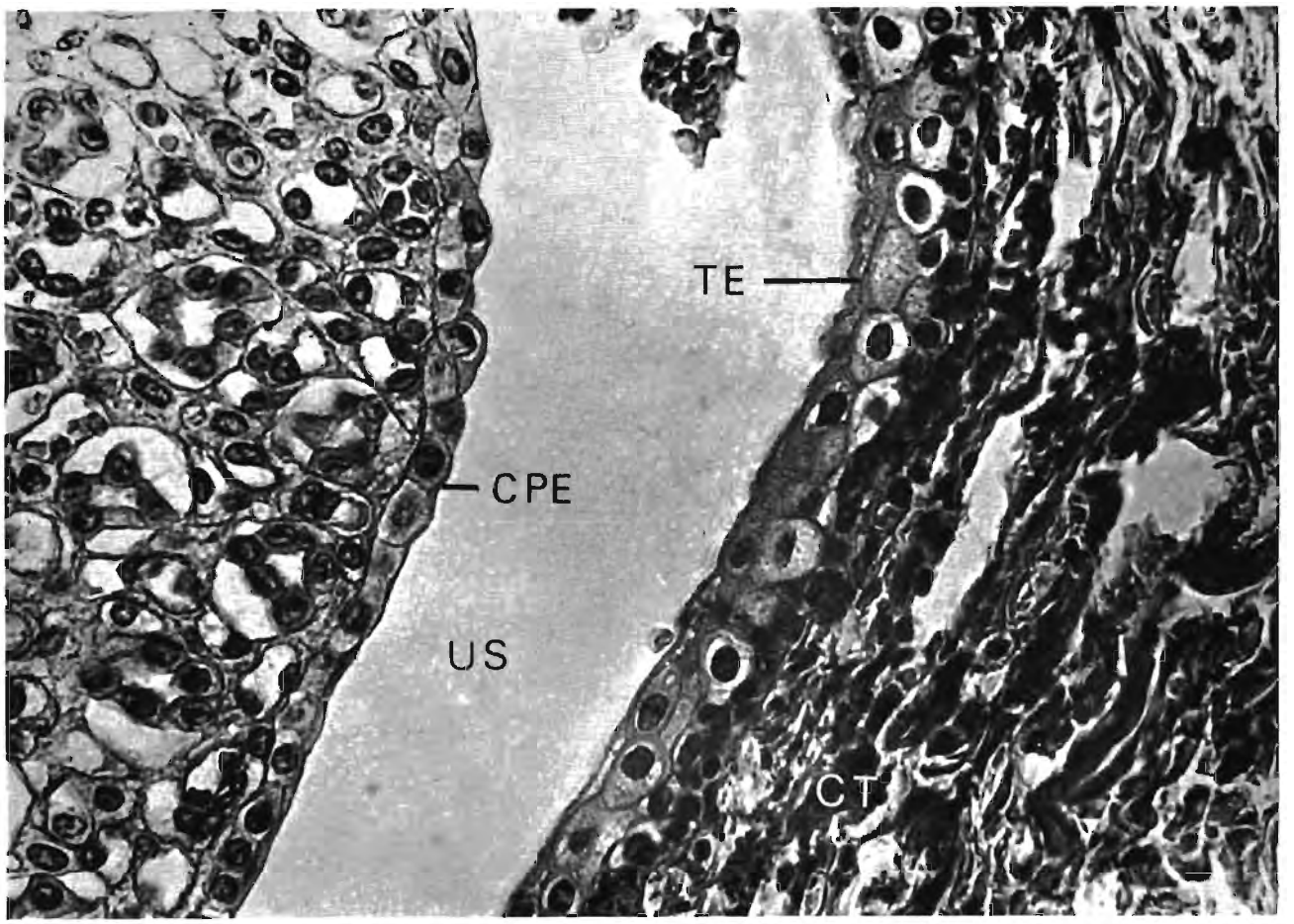
TE
CPE
US
CT


PL
VL
VP
MD
GLOM
CFS

Figure 12. Transverse section through calyx and medulla showing CPE = single layered papillary epithelium; US = urinary space; TE = transitional epithelium; CT = connective tissue.

Figure 13. Section through a glomerulus. CFS = capsular free space; GLOM = glomerulus; MD = macula densa; PL = parietal and VL = visceral layer of capsule of Bowman; VP = vascular pole.

superficial renculi within the cortex just beneath the capsula fibrosa (Figs. 8 & 9), receive blood by means of short venous roots (Venenwurzeln after Wrobel 1963) from the cortical plexuses of bordering renculi. Fig. 10 illustrates the superficial layer of the cortical plexus, the cortex corticis, which drains into the venulae stellatae. Continuous with the vv. renculares are the vv. subcorticales (vv. arcuatae) which are directly connected with the interrencular veins by means of radially disposed veins (vv. interlobulares, radial veins), which in turn drain the cortical plexus. In some superficial renculi, terminal branches of the vv. renculares were seen to connect directly with the venulae stellatae. Vv. rectae spuriae from the medulla drain into the vv. subcorticales.

From the above, therefore, it is clear that *A. pusillus* has an intrinsic as well as an extrinsic pathway of venous drainage from the renculi and that this is extended intrarenally into a v. renalis emerging from the hilus, similar to cetaceans, but unlike other pinnipeds described which only posses the extrinsic pathway, extended intrarenally (*Z. californianus*) or extrarenally (*Phoca*).

3.2.3. Histology

Histological descriptions of cetacean and pinniped kidney material are limited and confined to comparatively superficial inspections of sectioned material.

