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Renal cellular and tissue specializations in the bottlenose dolphin (*Tursiops truncatus*) and beluga whale (*Delphinapterus leucas*)

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Abstract

In this report selected examples of subcellular specializations and tissue structures of the bottlenose dolphin and beluga whale kidney are presented which illustrate some unique renal adaptations of cetaceans, and other subcellular structures are depicted for here the first time by electron microscopy. Large reservoirs of glycogen in the cortical proximal convoluted tubules, some unique bundles of medullary blood vessels, and the well-known sporta perimedullaris musculosa of the reniculi are considered to be specialized adaptations in the kidney which may facilitate cetacean diving behavior.

Introduction

The physiologic demands of diving, sometimes to great depths up to 1000 meters, and the associated extreme ambient pressures, have evolutionarily brought about many adaptations for cetaceans and other marine mammals. These specializations are apparent within physiologic, biochemical, and structural systems, and in the latter case anatomic changes at gross, histological, and ultrastructural levels have been recognized in whales, dolphins, and porpoises. In our laboratory we have been investigating at the cellular and tissue level a number of these mammalian adaptations related to diving or the aquatic environment in vascular (Pfeiffer & Kinkead, 1990), cardiac (Pfeiffer, 1990; Pfeiffer & Viers, 1995), integumentary (Pfeiffer & Jones, 1993; Jones & Pfeiffer, 1994; Pfeiffer & Rowntree, 1996), and gastrointestinal systems (Pfeiffer, 1993). Since most detailed physiologic monitoring of free and deep-swimming marine mammals is technically problematic, some clues to their diving-related specializations can be obtained by analysis of their unique fine structural features in diverse organ systems.

The kidneys of cetaceans have long been studied, and indeed the unique macroscopic appearance of the cetacean kidney as a grape-like cluster of small renal units was recorded as early as 1680 (Tyson, 1680) and in the following century (Bonnaterre, 1789). Each of these small units, termed 'renules' or 'reniculi; has the anatomic and physiologic characteristics of a single kidney as seen in typical terrestrial mammals. Reniculation is quite distinct from the simple lobulations observed in some terrestrial mammals, and the reniculi may number in the hundreds to thousands per kidney, depending upon species (Ommanney, 1932). Following these historic reports, cetacean renal anatomy has been reported by several workers for diverse cetacean species, and extensively characterized mostly at the macroscopic level (Gihr & Kraus, 1970; Cave & Aumonier, 1964, 1965, 1967; Arvy, 1973; Hedges *et al*., 1979). Recent detailed accounts have characterized both the macroscopic (Abdelbaki *et al*., 1984) and microscopic (Henk *et al*., 1986) structure of reniculi of the bowhead whale, *Balaena mysticetus*. Along with reniculation, a second characteristic, unique for cetaceans, has been identified in the kidney. This is an architectural component seen histologically which partially separates the medulla from the cortex by a basket-like layer of collagen, elastic fibers and smooth muscle, the sporta perimedullaris musculosa. The function of the sporta, which is devoid of smooth muscle in the bowhead whale (Henk *et al*., 1986), remains poorly understood. Furthermore, renal function of cetaceans in general remains a subject of considerable interest because few physiologic studies have been undertaken on these marine mammals (Malvin & Rayner, 1968; Telfer *et al*., 1970), and their usual access only to salt water imposes extraordinary functions in concentrating urine and in osmotic and water balance. Since very few ultrastructural studies and few histologic studies have been done on cetacean kidneys, the

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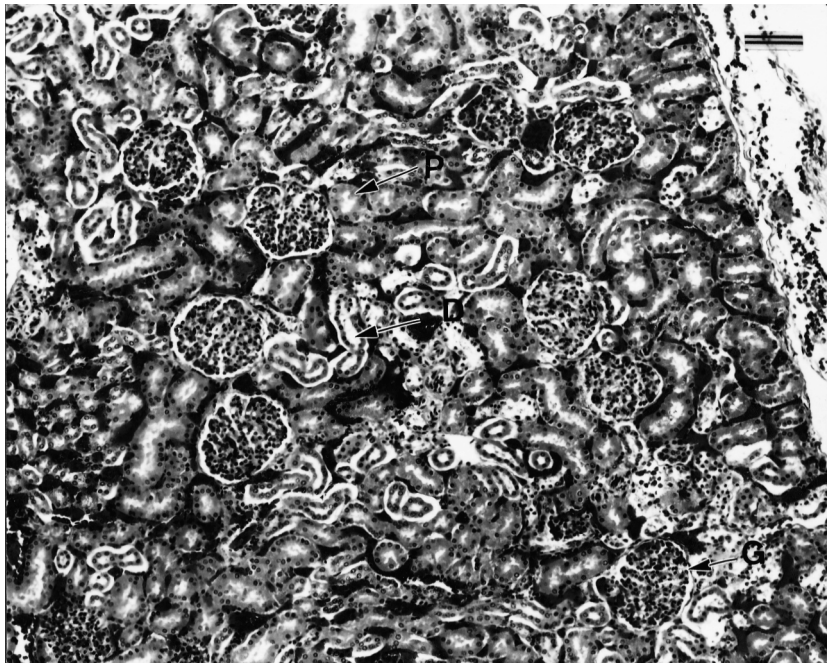

