1969 #38

ORIGIN OF THE SPINDLE CELL IN Myxine

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The marine cyclostome, Myxine glutinosa, appears to occupy a pivotal evolutionary position between invertebrates, in which the sophisticated and biologically complex immune mechanisms of the vertebrates are absent, and the other cyclostomes in which these mechanisms are relatively intact. Consequently, it has been of interest to examine in detail the immunologic capacity of this species from the cellular viewpoint.

Previously, it was established that the peripheral blood of Myxine contains a lymphoid cell which, by morphologic and electron micrographic criteria, resembled that of the lamprey and higher vertebrates. The objective of this investigation was to examine in further detail certain reations of the hagfish lymphoid cells. Two significant observations were made during 1969.

First, it was established that the spindle shaped cell which apparently derives from the pronephros rounds up and becomes lymphoid in character in vitro. This was found to be a temperature dependent process. At temperatures between 15° and 20° C the spindle shape was reacquired. In some ways the phenomenon resembled the deformation of a sickled erythrocyte under low oxygen tension in sickle-cell disease of man. The significance of this observation is yet to be understood, and cells taken under a variety of conditions are being examined by electron microscopy at the present time.

The other major endeavor was to determine whether mixed cell cultures of allogeneic peripheral lymphoid cells were capable of DNA incorporation in vitro. Cultures were established between a number of randomly selected animals in which 5×10^6 cells from each donor were mixed in a medium especially devised to support these cells. In those cultures which were kept 30 days at 18°C and pulsed for an additional 7 days with tritiated thymidine, a significantly higher incorporation of tritiated thymidine was observed in the allogeneic than in the syngeneic cultures in two out of three animals in which satisfactory experiments were possible. If these experiments are verified by additional similar studies, it would indicate there is an in vitro reactivity of lymphoid cells which corresponds to the chronic homograft rejection phenomenon which has been described recently by Hildeman and colleagues.

1969 #39

LOCALIZATION OF THE DILUTING SEGMENT IN THE DOGFISH NEPHRON: A MICROPUNCTURE STUDY

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To localize the segment of the dogfish nephron in which low water permeability permits the generation of hypoosmotic tubular urine, samples of fluid from progressively more distal segments were taken by micropuncture and analyzed for osmolality using a Clifton cryostat. The experimental animals were those described in report #40, this volume; 9 from seawater and 5 from diluted seawater environment.

Fish were prepared as described (see below); a lower midline incision was made and the

left kidney exposed by gentle retraction of the viscera. Using fine dissection forceps a window was opened through the capsule to permit direct visualization of the surface nephrons. Mineral oil covered the exposed surface to prevent desiccation. Micropuncture was performed using a Leitz micromanipulator and stereo microscope at a magnification of 60X. The kidney surface was illuminated with a Dolan-Jenner fiberlite system. Lissamine green-colored dogfish Ringer's solution was injected into a surface loop and its timed passage observed to permit identification of the distal segments for micropuncture. After sampling was completed, the punctured tubules were filled with latex or microfil for later microdissection. Microphotographs of the renal surface were taken at intervals during the passage of Lissamine green and after injection of latex or microfil.

Tubular fluid to plasma (TF/P) osmolar ratios selected from seawater and dilution experiments are recorded in Table 1.

Table 1

Exp. #	TF#	TF_{osm}	P_{osm}	TF/P_{osm}	U/P_{osm}
			Seawater		
12	1	1013	1010	1.0	0.87
	2	1008			
	3	1035			
	4	957		0.94	
	5	949		0.94	
11	1	948	989	0.96	0.9
	2	1006		1.02	
	3	1028		1.04	
	4	955		0.97	
	5	1000		1.01	
			Diluted Seawater		
H-4	1	725	755	0.96	0.45
	2	677		0.89	
	3	675		0.89	
	4	623		0.83	
H-5	1	685	683	1.00	0.45
	2	655		0.96	
	3	645		0.94	
	4	625		0.92	
	5	545		0.79	

In the seawater group no clearly hypoosmotic tubular fluid was recovered. Since the final urine to plasma osmolar ratios ranged between 0.8 and 0.9, demonstration of a TF/P osmolar ratio significantly below 1.0 is a priori, difficult. One group of data is suggestive. In exp. #12, 2 clearly distal samples taken from the same puncture site had TF/P values of 0.94, the lowest in the seawater group.