The present investigation showed that the rencular medullary pyramid in *A. pusillus* consists of an outer and inner zone (papilla), the latter prominently projecting into the calyx. The calyx has no processes (specialized fornices) and therefore the medulla constitutes an undivided primary pyramid (Fig. 3). A sporta perimedullaris musculosa is lacking as in a variety of pinnipeds examined by Cave and Aumonier (1964). This provides further evidence in support of these authors' postulate that this anatomical phenomenon could be an exclusive cetacean morphological character. The corticomedullary junction is distinct and medullary rays project outward through the cortical substance. The calyx, the proximal part of which is lagged by connective tissue, is lined by a thick transitional epithelium (Fig. 11) which becomes progressively thinner as it extends past the corticomedullary junction at the hilar extremity and is eventually reflected over the papilla as a single layered cuboidal epithelium (Fig. 12).

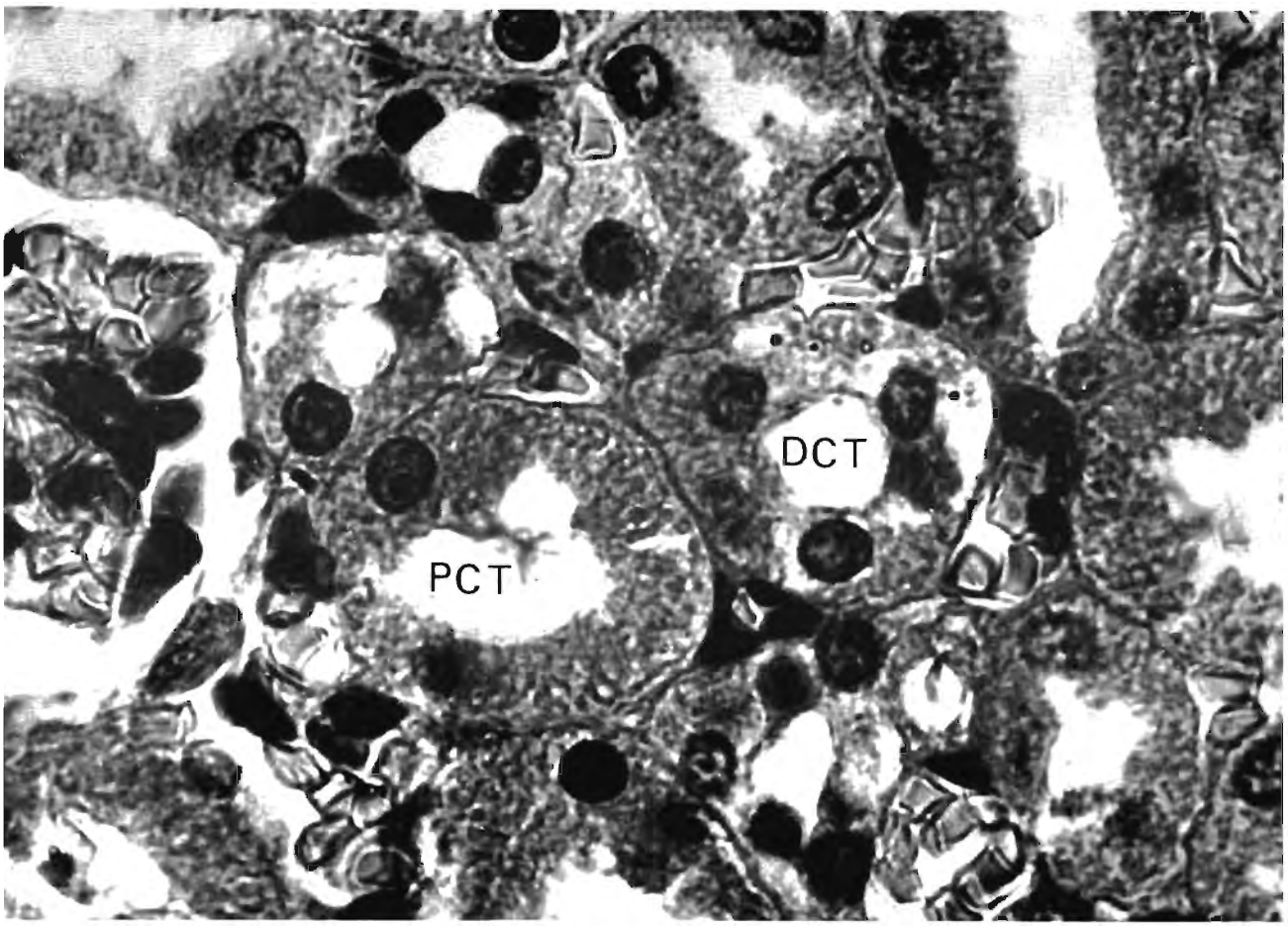

Figure 14. Section through cortex showing DCT = distal convoluted tubule; PCT = proximal convoluted tubule.