Figure 1. Renicular cortex of beluga whale. Glomerulus (G); proximal convoluted tubule (P); distal convoluted tubule (D). Bar = 73 μm.

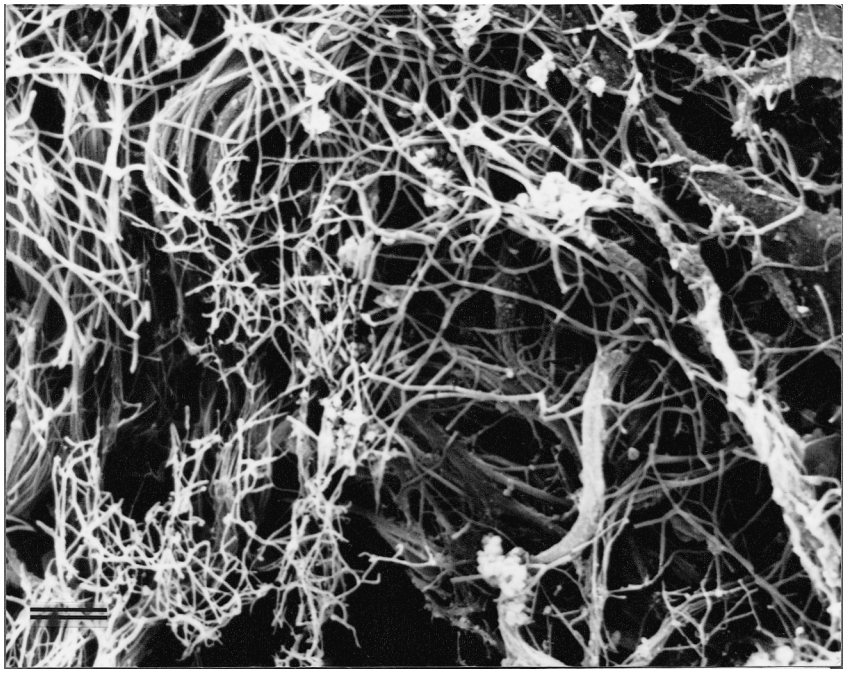

Figure 2. Scanning electron micrograph (S.E.M.) of renicular capsule of bottlenose dolphin, showing loose connective tissue. Bar = 2.3 μm.

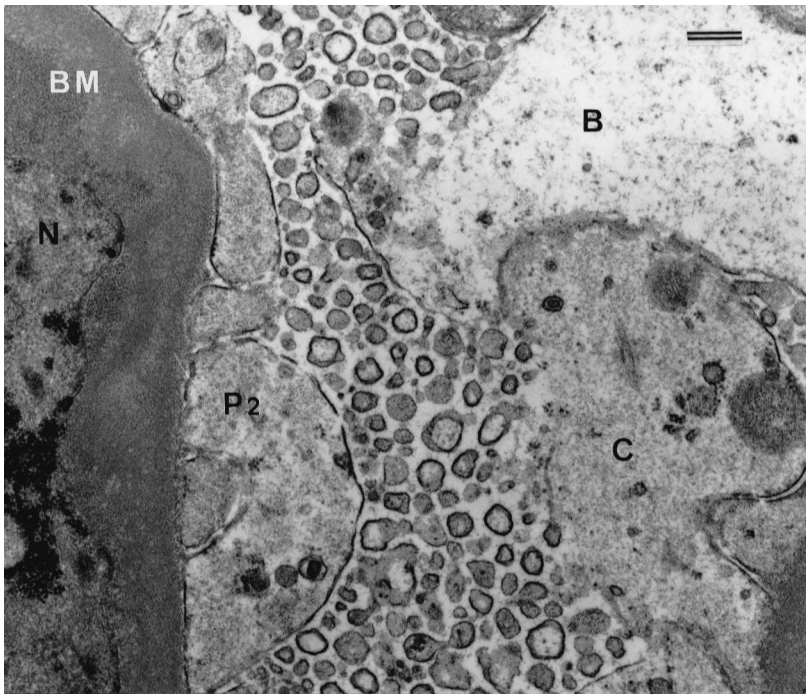

Figure 3. Transmission electron micrography (T.E.M.) of glomerulus of bottlenose dolphin, illustrating glomerular basement membrane (BM); secondary foot processes (P2) of podocyte; portion of nucleus of capillary endothelial cell (N); cytoplasm of podocyte (C); and Bowman's space (B). Bar = 350 nm.

present investigation was undertaken with several species in order to elucidate at the microscopic and subcellular level some renicular structural specializations which may facilitate the survival of theses aquatic mammals.

