temperature. The rate of rise of the upstroke of the action potential was about 10 V/sec. The action potential was found to be highly sensitive to the presence of sucrose in the central compartment. In fact the preparation showed deterioration signs such as shortening of action potential duration and appearance of graded contractile response within 30 minutes of exposure to the sucrose solution.

Prolongation of the action potential with the voltage clamp prolonged the contraction. The developed tension was maintained for the duration of depolarization. Tension developed at membrane potentials around –40 mV and the voltage tension relationship plateaued around +40 to +60 mV. No post-extrasystolic or post-clamp potentiation was observed. Such a direct relation between the membrane depolarization and development of tension suggests that movements of activator Ca^{+2} are under direct control of the surface membrane. Although in some preparations a phasic component of tension was recorded it could be shown that in these preparations the clamped potential was not spatially homogenous.

These results suggest that the contractile response of the dogfish myocardium is under direct control of surface membrane potential and that release and transport of activator Ca^{+2} occurs across the surface membrane. Although no structural results are as yet available specimens were embedded in collaboration with Dr. Karl Karnaky for electron microscopic examination. Based on the voltage clamp studies we predict that the dogfish myocardium will have no t-tubules and will show limited quantity of sarcoplasmic reticulum.

The voltage clamp experiments also revealed the existence of a slow inward phasic current which seemed to be responsible for the upstroke of the action potential. This current as well as the upstroke of the action potential were found to be insensitive to high concentrations of tetrodotoxin (10^{-5}M). These observations suggest the possibility that the inward current in this preparation may be carried by Ca^{+2}. If this finding is substantiated by future experiments it may reveal a mechanism for the transport of activator calcium across the cell membrane.

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1973 #34

THE CEREBRAL VENTRICULAR SYSTEM OF *Myxine glutinosa*

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Early anatomical reports suggested that the hagfish *Myxine glutinosa* differs from other vertebrates in lacking a well-developed ventricular system and a choroid plexus. As part of an effort to relate the structure of the hagfish brain to the physiology of the cerebrospinal fluid we have examined the ventricular system in a series of adult hagfish.

Most animals were fixed by vascular perfusion with phosphate buffered glutarldehyde-paraformaldehyde fixative for electron microscopy or with 10 percent formalin or Bouin's solution for light microscopy. Serial sections were made from brains processed for light microscopy.

The ventricular system consists of a much-reduced central canal extending from the spinal cord to the midbrain and two diencephalic cavities, the preoptic and the infundibular recesses (Figure 1).

The diencephalic cavities appear to be completely isolated from each other and from the rest of the ventricular system. The preoptic recess is a small cavity lined by typical columnar ependymal cells (Figure 2). A densely-packed group of small neurons, the preoptic nucleus, lies immediately adjacent to the preoptic recess throughout its length. The infundibular recess is closely associated on its ventral surface with the hypophysis (Figure 3). It is also lined with columnar ependymal cells.

The primary ventricular system exists as a narrow canal which gives off several irregular diverticula in the medulla and midbrain. These diverticula extend dorsally toward the surface of the brain and in some regions are quite extensive (Figures 4-6). Most regions of the ventricular system are lined with columnar ependymal cells which are similar to typical ependymal cells lining ventricles in higher vertebrates. They are ciliated cells joined near their apical surfaces by desmosomes (Figure 7) and having nuclei surrounded by filamentous rings. The diverticula however tend to be lined by cuboidal or more often by much attenuated epithelial cells. In the isthmic region of the brain the dorsal diverticula become quite extensive imparting a spongy, disorganized appearance to the roof of the brain (Figure 6). This region has been considered by Jansen (J. Comp. Neurol. 49, 1930) to represent a rudimentary choroid plexus but the observed lack of capillaries or secretory epithelium in this region argues against this interpretation.

The hagfish ventricular system thus appears to be unique in the degree of reduction in size, in the isolation of the preoptic and infundibular recesses, and in the apparent absence of a vascular choroid plexus. The retention of the diencephalic cavities in regions associated with neurosecretory nuclei, despite the reduction of the ventricular system as a whole, suggests that these cavities and the fluids which they contain must be of considerable physiological significance perhaps relating to neuroendocrine functions. Moreover the fluid contained in these isolated cavities must be derived from cerebral sources rather than from cerebrospinal fluid since the cavities are not continuous. The apparent absence of a choroid plexus further suggests that the entire ventricular fluid may be produced by the brain.

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Legends for figures on page 88.

Legends for Figures on page 86.

Figure 1. Section of hagfish brain showing capillary and adjacent neuron (N). Note wide lumen (L) of capillary, thick endothelial cytoplasm, and prominently stained basement membrane (arrow). One micron plastic embedded section, toluidine blue. 1000X

Figure 2. Cytoplasm of two adjacent endothelial cells (E). Note vesicles (v) and tubules (t), some of which appear to coalesce with the lumenal plasma membrane (bent arrow) and others which appear to communicate with the space between adjacent endothelial cell plasma membranes (straight arrows). Note also the area of apparent fusion between the two endothelial cells (star). 88,000X.

Figure 3. Endothelial cytoplasm with many vesicles. Note prominent basement membrane (arrow). 20,000X

Figure 4. Complexly interdigitated junction between two adjacent endothelial cells (star). 56,000X

Figure 5. Tubules in endothelial cytoplasm (arrows) communicating with basement membrane (BM). Note trilaminate structure of basement membrane. 88,000X.

Figure 6. Spoke like processes of basement membrane (arrow) extending into brain tissue. Note small dense granules (g), presumably glycogen. 34,000X

Figure 7. Clefts between adjacent glial cells contacting ablumenal surface of basement membrane. 88,000X

Figure 8. Endothelial cells process extending into lumen which contains glycogen granules similar to those found in ablumenal tissue. 88,000X

1973 #36

FURTHER STUDIES ON THE EFFECTS OF DIURETIC DRUGS ON RENAL FUNCTION IN *Squalus acanthias*.

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In 1971 data were presented which established that furosemide and ethacrynic acid in doses of 40 mg and 50 mg per dogfish respectively produced a striking diuresis with marked augmentation of sodium, urea, and osmolar outputs. Further experiments have been conducted utilizing the same general procedures to determine the effects of smaller doses of the diuretics with particular emphasis on furosemide. Dose-response curves have been derived.

Control observations, using the same experimental protocol but without drug, have been expanded. In the control studies dogfish Ringers solution, 4 ml intravascularly, were injected instead of diuretic drug and the usual sequence of clearance periods was followed.

The control data are presented in Table 1. Urea excretion does increase with time to a moderate degree but is associated with no significant change in plasma urea concentration. The calculated urea clearance is moderately increased. In the face of a slight fall in inulin clearance and in filtered loads of urea and sodium, the ratios $U_{urea}V/F_{urea}$ and $U_{Na}V/F_{Na}$ are increased. Otherwise all measured parameters are steady.

Table 2 provides the data for the various doses of furosemide. A striking diuresis, natruresis, and increase in urea excretion occurred with all doses from 0.1 mg through 40 mg but diminished effect was noted on a total dose below 1 mg for water and sodium and below 2 mg for urea. Plotting