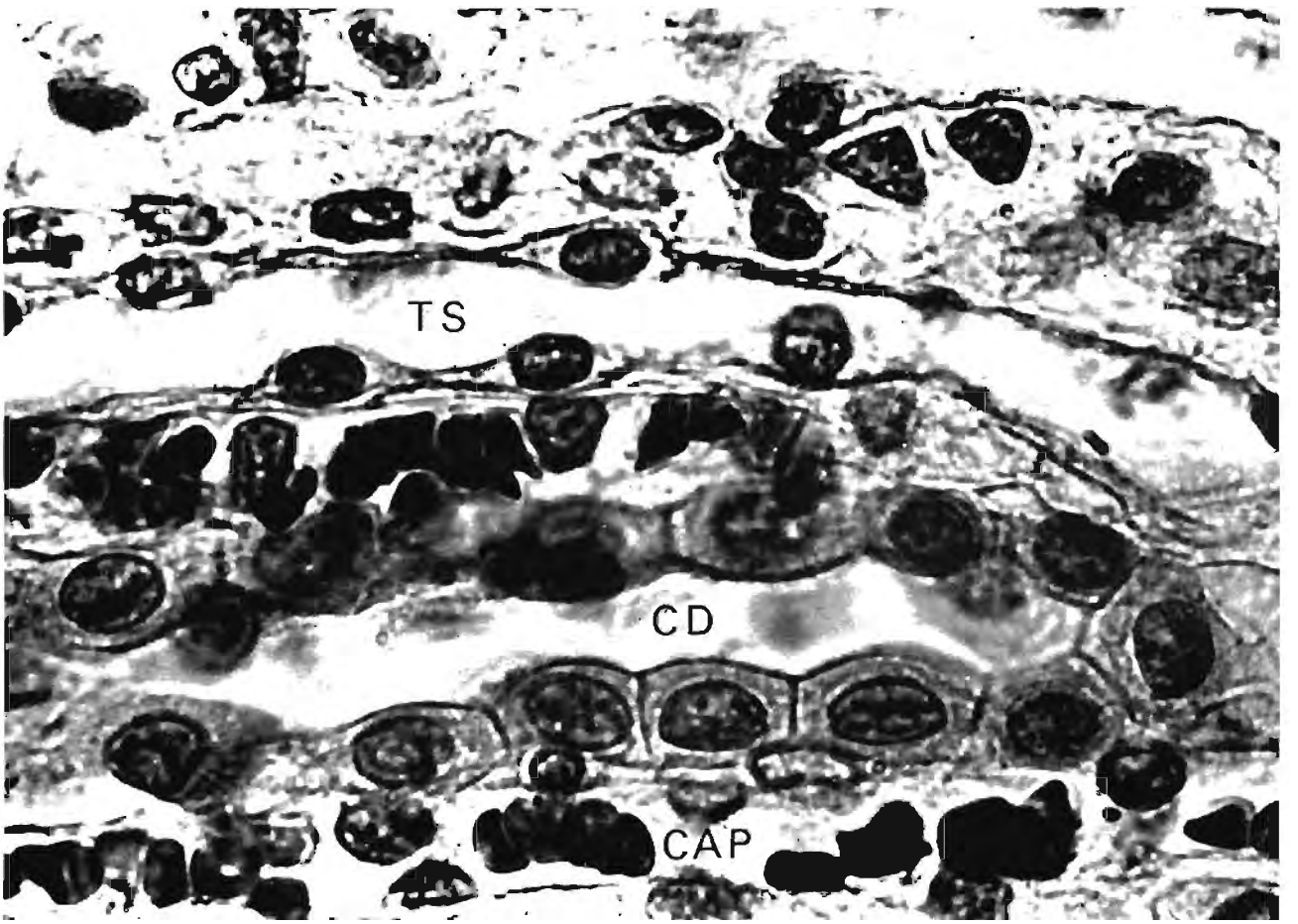

Figure 15. Longitudinal section through medulla indicating CAP = capillary; CD = collecting duct; TS = thin segment of loop of Henlé.

There is little connective tissue separating this epithelium from the underlying papillary tubules and vasa recta, therefore very little tissue is interposed between the latter and the urinary space of the calyx. According to Sperber (1944) a well developed inner zone (papilla) is associated with a preponderance of long looped nephrons (deep nephrons) which was apparent in *A. pusillus*. Long loops of Henlé are present and the thin segments in these loops turn in the area cribrosa. This is in agreement with the findings of Sperber (1944) who found in *P. barbata* that long looped nephrons constituted 68 ± 3,4% of the total number of nephrons.

The glomerulus has a crenated (lobulated) appearance and is closely invested by the flattened epithelium of the visceral layer of the capsule of Bowman with its slightly projecting nuclei. The parietal layer is a squamous epithelium with flattened nuclei. There is a pronounced capsular free space (Fig. 13). The tortuous proximal tubule has an irregular lumen, mostly triangular in cross section, which is lined by a single layered epithelium with obscured cell boundaries. The free surfaces of these cells appear to possess a brush border which is not found in the other segments of the nephron. The spheroid nuclei are situated towards the bases of the cells (Fig. 14). The thin segment of Henlé's loop has a very characteristic flattened epithelium with pronounced oval nuclei that bulge into the lumen (Fig. 15). The thin segment gives way to the thick segment with its thicker epithelium. The distal convoluted tubule has a regularly shaped lumen when compared to that of the proximal convoluted tubule and possesses a thinner epithelium. Round to oval nuclei are placed closer to the lumen (Fig. 14). Collecting tubules are easily recognised and have characteristic features i.e. a lumen lined with cuboidal epithelium and distinct cell boundaries. The large nuclei are centrally situated (Fig. 15). The collecting ducts gradually merge into larger papillary ducts that terminate at the tip of the papilla to form an area cribrosa. The cuboidal epithelium of the collecting ducts is continuous with that clothing the papilla.

On evaluating the morphological data a number of physiologically significant factors become evident. It is also important to note that the renculus in *A. pusillus* is practically identical in general architecture to the unipapillate (unilobar) kidney possessed by almost all small mammals. This allows one to compare the functional status of the two types of kidney. For example, Hendrikx and Epstein (1958), Crawford *et al.* (1959) and Jaenike (1960) found that urea administration in dogs and rats enhanced water reabsorption in the renal medulla, thus increasing the renal concentrating ability. Schmidt-Nielsen *et al.* (1961, 1966), and Pfeiffer (1968) found that a correlation existed between this effect of urea on the osmotic ceiling of urine and the