Materials and methods

Tissue collection

In our larger study, renal tissue has been collected and studied over a period of years from single or multiple, adult specimens of either sex of the following species: long-finned pilot whale (*Globicephala melaena*), the bottlenose dolphin (*Tursiops truncatus*), the harbor porpoise (*Phocoena phoecoena*), the striped dolphin (*Stenella coeruleoalba*), the beluga whale (*Delphinapterus leucas*), and the common dolphin (*Delphinus delphinus*). The cetaceans were either freshly stranded on the Atlantic coast of the USA, or were collected after being inadvertently caught in commercial fishing nets, except for the two beluga whales, which were necropsied oceanarium specimens examined by light microscopy only. The field collected specimens were placed as immediately as possible in cold 5% glutaraldehyde/3% formaldehyde in 0.1 M Na cacodylate buffer at pH 7.4, or in 10% buffered formaldehyde. This report focuses upon samples obtained from the bottlenose dolphins and beluga whales.

Electron and light microscopy

The fixed samples for electron microscopy were washed in 0.1 M cacodylate buffer, post-fixed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h, washed in buffer again and dehydrated in a series of ethanol. Formaldehyde preserved samples for routine light microscopy were stained with H & E stain.

For orientation of specimens and preliminary study by light microscopy, semithin sections (1 μm) were cut from samples embedded in Poly/Bed 812 (Polysciences) by standard methods we have used for cetacean tissue (Pfeiffer & Kinkead, 1990). These sections were stained with 1% toluidine blue in 1% sodium borate for 30 s, followed by 0.5% safranin in 0.5% sodium borate for 10 s. Thin sections were subsequently cut and doubly stained with lead citrate and uranyl acetate and were studied with a JEOL 100 CX-II transmission electron microscope operating at 80 kv. Multiple sections from multiple tissue blocks were prepared

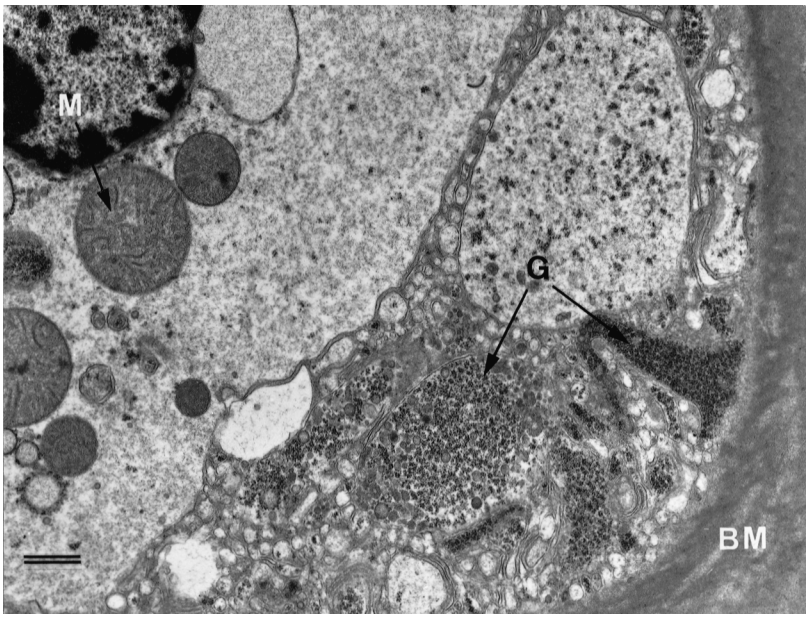

Figure 4. Reservoirs of concentrated glycogen were commonly seen near the basal region of renicular convoluted tubular cells. Glycogen (G); basement membrane of tubular cell (BM); mitochondrion of tubular cell (M). Bottlenose dolphin. Bar = 714 nm.

