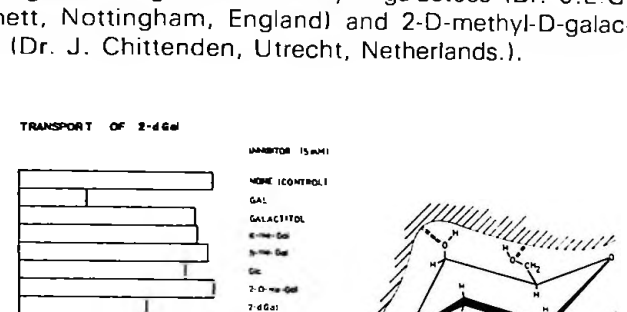
USPHS Grant AM-12619. The authors gratefully acknowledge generous gifts of 3-deoxy-D-galactose (Dr. J.E.G. Barnett, Nottingham, England) and 2-D-methyl-D-galactose (Dr. J. Chittenden, Utrecht, Netherlands.).



29 • 1975

The Specificity of the Transport Systems for Glucose in the renal tubular cells of the Flounder (*Pseudopleuronectes americanus*)

Arnost Kleinzeller, George R. Dubyak and James F. Mullin, University of Pennsylvania School of Medicine, Philadelphia, Pa.

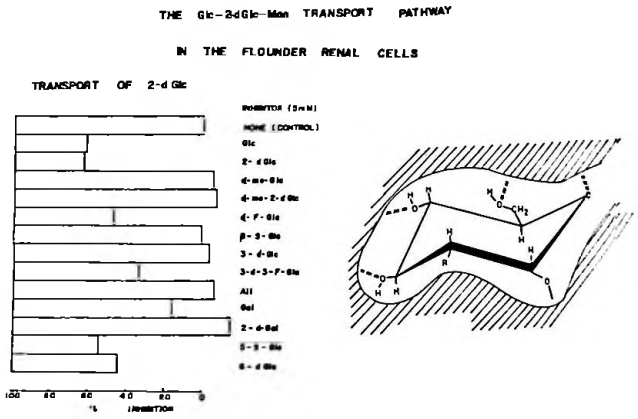
Data on the specificity of both transport pathways for D-glucose found at the antiluminal (basal) face of the renal tubular cells of the winter flounder (*pseudopleuronectes americanus*) have been presented previously (*Kleinzeller, and McAvoy, J. Gen. Physiol. 62:169, 1973; Kleinzeller, Rittmaster, Griffin and McAvoy, Bull., Mt. Desert Island Biological Lab. 14:60, 1974*). These studies have been extended using a broader selection of structural analogs of D-glucose. As in previous investigations, an inhibition analysis of model sugars by teased renal tubules served as the principal technique.

Using 0.5 mM methyl-x-D-glucoside-^{14}C as a model substrate for the transport system shared by D-glucose and both methyl-D-glucosides, the following newly tested sugars (5 mM) were found to be potent inhibitors: -Fluoro-D-glucose, 3-deoxy-3-fluoro-D-glucose. The sugars -thio-D-glucose and 3-deoxy-D-glucose had no effect. These data, taken in conjunction with previously recorded evidence, indicate that an interaction between the transported sugars and the carrier takes place at the following points:hydrogen bridges may be established between the oxygens at C_1, C_3 (in the axial configuration) and C_4 (in the equatorial configuration), a pyranose ring structure appears to be essential; finally, a bond of a firmer nature (covalent ?) may be formed at C_2-OH (in the equatorial configuration).

The specificity of the transport pathway shared by D-glucose, 2-deoxy-D-glucose and D-mannose was studied using the last two sugars as models. Figure 1 summarizes data obtained in previous and the present studies by an inhibition analysis of the tissue uptake of 2-deoxy-D-glucose. The inhibition pattern was identical with that for D-mannose and D-glucose (details not given here). Of the newly tested analogs of D-glucose, only -F-glucose, 3-deoxy-F-glucose, 5-thio-glucose and 6-deoxy-glucose were inhibitory. The scheme given in Figure 1 summarizes the possible points of interaction between the carrier and the transported substrates as follows:

A bond of a relatively firm nature (covalent link to a phosphoryl group at the carrier? See Dubyak, Mullin and Kleinzeller, Bull. Mt. Desert Island Biological Lab 15:000. 1975) is established at C_1-OH; fluoride can replace the hydroxl. Hydrogen bonding at C_3-OH in the axial configuration is indicated by the fact that 3-deoxy-D-glucose, D-allose and D-altrose were not inhibitory, whereas 3-deoxy-3-F-glucose was inhibitory. C_4-OH in the equatorial configuration is essential. A pyranose ring structure is mandatory but no hydrogen bond appears to be formed between the ring oxygen and the carrier in the light of the inhibitory effect of 5-thio-D-glucose.