presence of an inner medullary zone and specialized fornices (secondary pyramids). This phenomenon, of urea enhancing the osmotic ceiling in animals which possess these structures, was also observed by Plakke and Pfeiffer (1964, 1965) in animals which exhibited zonation of the vasa recta (e.g. *Dipodomys*). However, a curious exception exists in the species *Psammomys* (Schmidt-Nielsen *et al.* 1961) which also has a pronounced inner medullary zone as in *Dipodomys* but has a fixed osmotic ceiling unaffected by protein intake. Therefore it is similar to the response found in the beaver, pig (Schimdt-Nielsen *et al.* 1961) and *Aplodontia* (House *et al.* 1963), which all lack zonation of the vasa recta and medulla and do not possess secondary pyramids (Pfeiffer *et al.* 1960; Plakka and Pfeiffer 1964; Pfeiffer 1968). Plakka and Pfeiffer (1968, 1970) presented the following possible reason for the increase in concentrating ability after protein intake: the inner zone of the medulla and/or the secondary pyramids assist in raising the urea concentration of the papilla, and therefore the efficiency of the urine concentrating mechanism, by recycling of urea through the single layered cuboidal epithelium lining these structures. Since the thin loops of Henlé and the vasa recta are separated from the pelvic urine by only a little interposed tissue, and as the presence of a well developed inner zone and secondary pyramids increases the interface between the pelvic urine and the underlying medullary tissue, this would be possible.

In this respect the renculus of *A. pusillus* possesses the characteristics for the recycling of urinary urea in order to increase water reabsorption in the renal medulla after protein intake. However, although secondary pyramids are lacking as in *Dipodomys*, all the other prerequisites are present for the recycling of urea. Whether this in fact happens, or has a perceptible effect on the concentrating ability, is not certain in view of the exception of *Psammomys*. A final answer would require the calculation of the relative concentrations of urea and electrolytes in the urine on a low, medium and high protein diet. In seals we are, however, dealing with a different renal response since infusion of urea in *P. vitulina* increases the urea content of the plasma (Schmidt-Nielsen *et al.* 1959) as found in the animals in which the osmotic ceiling is affected by protein or urea intake. However, the urinary urea level was considerably lower than the level maintained during fasting. The critical factor seems to be the ingestion of protein which raises the urinary urea concentration above the fasting level, but, as this causes an increase in urine volume, it is doubtful if recycling of urea does reduce the renal water requirements substantially.

An histological feature in the collecting duct system which can be considered as characteristic of kidneys of mammals exposed to severe problems of water economy, is present in desert rodents. For example, Vimtrup and Schmidt-Nielsen (1952), Abdallah and Tawfic (1969) and Khalil and Tawfic (1963) found that in *Dipodomys*, *Psammomys* and *Jaculus* the epithelium of the collecting ducts changed from cuboidal to flattened cells in the middle of the outer zone of the medulla. The flattened cells had distinct borders with rounded nuclei which project towards the lumen. In the inner zone the epithelium changes back to cuboidal. This arrangement is not present in other rodents such as the albino rat or those examined by Young and Wissig (1964) and Bulger and Trump (1966). In this respect *A. pusillus* renculi conform to the pattern of the albino rat kidney in that the collecting duct epithelium remains cuboidal throughout.

The blood vascular system of a kidney constructed on the renculate plan is characterised by the feature that ample provision is made for the return of rencular venous blood to the general circulation. An extraglomerular pathway for circulation through the renculus is present and is particularly evident in *P. phocoena* (Van der Spoel 1963), where some arteriolae rectae spuriae originate in the vasa afferentia and the plexuses of the medulla and pelvic cavity also act as an extraglomerular blood circuit. This in itself does not differ from the situation in the dog where Kügelgen and Passarge (1960) estimated that ± 60% of the blood flowed extraglomerularly through the kidney. Van der Spoel (1963) considered the arterial and venous system of the dog kidney to be similar to the blood system in the kidney of *P. phocoena*. However, an additional venous system is situated outside the renculi in cetaceans and pinnipeds. It is clear, therefore, that the interrencular system has developed as a supplement to the more primitive rencular system, as pointed out by Cave and Aumonier (1965). It is probable that the interrencular system can be utilised as a blood reservoir (Van der Spoel 1963) which can modify its volume in response to the change in blood distribution on diving (Diaconescu and Veleanu 1967).

Harrison and Tomlinson (1956) and Simpson *et al.* (1970) in support of previous authors calculated the blood volumes of diving mammals and found the volumes to be greater than in non-diving mammals and it is possible that the interrencular (perirencular) plexus, evident in cetaceans and pinnipeds, can contribute to blood storage.

The absence of a porta perimedullaris musculosa in *A. pusillus* refutes any idea that the sporta is an essential part of the renculate kidney.

3.3. Dimensions and thickness of kidney regions

The counter-current multiplication theory of urine concentration, as summarised by Schmidt-Nielsen *et al.* (1961) and Young and Wissig (1964), postulates that the loops of Henlé act as a counter-current multiplier system, thus creating an increase in osmotic concentration in the kidney tissue from the cortex towards the papilla, while the vasa recta acts as a counter-current diffusion exchanger. Co-ordinated operation of these two systems produces an osmotic gradient of salt concentration in the interstitium of the papilla with maximum concentration at the apex. The maximum concentration that this system can achieve is directly related to the length of the multiplier system (thickness of medulla) and in kidneys with an outer and inner zone of the medulla the sodium and urea concentration con-

tinues to increase throughout both zones, reaching the highest values in the kidney with the thickest medulla (Schmidt-Nielsen *et al.* 1961). A hypertonic urine is thus produced by active water reabsorption in the collecting ducts and the urine in the collecting ducts attains a final osmotic concentration similar to that of the tissue surrounding it.