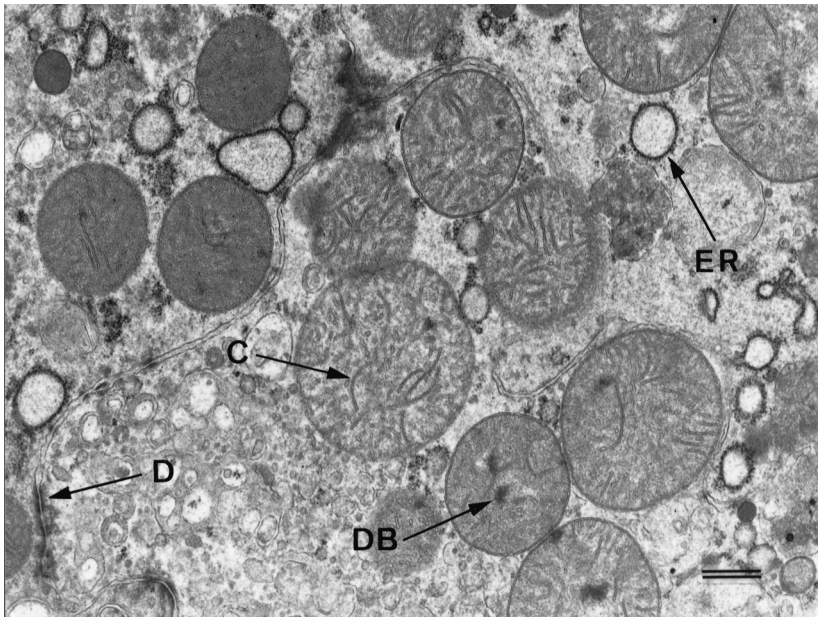

Figure 5. Portions of two convoluted tubular cells of bottlenose dolphin are illustrated here by T.E.M., revealing thin cristae (C) and dense bodies (DB) of the mitochondria, rough endoplasmic reticula (ER), and desmosomes (D) at the cellular junction. Bar = 500 nm.

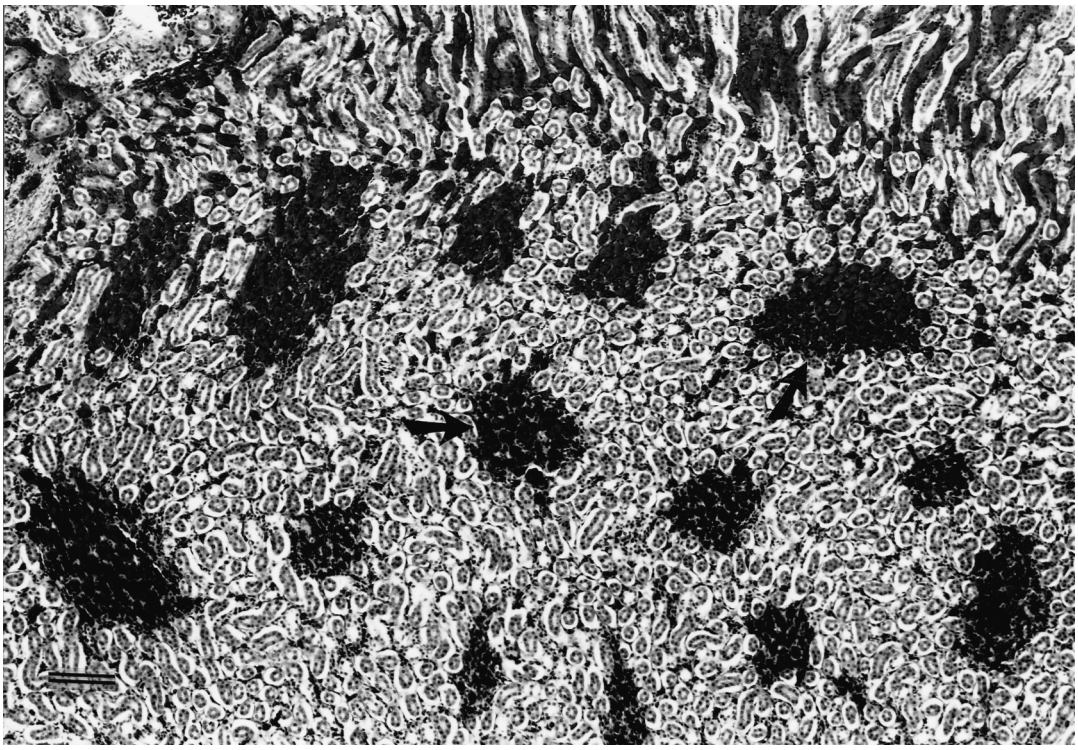

Figure 6. Light micrograph of corticomedullary junction of beluga whale reniculus, showing at low magnification unique bundles (arrows) of vasa recta, blood vessels in the outer medullary zone. These are readily discernible because of congestion related to the animal's mode of death. Bar=40 μm.

from each animal. Selected specimens were similarly fixed for examination by scanning electron microscopy. These specimens were critical point dried, mounted and coated with approximately 1500 Å gold in a SPI sputter coater for 5 m and examined in a JEOL JSM 35C scanning electron microscope operating at 10 kv.