This investigation was supported in part by USPHS Grant AM-12619.



30 • 1975

Configuration of the Skate (*Raja Erincea*) Nephron and Ultrastructure of Two Segments of the Proximal Tubule

Eric R. Lacy, Bodil Schmidt-Nielsen, Rainer Galaske and Hilmar Stolte. Mount Desert Island Biological Laboratory SalsburyCove, Maine; Medizinische Hochschule Hannover, Germany

The micropuncture study of skate kidney (*Stolte et al., MDIBL Bulletin, #42, this issue*) presented evidence that the principal site of Mg, Phosphate and Sulphate secretion is the proximal tubular segment II (PTS II) which is located primarily on the ventral surface of the kidney. Since this is the first time that a specific site for secretion of these divalent ions has been localized, it was of interest to study the ultrastructure of this and other segments of the skate renal nephron. The nephron and

eport 30, Table 1

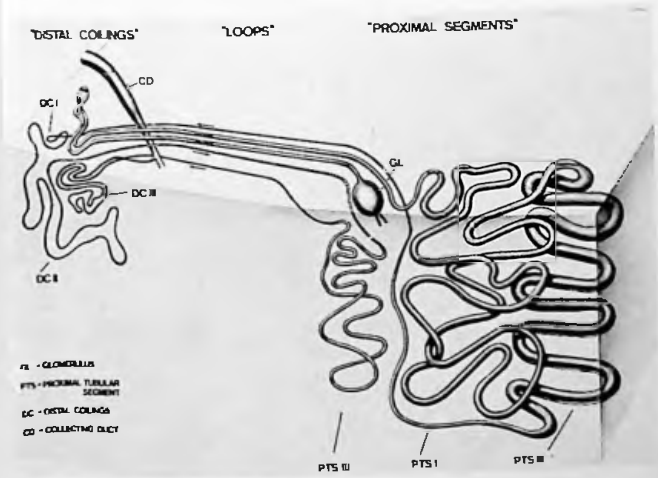
Table I

Nomenclature diagrammatic drawing	Sequence of tubular segments	Length of tubular segments in mm	% of total length	Corresponding numbers assigned by Deetjen and Antkoviak
DC I	1	8	9.8	I & II
DC II	4	12	14.6	VI
DC III	6	9	11	VIII
PTS I	2	14	17	III
PTS II	3	25	30.5	IV
PTS III	5	14	17	V
		82 mm	100%	

Length measurements of the different segments of the skate nephron.

rious parts have previously been described by Deetjen d Antkoviak (*MDIBL Bulletin 10: 5-7, 1970*). In the esent study it was examined by *in vivo* dye injection. he passage of the dye was followed by surface micro- opy and time lapse photography. Pictures were taken th intervals of 4 to 5 seconds. The various coils of the ephron were also studied by latex injections. From these udies the nephron was reconstructed as shown in gure 1. Our reconstruction differs in some important spects from that of Deetjen and Antkoviak as shown in able 1.

Excised tissue was immersed in phosphate buffered utaraldehyde, postfixed in OsO_4, dehydrated in graded thanols and embedded in epoxy resin. One micron thick ections were cut on glass knives, stained with a 1% oluidine blue-1% Borax solution and examined with the ght microscope. Thin sections (800A) were cut ltratome III) on diamond knives, stained with uranyl etate and lead citrate and examined in a Hitachi HUll-C ectron microscope.



igure 1

In sections from the dorsal surface of the kidney the oils of the proximal tubular segment (PTS I) were seen ogether with bundles of five tubules surrounded by onnective tissue. The five tubules in a bundle orrespond to the straight tubules seen in the drawing. These will be described in greater detail later. The length of PTS I is about 14 mm, the lumen diameter 35-40 . A few ciliated cells are seen in this segment. The brush border cells are on the average 17 tall and 10 wide (Figure 2). The highly irregular apical plasma membrane with sparse microvilli projects blunt cytoplasmic extensions into the lumen. Membrane bound spheres are present in the tubular lumen. The lateral cell junction has a typical but short terminal bar. Adjacent cells interdigitate along the lateral borders with fingerlike projections oriented perpendicular to the long axis of the cell. The basal membrane is relatively straight and rests on the basal lamina. The basal lamina shows some blunt projections toward the cell. The nucleus is round and centrally located. The most conspicuous cell organelles are numerous, typically shaped mitochondria. These are randomly and rather evenly distributed throughout the cytoplasm. except for a few small microfilaments and a small Golgi apparatus, no other organelles are prominent.