This system therefore suggests certain anatomical prerequisites for the kidney to produce an hypertonic urine, i.e. nephrons with long loops and vasa recta as well as collecting ducts in close apposition to them. The relative number of long looped and short looped nephrons do not have a significant effect on concentrating ability (Schmidt-Nielsen *et al.* 1961). For example, the dog and cat have 100% long looped nephrons but cannot concentrate urine to the level attained by *Dipodomys* and *Jaculus* which have 27% and 33% long looped nephrons respectively. Sperber (1944) found that the relative thickness of the medulla (relative to kidney size) was related to the habitat of animals in such a way that those living under arid conditions had the greatest relative medullary thickness. The desert rodents *Jaculus*, *Dipodomys* and *Psammomys* (Khalil and Tawfic 1963; Vimtrup and Schmidt-Nielsen 1952; Abdallah and Tawfic 1969) have the anatomical prerequisites required for maximal concentrating ability (5 000—6 500 mOsm/l). These are a long papilla renis which extends into the ureter with an accompanying high relative medullary thickness (8,5—10,7), long collecting ducts and very long thin segments of the long looped nephrons which reach far down into the papilla. In comparison non desert forms, for example the albino rat, has a small pointed papilla, a lower relative medullary thickness (5,8) and shorter thin segments with a lower concentrating ability (3 060 mOsm/l). The other extreme is occupied by *Aplodontia* (Pfeiffer *et al.* 1962) which lacks all these anatomical prerequisites (maximum urine concentration 820 mOsm/l). This species range is restricted to areas of heavy rainfall and water conservation is unnecessary.

Kidney type appears to be related to kidney size according to Sperber (1944). For example, large kidneys with a mean size of 84 mm belong to the renicular type. The large kidneys of *A. pusillus*, with an average adult size of 87,2 mm, conform to this pattern. A direct relationship exists between kidney weight and size (Table 5). In a large number of mammals with diverse kidney types and sizes, Sperber (1944) found that, in addition to a change in its structure, larger kidneys were accompanied by a change in the relative layer thicknesses. The large renculate kidneys of cetaceans and pinnipeds for instance have lower relative layer values, especially for the medulla, than other mammals of equivalent size. Table 6 shows the relative layer values for two different age groups within *A. pusillus*. Since renicular measurements are influenced by frequent distortion due to mutual renicular pressure and the fact that the borders between renculi are indistinct, the best estimates of kidney size/layer thickness relationships can adequately be illustrated only by comparing large (adult) kidneys with values obtained from pups. On percentage basis the actual cortical thickness increases from 13,6% of renicular length in the small kidneys to 15,8% in the large ones. The medullary thickness decreases from 86,4% to 84,2%. This is probably attributable to age changes since in the young the medulla grows initially but ceases later while the growth of the cortex is maintained (Sperber, 1944). The layer thickness relative to kidney size, however, shows a decrease of 1,65 to 1,31 for total layer thickness (cortex and medulla), from 1,42 to 1,10 for medullary thickness and 0,23 to 0,21 for cortical thickness with increasing kidney size. This agrees with Sperber's (1944) conclusions that the relative thickness of the layers diminishes with increasing kidney size when different mammals are compared with one another. This therefore holds true also for size incerase with age within the same species. The low average relative medullary thickness recorded for the mature *A. pusillus* kidneys reflects their modification (renculation) and is comparable to those found in *Phoca* (1,1—1,5) and *Otaria* (1,2).

When one, however, compares the relative medullary thickness of a renculus in *A. pusillus* (as calculated relative to kidney size) with urine concentrating ability a serious discrepancy appears. For example, when the value (1,1) obtained for *A. pusillus* is compared to the data in Table VIII in Chew (1965), it should have a concentrating ability of less than 520 mOsm/l, or a concentrating ability less than the maximum recorded for the beaver and *Aplodontia*. This, however, is not the case as *A. pusillus* can concentrate urine to 2 364 mOsm/l and possesses the structure required for producing a concentrated urine. When, however, the renculus is considered as a single entity, which appears to be the situation, the situation changes entirely. The medullary thickness must then be calculated relative to renicular size and not kidney size. The value of 10,5 thus obtained gives a relative medullary thickness which places this species in the class of desert

Table 5. Relationship between kidney weight and kidney size.

	Adult Bulls					Pups		
Weight (g)	540	420	405	330	320	46	38	31
Size* (mm)	93	89	88	84	82	42	40	38

* calculated as $\sqrt[3]{\text{length x breath x depth}}$

Table 6. Relationship between kidney size and thickness of the various zones.

Age group	Mean kidney size*	Absolute thickness				Relative thickness	
		Cortex mm	%	Medulla mm	%	Cortex	Medulla
Bulls	87,2	1,8	15,8	9,6	84,2	0,21	1,1
Pups	40,0	0,9	13,6	5,7	86,4	0,23	1,42

* calculated as $\sqrt[3]{\text{length x breath x depth}}$

Table 7. Comparative kidney sizes and relative thicknesses of the various zones.

	Kidney size* (mm)	Absolute thickness of:						Relative medullary thickness
		Cortex		Outer Zone		Inner Zone		
		mm	%	mm	%	mm	%	
White rat	12,9	2,8	27	2,6	25	5,0	48	5,9
Jaculus	7,5	1,3	15	1,9	22	5,4	63	9,7
Arctocephalus	5,6**	0,9	13	1,9	28	4,0	58	10,5
Gerbillus	6,3	1,4	18	1,8	22	4,8	60	10,5
Psammomys	12,8	2,2	12	3,3	18	13,2	70	12,9

* calculated as $\sqrt[3]{\text{length x breath x depth}}$

** rencular size.

rodents (Table 7). Sperber's (1944) calculation of relative medullary thickness to total kidney size is therefore not justifiable in assessing the functional ability of the renculate kidney.

This is further conifrmed by comparing the kidney structure of *A. pusillus* with other kidney features of desert rodents included in Table 7. The latter have relatively thick inner medullary zones and therefore long thin segments in the long looped nephrons. The renculi in *A. pusillus* also possess long loops, in fact, the inner zone exhibits only thin segments, collecting ducts and vasa recta. The relative thickness of this zone (58%) is indicative of long thin segments and resembles the condition in desert rodents (60—70%) and is higher than that recorded for the non desert form (48%). The medullary thickness, relative to total layer thickness (84,2—86,4%), is approximately the same as found in deesrt rodents (82—88%) which is associated with long collecting ducts (Tables 6 & 7).