Results

The following results are not intended to illustrate the fine structure of all renicular components of the species examined, but rather to illustrate some specialized features unique to cetaceans, or some non-unique characteristics of the cetacean kidney which are shown for the first time at this high resolution.

Cortical structures

The renicular cortex of cetaceans is structurally non-distinctive at the light microscopic level, as illustrated by the beluga whale (Fig. 1), except that here and in most species the cortex is thinner relative to the medulla in cetaceans vs terrestrial species. Typical glomeruli and thick proximal convoluted and thin distal convoluted tubules are observed in the cortex (Fig. 1). The cetacean reniculus is surrounded by a thin fibrous capsule composed of loosely-arranged collagenous and reticular fibers as shown by scanning electron microscopy (Fig. 2) of a bottlenose dolphin renicular capsule. The fine structure of the cetacean glomerulus reveals, as shown by an example from the bottlenose dolphin (Fig. 3), the key morphology associated with filtration as commonly seen in other mammalian kidneys. A prominent, electron dense glomerular basement membrane upon which secondary foot processes of podocytes rest, and vesicular components within the podocyte cytoplasm are evident (Fig. 3). Much evidence was found of multiple, large storage reservoirs of glycogen within the basal cytoplasm of proximal convoluted tubules (Fig. 4). These storage depots were spheroid or irregular in shape, and were sometimes, but not always observed in close proximity to the elaborate basal plasma membrane infoldings of these cells. Other organelles within the proximal convoluted tubule cytoplasm included rough endoplasmic reticula, small vesicles, and numerous mitochondria (Fig. 5). The latter contained narrow,

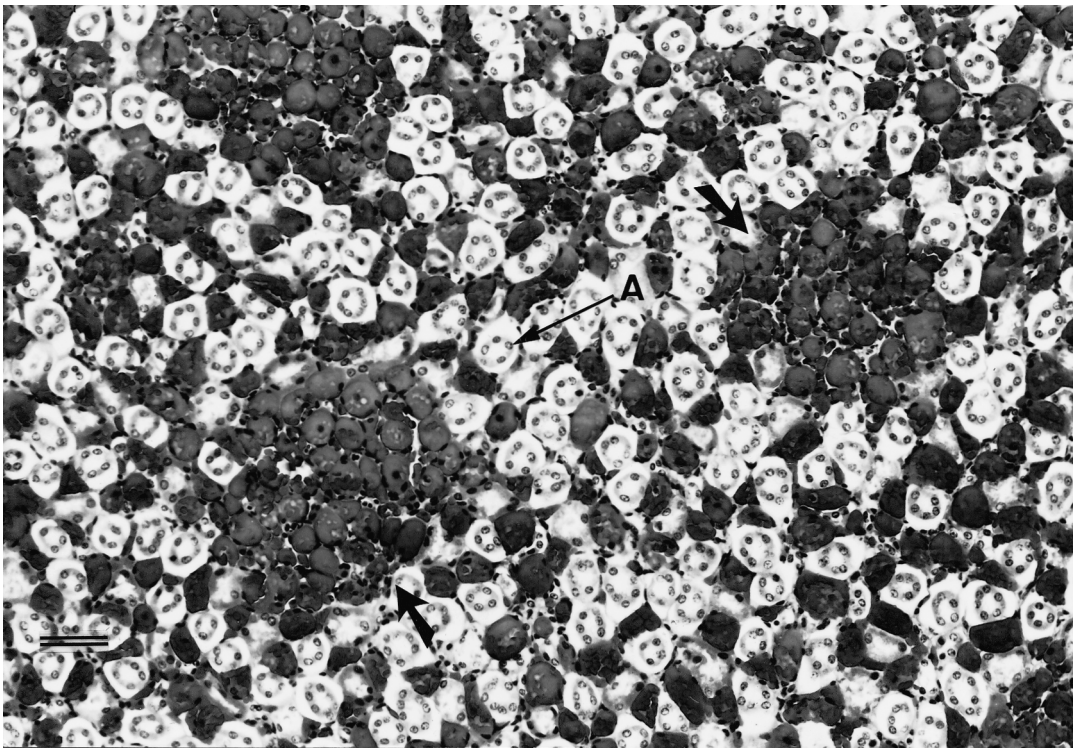

Figure 7. Cross section light micrograph of beluga whale reniculus at outer medulla, illustrating higher magnification of vasa recta bundles (arrows). Note also numerous ascending limbs of the loops of Henle, consisting of low cuboidal epithelium (A). Bar=22 μm.

widely-spaced cristae and occasional dense bodies. Desmosomes between adjacent tubular cells were evident (Fig. 5).