Definite hypoosmotic tubular fluid was demonstrated in the diluted group of animals in the most distal visible portions of the nephron to be colored by the passage of dye. The lowest values (Hypo 5, Table 1) were found in a thin segment, probably Segment 6 of Ghouse et al (Bull. MDIBL, 8:22, 1968) as it returned toward the vicinity of the glomerulus. This segment, owing

to its thin-walled structure, could be visualized only during the passage of Lissamine green; it was not at the kidney surface but the thickness of one tubule below. To insure localization of the tip of the micropipette it was necessary to puncture this segment during the passage of Lissamine green. Because at the concentration used this dye contributes significantly to the osmolarity of the sample, the ratio 0.79 found in TF_6 (Table 1) is factitiously high. Application of an appropriate correction yields a TF/P_{osm} for this sample of 0.65; final urine U/P_{osm}, 0.45.

Microdissection of the nephrons punctured in Hypo 4 and 5 confirms that the most dilute tubular fluid came from late Segment 5 and Segment 6. The greater part of Segment 5 contained isomotic fluid. As it approaches the glomerulus of origin Segment 6 courses parallel with and closely adherent to Segments 1 and 2. The three segments have been noted in intimate apposition for a considerable distance, forming a coiled helix (Figure 1). These groupings are apparent in the histological studies of Ghouse et al (Bull. MDIBL 8:22, 1968) and their countercurrent arrangement is suggestive of a functional role in the unique handling of urea by this kidney. This countercurrent arrangement of tubular segments belonging to the same nephron unit was observed in each nephron investigated. Its localization in relation to the rest of the nephron is noteworthy. It is not situated with the tubular convolutions but extends either cranially or cau-