It is therefore evident that the renculus of *A. pusillus* has all the anatomical prerequisites necessary for the production of a highly concentrated urine. Moreover, the kidney as a whole is composed of units (renculi), each of which is anatomically equivalent to an exceptionally efficent kidney of a desert rodent.

The question, however, arises as to what the evolutionary advantages of the renculate type of kidney could be. Renculation may bring about a more effective organisation of the components of the kidney. For example, from comparative data it is evident that the ratio of cortex: medulla, and therefore the relative medullary thickness, is increased when measured within the renculus. However, that this provided the main selection pressure towards renculation is debatable because the renculate kidney does not attain the concentrating ability expected from a kidney with such excellent anatomical credentials. Also the morphology of the kidney is not related to the maintenance of function during diving because urine production ceases immediately and completely in *P. vitulina* during diving (Murdaugh *et al.* 1961) as a result of the general arterial constrictor response. Sperber (1944) thought it possible that the secondary kidney types, for example the renculate type, had developed as a result of the inability of the nephrons to lengthen with the increase in kidney size. He therefore assumed an upper limit to nephron length. However, the modified kidney types found in certain fresh water mamamls (Lutrinae) are thought to have evolved as a result of the decreased thickness of the medulla.

Diaconescu and Veleanu (1967) and Cave and Aumonier (1964) are of the opinion that the development of a renculate kidney is ontogenetically dependent upon the formation of the ureteral system, and the former authors thought that differences in renculi number in different aquatic mammals were apparently not entirely dependent on the body mass but were related to the physiological effect of diving depth on these animals. However, as pointed out previously, the harbour seal kidney does not function during diving and therefore the latter assumption cannot be accepted. Nevertheless, it is significant that renculate kidneys are limited to diving mammals. Any possible relationship between number of renculi, body size and relative medullary thickness, and therefore functional ability, has not yet been elucidated. Blessing (1969), however, suggests that the number of renculi may be associated with concentrating ability in view of the small number of renculi found in the freshwater Ganges dolphin. Another possibility suggested by Blessing (1969) was that renculation could be an adaptation related to the cessation of bloodflow to the kidney during diving.

It is therefore apparent that very little is known about the evolution of the renculate kidney and the environmental factors which provided selection pressure for this evolution. The present investigation favours the hypothesis that true renculation is associated with large body size, which *ipso facto* requires large kidney size, and the marine environment which demands efficient renal function. These arguments, however, remain speculative.

3.4. Glomerulometrics

As mentioned previously, it has been found that deep nephrons (juxtamedullary nephrons) with long

thin segments are associated with a well developed medulla (inner zone) and that this is an advantage in the case of kidneys which are compelled to excrete a highly concentrated urine. In general juxtamedullary glomeruli are larger than the cortical glomeruli and this has been established in numerous forms, e.g. man and albino rat (Zolnai and Palkovits 1965a, 1965b), *Aplodontia* (Pfeiffer *et al.* 1960), *Jaculus* and *Gerbillus* (Khalil and Tawfic 1963), all of which differ substantially in their water requirements. This is, however, by no means the case in the dog (Lange 1965), muskrat and water vole (Batenko 1972). In these mammals glomeruli do not differentiate into cortical and juxtamedullary types and no size difference has been found. Moreover, the difference in size varies according to species and age. The glomerular dimensions as a whole also vary with sex, diet and phylogenetic position (Palkovits and Zolnai 1965b).

Nevertheless, since the larger juxtamedullary glomeruli in mice represent an increased surface area available for filtration (Hanssen 1961), and as they are thought to be correlated with filtration demands in various mammalian species as an adaption to their environment, Munkasi and Palkovits (1965) made comparative volumetric analyses of the glomeruli of various species in relation to their different habitats. In the desert rodent *Jaculus* and the semi-desert subprimate *Galago* the juxtamedullary glomeruli were 101% and 169% larger in volume than the cortical glomeruli respectively. The volume increase was only 21% and 28% in the albino rat and *Cercopithecus* (grivet monkey) respectively. The former is a manifestation of adaptation to an arid environment.

The renculi of *A. pusillus* examined in the present investigation possessed relatively small glomeruli with an average volume of 177 300 μ^3 for a single renculus and 124 900 μ^3 as an average of five renculi (Table 8). Rytand (1938) found that glomerular size was closely related to kidney and body weight in mammals ranging in size from small rodents to the elephant. Munkacsi and Palkovits (1965) in contrast found no relation between average glomerular volume and body and kidney weights in different species they examined. The correlation was, however, quite close between *Jaculus* and the albino rat. For example, the kidney weight of the albino rat was 3,87 times greater and the average glomerular volume 3,47 times greater than in *Jaculus*. In *A. pusillus* the weight of each renculus in a kidney weighing 405 g is estimated to be $\pm$ 1,75 g, there being 231 renculi per kidney. The average rencular weight of *A. pusillus* is therefore 2,8 times greater than that of the albino rat and the average glomerular volume 3,8 times greater. Therefore no close relationship of rencular weight: glomerular volume exists between these phylogenetically distinct species. When compared with kidney weight (405 g), however, the difference was 653 times greater and it is evident that glomerular volume followed rencular weight rather than kidney weight. Rytand's (1938) relationship therefore is not applicable to *A. pusillus* and further confirms the individual nature of renculi which were established on an anatomical basis.

The average ratio of glomerular volume to cortex volume of 5,8% (6,7% for a single renculus) established for an adult specimen of *A. pusillus* is higher than that found in a 15 week old albino rat (3,7%) and approximates the value established for adult humans (Zolnai and Palkovits 1965a, 1965b). It is, however, lower than values recorded for *Jaculus* and other species described by Munkacsi and Palkovits (1965) which ranged from 6,9% to 7,73%. Since age has a pronounced affect on this ratio (relative growth of glomeruli and cortex) and as the precise age of the above species was not known, no final conclusions can be drawn.