Medullary structures

At the cortico-medullary junction of the reniculi as revealed by light microscopy of beluga whale material (Fig. 6), an array of straight, collecting ducts and loops of Henle can be seen in the deepest portion of the cortex, and medullary rays composed of collecting ducts and tubules can be seen in the medulla. This is similar to the terrestrial mammalian kidney model. Prominent bundles of parallel blood vessels (vasa recta) can be seen in the outer medulla, as shown in cross section at low (Fig. 6) and high (Fig. 7) magnification of the beluga reniculus, and in longitudinal section (Fig. 8). These constitute a rete mirabile and, although rete mirabile have been observed in terrestrial mammalian kidneys, the tight, parallel bundling of these blood vessels as seen in the cetacean reniculus is unique. The well-known sporta perimedullaris musculosa, a basket-like network dividing the cortex from the medulla in all cetacean reniculi, was observed in all of our specimens. As viewed by scanning electron microscopy (bottlenose dolphin) for the first time, this network resembles a fibrous sheath of loose connective tissue with collagenous and elastic fibers aligned unidirectionally (Fig. 9).

Scanning electron microscopy of the renicular medulla, as shown by examples from the bottlenose dolphin, revealed thin descending limbs of the loops of Henle as well as larger collecting tubules and an interstitium of loose connective tissue (Fig. 10). Higher magnification of the collecting tubules revealed the fibrous adventitial covering of those ducts in the reniculus (Fig. 11).

Discussion and conclusions

The data presented here, while not surveying the overall ultrastructure of the cetacean kidney, have focused upon several renicular structures, some of which show adaptive specializations in these aquatic mammals. Few prior reports have presented electron microscopic perspectives of the cetacean kidney (Hedges *et al.*, 1979; Henk *et al.*, 1996) although our laboratory has reported a number of cardiovascular and integumentary subcellular specializations which apparently have evolved to

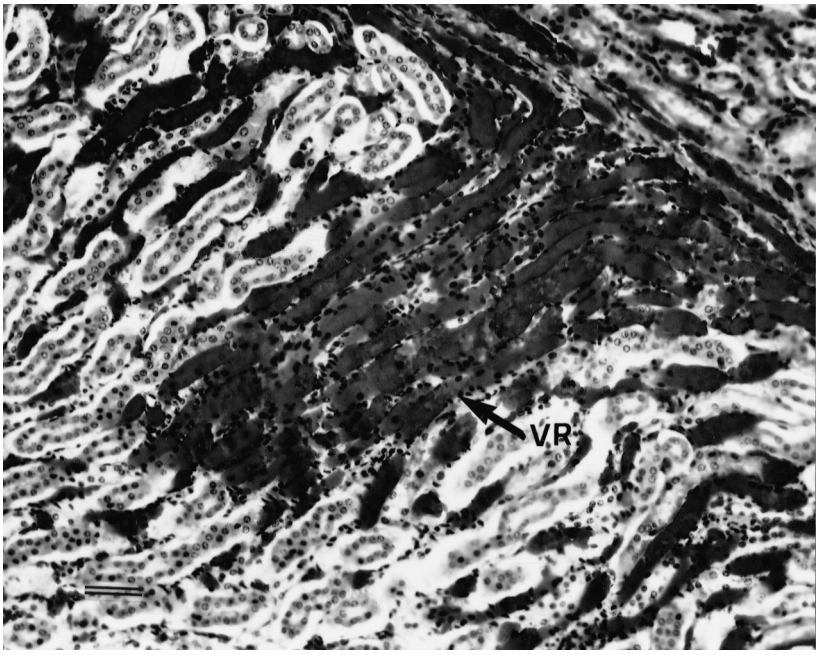

Figure 8. Longitudinal, high power section of vasa recta bundle of beluga whale renicular medulla (VR). The blood vessels turn at the corticomedullary junction, where arcuate arteries and veins are also present. Numerous, small open collecting tubules are also evident. Bar=22 μm.