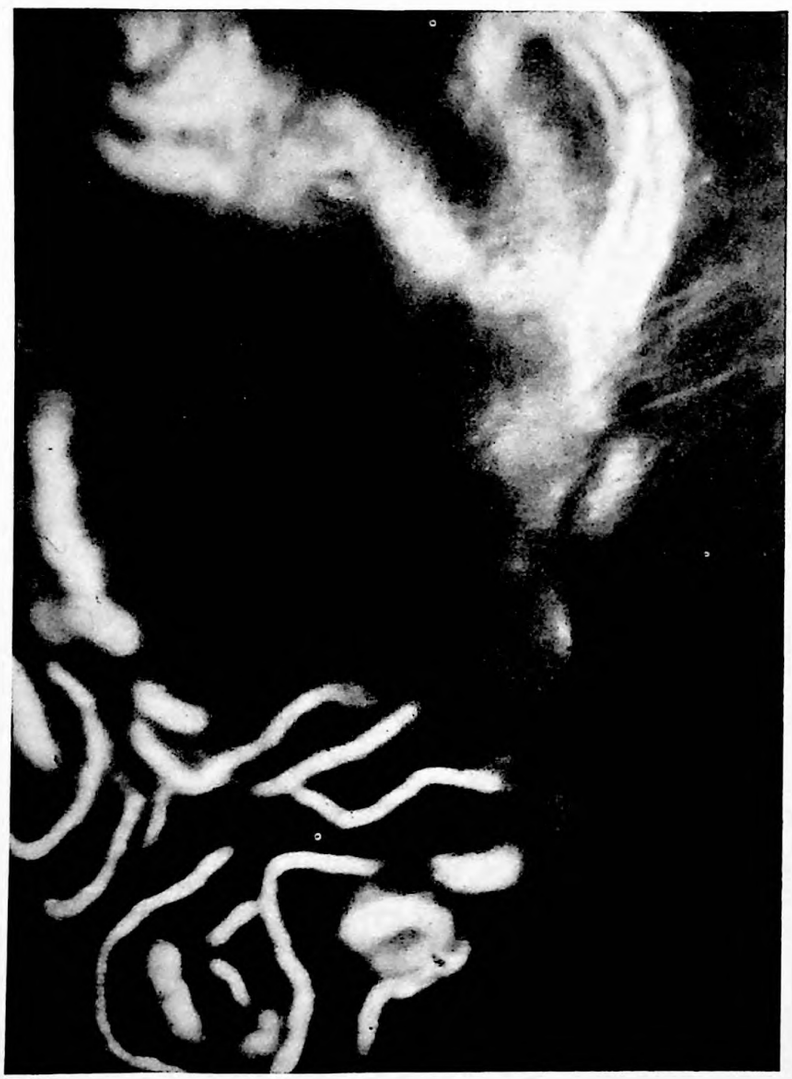

Figure 1.

dally from the convoluted area. This systematic grouping leads to segmental subdivisions of the kidney along its longitudinal axis, in that areas composed largely of tubular convolutions alternate with those composed primarily of countercurrent arrangements.

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1969 #40

DEMONSTRATION OF A RENAL OSMOREGULATORY MECHANISM IN THE SPINY DOGFISH, Squalus acanthias

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We examined the renal response to bypoosmotic environment in 5 female dogfish (1.6 - 4.2 kg BW) maintained in an aerated tank thermostated at 10-12° C for periods of 24 to 42 hours. Tank osmolarity lowered by the addition of fresh water, ranged from 320 to 630 with an average of 459 Mosmol L^{-1}: The pH of the tank was checked twice daily and Tris buffer added to keep this value at about 7.6. The fish appeared vigorous throughout the period of observation (hypoosmolarity). Control data are presented from 9 fish treated similarly in undiluted seawater. Both control and diluted animals received 200 mgs inulin as a 10% solution intravenously 24 hours prior to study.

Since these observations were incidental to micropuncture, data were not collected in the free swimming state. The animals, anaesthetized with nembutal and curare, were placed ventral side up on a V-board, the gills being perfused with seawater (control group) or from the tank (diluted group). Urine for clearance periods was collected via a polyethylene catheter in the urinary papilla. A single blood sample was drawn from the dorsal aorta at the conclusion of the urine collection periods.

Urine and plasma were analyzed for inulin (resorcinol-thiourea method), urea (modified Berthelot reaction) and total osmolarity (Adv. Instr. Osmometer). Sodium and potassium concentrations in these samples were read in a Baird KY2 flame photometer.

Mean plasma and urine concentrations and the derived parameters of renal function for control (seawater) and experimental (diluted seawater) groups are set out in the table.

The normal difference in osmolar concentration between dogfish plasma and the sea (about 50 mosmol/L) was increased to an average of 300 mosmol/L. In response, urine osmolality, normally about 150 mosmols/L below plasma (U/P osm 0.85), fell to an average of 360 mosmols/L below existing plasma osmolality in the diluted group (U/P osm 0.53).

Reduction in concentration of the measured plasma constituents (Table 1) may be accounted for in part by the entry of free water down its activity gradient into the fish but it is apparent from the table that excretion of these substances is markedly augmented.

The increase in urine flow (1 ml/hr x kg) in the hypoosmotic group was effected by an increment in GFR (0.6 ml/hr x kg) as well as by reduction in tubular reabsorptive rate (0.4 ml/hr x kg). At the time of measurement urea excretion averaged over 400% above control values and plasma urea concentration had fallen proportionately to the fall in plasma osmolality ($\Delta[P_{osm}] = \Delta[P_u] = -21\%$. Sodium excretion averaged 57% above control and plasma conc. of Na with its at-