The distribution curves (Fig. 16) of the cortical and juxtamedullary glomerular volumes of *A. pusillus* are steeply sloped indicating uniformity of size. Both curves are equal in width therefore exhibiting the same range in volume. The juxtamedullary curve, however, is positioned to the right of the cortical curve indicating that the juxtamedullary glomeruli have a greater average volume (Table 8). The average difference in volume between the cortical and juxtamedullary glomeruli is not great, the latter being only 35% larger and comparable to the values obtained for man and the albino rat (20% and 25% respectively) but are nowhere near the values calculated for arid adapted mammals (101% and 169%).

The results suggest that a relationship exists between concentrating ability and the ratio of juxtamedullary: cortical glomerular size. This is lower on the average in mammals with an inferior concentrating ability when compared with those which are arid adapted. In this respect adult *A. pusillus* is similar to the former group and does not even approach the values obtained for arid adapted mammals.

Table 8. Glomerular volumes in *A. pusillus* at magnification 250 X.

	Log volume												Mean	
	4,4	4,5	4,6	4,7	4,8	4,9	5,0	5,1	5,2	5,3	5,4	5,5	log	μ^3
Cortical glomeruli	2	1	2	7	5	14	19	25	30	3	—	—	5,0017	100 400
Juxtamedullary glomeruli	—	1	1	1	2	6	15	22	19	19	3	—	5,1307	135 100
Mean, all glomeruli	1	1	1	5	3	8	18	24	25	11	2	1	5,0693	117 300

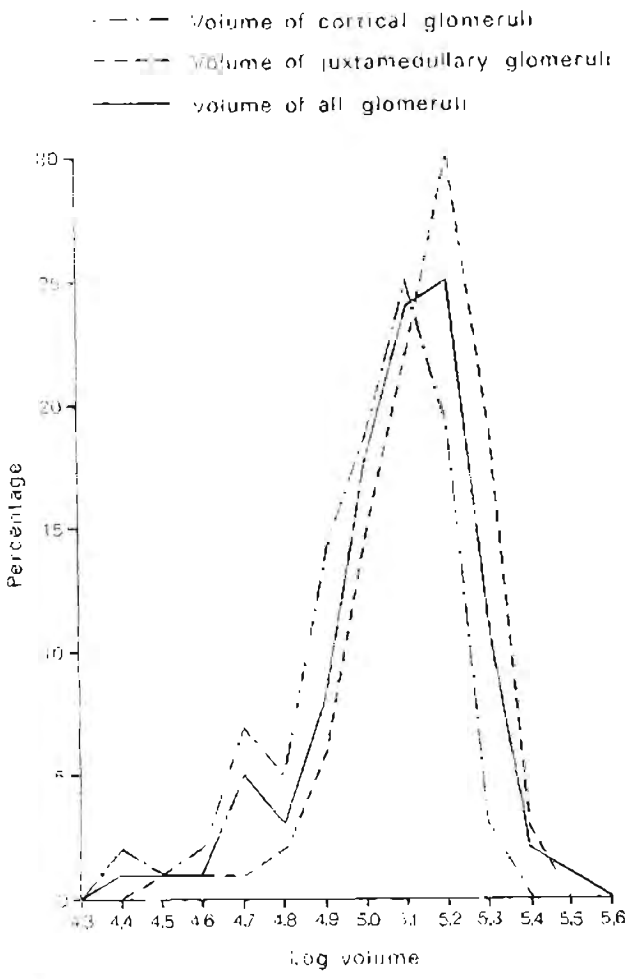

Figure 16. Distribution curves of glomerular volumes in *A. pusillus*.

3.5. The percentage distribution of the thin segments of the loops of Henlé in the renicular medulla

Munkacsi and Palkovits (1966) investigated differences in kidney characteristics among four species of mammals two of which originated from an arid environment and two from a mesic environment. The characteristics investigated were: the ratio between cortex width and medullary length, the length and type of the loops of Henlé and the percentage distribution of the thin segments of the loops of Henlé at various levels in the renal pyramid. The determining factors in the percentage distribution of the thin segments were their length, diameter and number of the loops of Henlé. They showed that kidney efficiency could not be evaluated by only taking the number of the loops of Henlé or the lengths or the volume (length x diameter) of the loops into account, but that the percentage distribution of the thin segments, which also include all the other factors, is a truer reflection of efficiency. Their results showed that *Jaculus*, which had the most efficient urine concentrating ability, exhibited the highest relative value in terms of the percentage of thin segments in the long loops. The mean percentage of thin segments in the middle and high loops was, however, similar in all the species which they studied (albino rat, *Galago*, *Cercopithecus*).

As a result of these findings it was not necessary to determine the number of loops of Henlé nor the length or diameter of their thin segments in the renculi of *A. pusillus*. It was, however, necessary to determine the relative percentage distribution of the thin segments as an index of concentrating ability. It should, however, be mentioned that the method of calculation and the preparation of the material in this investigation differed somewhat from that employed by Munkacsi and Palkovits (1966). For example, the renculus was fixed in a stronger formaldehyde solution (10%) to ensure hardening of the tissue thus minimising shrinkage during processing. Sections were cut at 7 μ in an attempt to retain erythrocytes in the capillaries of the vasa recta. This was done to aid in distinguishing between the capillaries and the thin segments which proved to be no easy task. Moreover, during sectioning and processing erythrocytes were transferred by accident into the tubules, thus complicating identification further. The most important criterion employed for identification was that the nuclei of the epithelial cells of the thin segments extend further into the lumen and that they are closer together than those in the larger capillaries. Fig. 15 illustrates the similar appearance of these two structures.