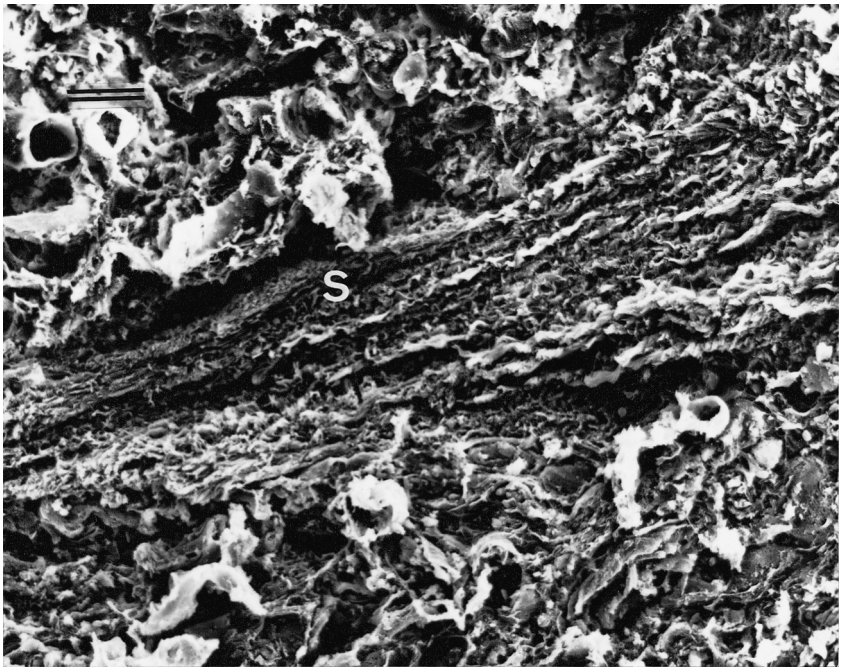

Figure 9. S.E.M. of the sporta perimedullaris musculosa (S), the fibrous network between the cortex and medulla. Elastic and collagenous connective tissues are present here. Bottlenose dolphin. Bar=45 μm.

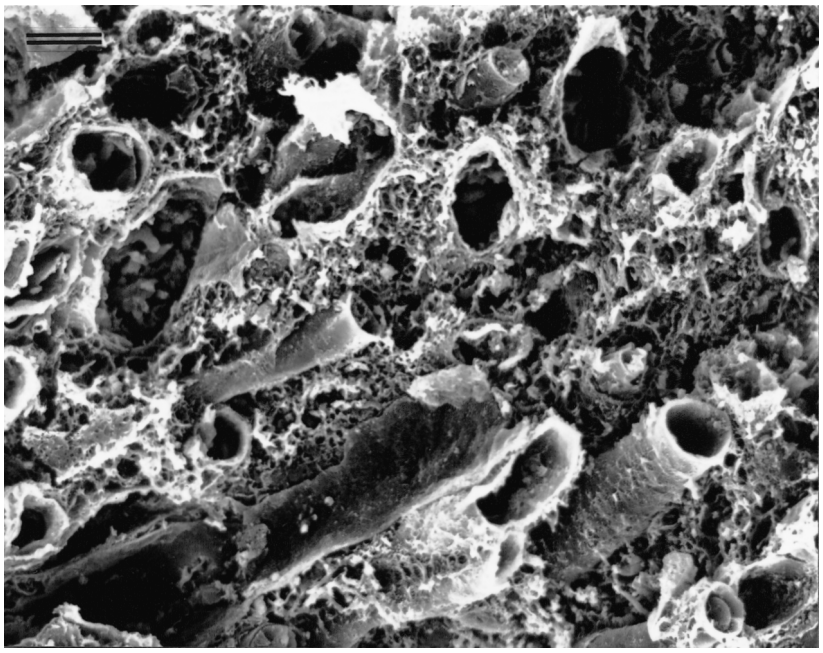

Figure 10. From the perspective of S.E.M., the bottlenose dolphin renicular medulla shows the parallel collecting tubules and smaller limbs of loops of Henle, all separated by a significant stroma of connective tissue. Bar = 20 μm.