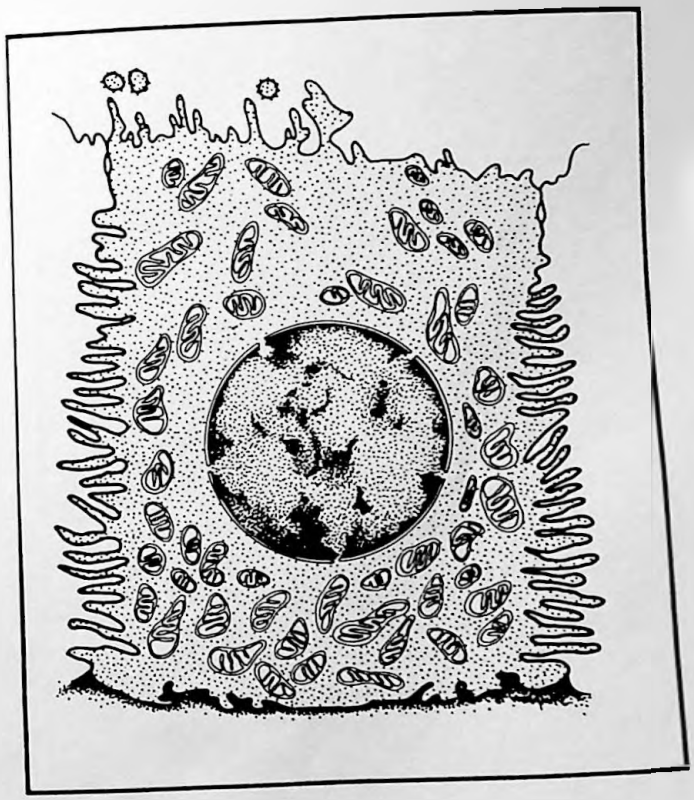


Figure 2

In sections from the ventral surface of the kidney the large coils of the PTS II are dominant. The length of this segment is 30 mm (Table 1). The lumen measures 35 in diameter. There are two cell types in this segment: ciliated and more numerous brush border cells. The ciliated cells are narrower than the brush border cells and have a basally located nucleus. The brush border cells (twice as tall as the cells in PTS I) are 30-35 tall and 10 wide (Figure 3).

The apical plasma membrane is undulating and has a pronounced brush border with branched microvilli. The

microvilli are 2-3 times as long as those in PTS I and more densely packed. At the base of some microvilli there appear to be small pinocytotic vesicles. The lateral cell junction has a terminal bar approximately twice as long as that seen in PTS I. The adjacent lateral membranes interdigitate with long fingerlike projections arranged parallel to the long axis of the cell. In contrast to PTS I the lateral membranes are not always closely opposed. Occasional desmosomes are seen on the lateral cell surfaces. The basal membrane rest evenly on the basal lamina which is smooth and appears thinner in PTS I. The round and centrally located nucleus is of the same size as in PTS I. Again the most prominent cell organelles seen are mitochondria which are conspicuously absent just under the apical membrane but otherwise evenly and densely distributed. Rough endoplasmic reticulum and Golgi apparatus are not prominent.

In summary, the segment of the nephron which secretes the divalent ions (PTS II) differs in several respects from PTS I. The cells are much taller, the brush border more pronounced, and lateral cell borders interdigitate in different patterns.