A projection apparatus was not used to enlarge the sections since the resolution at a magnification of 1 000 proved to be inadequate to reliably identify the thin segments and calculate the percentage distribution. The counts were therefore made with a light microscope using an ocular grid at the required magnification of 1 000, which according to Palkovits and Zolnai (1963) decreases the accuracy of the calculation.

The distribution curve of thin segments established for a renculus of *A. pusillus* (Fig. 17) shows that it coincides more closely with the curve for *Jaculus* than that of the albino rat (Munkacsi and Palkovits

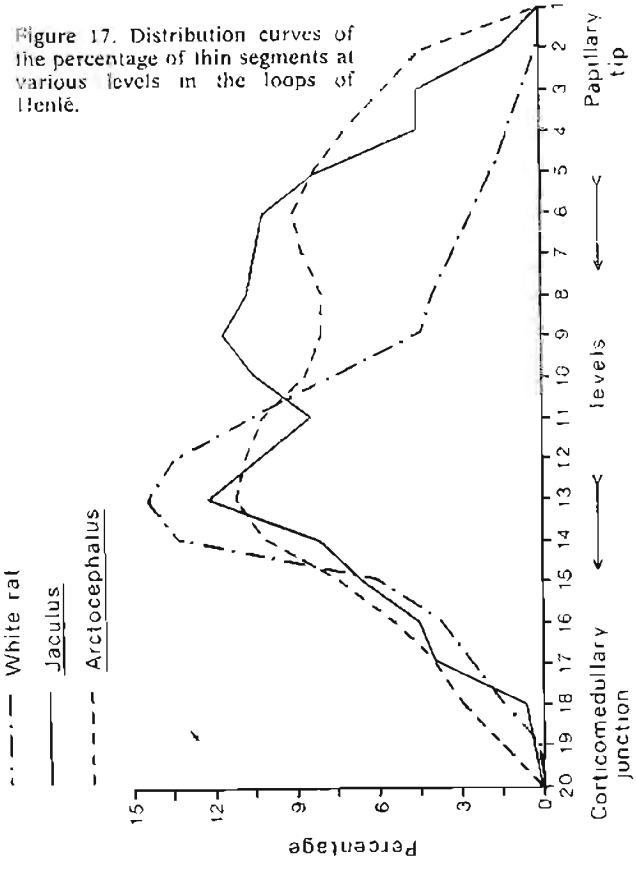

Figure 17. Distribution curves of the percentage of thin segments at various levels in the loops of Henlé.

1966). The mean percentage of the thin segments in the inner zone (8,3%) was 2,2 times greater than the value in the albino rat inner zone, but only slightly greater than the percentage distribution in *A. pusillus* (7,4%). Furthermore, the highest percentage distribution (11,1%) in *A. pusillus* was found at the junction between the inner and outer zone as in other species (Fig. 17). The distribution curve also has two peaks; the first on the above-mentioned junction and the second within the inner zone. In addition, a high percentage of thin segments extends closer to the tip of the papilla than in *Jaculus*.

In general the results are typical of a kidney with a high urine concentrating capacity and therefore corroborate the other anatomical findings in the present study.

SUMMARY AND CONCLUSIONS

Chemical analyses of plasma samples obtained from *A. pusillus* showed that the plasma osmolality and concentration of various constituents, including electrolytes, were typically mammalian. Urine analyses, however, showed that *A. pusillus* possesses very efficient renal function although not as spectacular as certain desert rodents. For example, the mean plasma osmolality of adults was 326,6 mOsm/l while the maximum urine osmolality was 2 364 mOsm/l. This provides a urine: plasma ratio of 7,2 which is considerably less than that obtained in the camel and hyrax, namely ± 10, and far less than that obtained in *Jaculus* namely ± 16.

Morphological studies showed that the kidney is typically renculate with each renculus conforming in general architecture to the unilobar kidney found in e.g. rodents. The percentage distribution of the thin segments of the loops of Henlé and the relative layer thicknesses, relative to rencular size, are quantitatively the same as found in the desert rodents which have very superior concentrating ability.

The absence of flattened epithelium in the collecting duct system as well as the low ratio of the juxtamedullary glomerular volume: cortical glomerular volume are, however, similar to the condition found in non desert forms. The renculli also possess the necessary anatomical characteristics for the recycling of urea to enhance renal water conservation. The blood vascular system comprises an intrinsic pathway, typically found in cetaceans, but hitherto not described for pinnipeds, as well as an extrinsic pathway which extends intrarenally to form a vena renalis. The rencular blood vascular system, apart from that portion situated outside the renculus, does not deviate markedly from the condition found in terrestrial mammals. The interrencular plexuses which were found, are thought to be exclusive to a kidney constructed on the renculate plan.

In the final summary therefore it is clear that both renal function and renal morphology in *A. pusillus* is more than adequate to allow this species to be independent of fresh water. Renal efficiency is, however, not nearly as spectacular as in certain desert rodents and can only be considered adequate when judged against the normally cool and moist environment in which these animals live.

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ERRATA

The functional morphology of the kidney of the Cape Fur Seal, *Arctocephalus pusillus* (Schreber) by Marthán N. Bester.

Circumstances prevented the author from reading this paper before publication. While several errors exist, only those which effect the meaning have been noted.

P. 70 — col. 2 — para. 3 — line 3:	celloidin for colloidin
P. 74 — col. 1 — table 4 — group 4:	Bulls **142** for 152
P. 79 — col. 1 — para. 2 — line 15:	plexus **or** for plexus of
P. 79 — col. 2 — para. 1 — line 12:	**A** v. renalis for *A. v. renalis*
P. 85 — col. 1 — para. 2 — line 12:	urine **of** *A. pusillus* on for urine on
P. 85 — col. 2 — para. 2 — line 8:	rectae **verae** for rectae spuriae
P. 88 — table 8 — col. 10:	Values 30 and 19 reversed