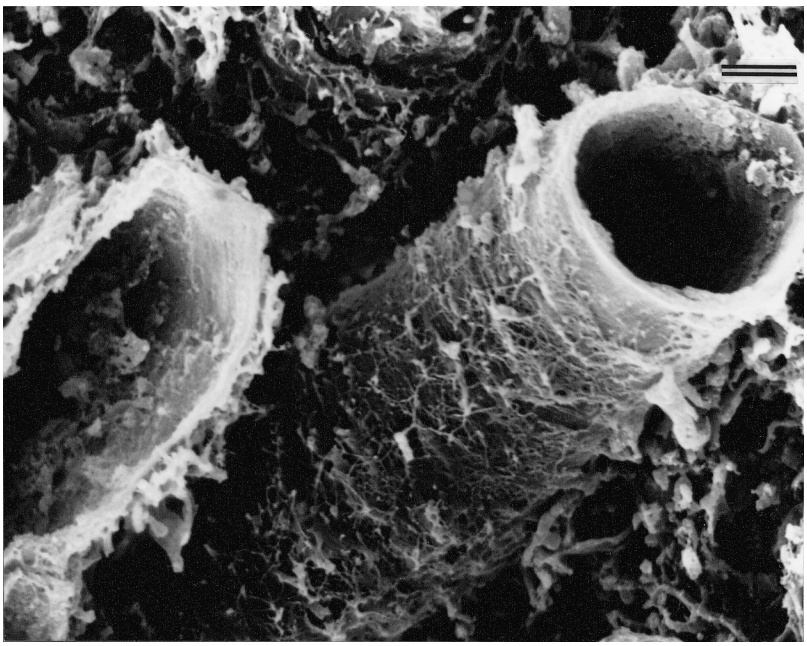

Figure 11. A higher magnification by S.E.M. of the medullary tubules also reveals the fibrous character of the adventitia surrounding the tubules. Bar = 50 μm.

support the diving or aquatic behavior of cetaceans (Pfeiffer, 1990; Pfeiffer & Kinkead, 1990; Pfeiffer & Jones, 1993; Pfeiffer & Rowntree, 1996) and pinnipeds (Pfeiffer & Viers, 1995). Considerable data have been published elsewhere for comparison purposes on the renal ultrastructure of terrestrial-based mammals (Crayen & Thoenes, 1978; Kerjaschki, 1978; Kaissling, 1980; Bachmann *et al*., 1986; Tisher & Madsen, 1986).

One observation of this study, i.e. the presence of circumscribed glycogen reservoirs within proximal convoluted tubule epithelial cells, was quite similar to earlier reported findings of intracellular zones of glycogen concentration within ringed seal and harp seal myocardium (Pfeiffer & Viers, 1995) and findings of intracellular reservoirs of glycogen in the bowhead whale heart (Pfeiffer, 1990). Vogl & Fisher (1976) also reported glycogen pools in the arterial thoracic retia of the narwhal, *Monodon monoceros*. In each case such enlarged reservoirs may help support anaerobic glycolysis of the cells involved during conditions of systemic hypoxia associated with prolonged submersion. This morphologic evidence of specialized glycogen reservoirs in the cetacean kidney cells and elsewhere supports the chemical finding of two to three-fold enhanced glycogen levels observed in Weddell seal (*Leptonychotes weddelli*) cardiac tissue (Kerem *et al*., 1973). As part of the diving reflex, both renal and cardiac function are greatly reduced during submersion. However, these organs have inherent high metabolic demands and must remain resistant to reperfusion, free radical-induced injury, and in the case of the kidney, to the cytotoxic effects of concentrated urea.

The present study has also identified another unique feature of the cetacean renicular unit in respect to the medullary vasculature. Early comparative studies (Plakke & Pfeiffer, 1964) of the blood vessels of terrestrial mammalian kidneys have clarified a range of medullary vascular patterns, from highly zonated patterns (opossum, gerbil, cat, kangaroo rat) to a lack of zonation (pig, beaver). In none of these species, nor in the rat (Moffat & Fourman, 1963), are the vasa recta of the renal medulla as concentrated into bundles as they are in cetaceans. Further, dense capillary plexuses were not observed in the cetacean sub-cortical medullary, or outer medullary zones, as they have been reported for rats (Moffat & Fourman, 1963) or other terrestrial mammals (Plakke & Pfeiffer, 1964). In this respect, the blood vessels of the outer medulla of the cetacean renicules somewhat resemble the retia mirabilia as observed in other regions of the cetacean body (Vogl & Fisher, 1976; Pfeiffer & Kinkead, 1990). This concentration of medullary blood vessels into bundles would seem to reduce the countercurrent exchange mechanism at this site as available to kidneys of terrestrial mammalian species. This vascular adaptation, along with the cetacean phenomenon of reniculation of the kidney, and the unique presence of a sporta perimedullaris musculosis found only in cetaceans are all renal specializations which have evolved for an aquatic existence. However, the relative large ratio of medulla to renal cortex, as observed in most cetacean reniculi (Arvy, 1973), can be considered as an adaptation for great efficiency in concentrating urine due to enhanced area for tubular reabsorption. Hedges and associates (1979) have earlier described, elsewhere, comparative aspects of cetacean renal vasculature.

The function of the sporta perimedullaris musculosis, termed 'sporta perimedullaris' in the bowhead whale because of a lack of contained smooth muscle in this species (Henk *et al*., 1986), has not been clarified in this study. It is possible, however, that its obscure and debated function may be to empty rapidly the medulla of urine at the moment of diving, as concentrated urea would be cytotoxic during prolonged submersion, and in most cetacean species the sporta does contain smooth muscle.

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