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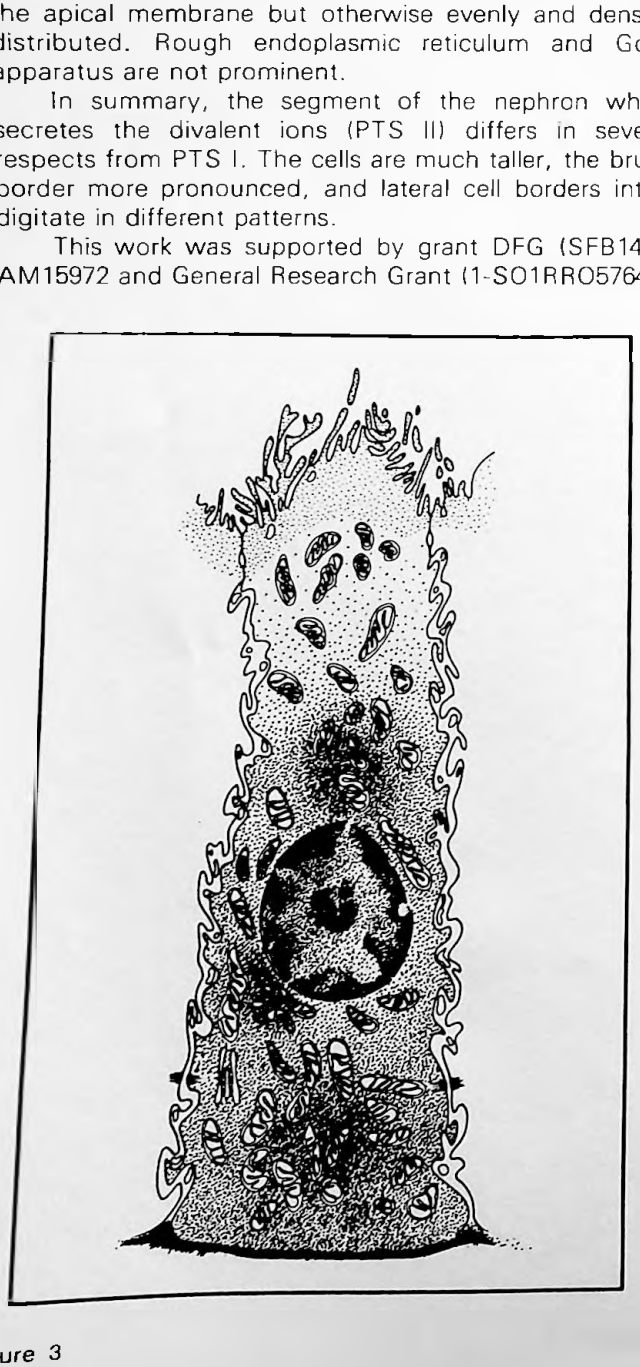

Figure 3

The Urinary Bladder in the Little Skate, *Raja Erinacea*

Eric R. Lacy, Bodil Schmidt-Nielsen, Erik Swenson and Thomas Maren. Mount Desert Island Biological Laboratory, Salsbury Cove, Maine, and University of Florida, Gainesville, Florida.

The gross anatomy of the lower urinary tract of the little skate, *Raja erinacea*, was investigated. By injecting phenol red into the tail vein 5-12 hours prior to sacrifice the urine became deeply red or yellow. Only the finding in the female skate will be reported here. The mature female has a distince, rather large, bilobed urinary bladder (u. bl.) (Figure 1), attached caudally and dorsally to the urogenital sinus (u.s.). It is normally distended with urine (2 to 5 ml). It empties through a short urethra into the urogenital sinus via a small orifice. Eight to ten ureters (Figure 2) lad from each kidney to the dorsal surface of the bladder where they penetrate the epithelium individually. In immature female skates the bladder and urogenital sinus are much smaller. In very young specimens the urine is stored in the cloaca. Urine taken from the bladder of mature female skates is acid (pH 4.90 ± 0.08, n = 6), while urine taken from the cloaca of immature specimens is less acid (pH 5.14 ± 0.05, n = 4). The difference is significant, P 0.05.

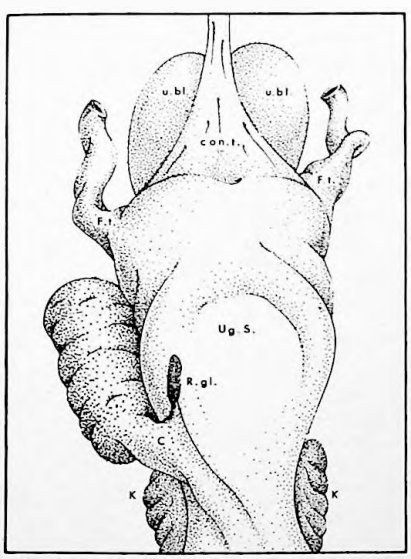

Figure 1

The mucosae of the bladders were dissected and analyzed for carbonic anhydrase content by the micro-method of Maren (*J. Pharm. Expt. Therap. 130: 1960*] using barbital buffer. The tissue contained small but definite amounts of enzyme, about 3 unite/gram mucosa (on this scale shark red cells have about 40 units/ml). The enzyme activity was entirely abolished by 4 M Methazolamide.

Bladders from mature female skates were removed from the animal and fixed with a phosphate buffered glutaraldehyde-papaformaldehyde fixative, embedded in Epon plastic and aectioned for light and electron