

surface (mucous coat and serosal connective tissue). The flux ratio peaks around 60 min, and declines toward unity with time, indicating a change in relative urea permeability with time of exposure to zero-urea solutions.

In a further series, steady-state urea flux was determined by maintaining the urea flow from a standard serosal solution into urea-free mucosal solution. The results are shown in Figure 2. Following the initial fall

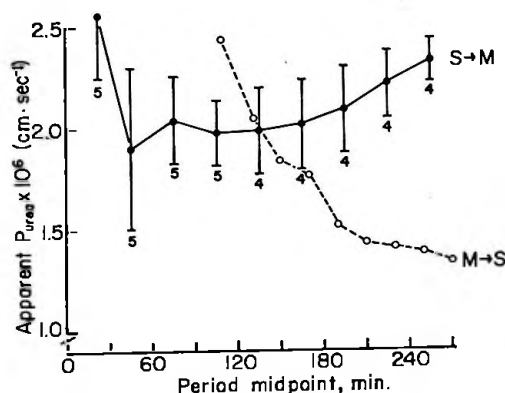


Figure 2

(presumably due to removal of trapped urea) the permeability appears to rise steadily, although the increase is not statistically significant. The values obtained are not far from those reported by Hogben in urea-free solutions. However, the reverse experiment, performed in only one tissue, shows a rapid drop in  $P_{urea}$  to rather low values.

One would like to believe that the osmotic properties of the solution bathing the mucosal face of the gastric mucosa were not reflected in cellular osmolarity or changes in membrane properties. The data here presented suggest that this may not be the case, but that the urea permeabilities of both membranes can be altered by gross changes in the urea content of either solution. This question can best be answered by studies with  $^{14}\text{C}$ -urea, so that fluxes can be measured into urea-containing solutions.

In light of the permeability data, one cannot be sure that the decrease in  $J_H$  seen in Figure 1 represents a thermodynamic loading of the pump by a large reverse  $\text{H}^+$  gradient. If it does, the dogfish gastric mucosa is capable of producing a primary secretion of 1475 mOsm, which if it consists entirely of  $\text{HCl}$  and  $\text{H}_2\text{O}$ , should have a pH of 0.13 and produce a gradient of more than  $10^7$ -fold with respect to cell cytoplasm at pH 7.4. Supported in part by the National Science Foundation Grant PCM77-03336.

#### THE MDIBL SEAWATER SYSTEM: SOME CHARACTERISTICS AND DATA

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The seawater system consists of pumps which draw water from Eastern Bay, pipes and valves (largely plastic) which distribute the water to laboratories and aquaria, and drains which return the water to the bay. There are three similar pumps and intake lines, two different but interconnectable distribution systems, and many separate drains.

The intake lines are approximately 120 feet long and 3 inch I.D. Both the continuous and intermittent pumps deliver through 3" lines to a central distribution point at the sea water storage tank which is about 150 feet from the pumphouse and 20 feet above the pumps. At this point the systems can be interconnected to supply all lines from a

single pump. From the central distribution point 2 inch or 3 inch lines run to the various laboratories and aquarium areas. Some outlets to aquaria are supplied by gravity from a storage tank, which is replenished by the intermittent operation of one pump, and is therefore termed the "intermittent" system. Other outlets are driven directly by a continuously-running pump, and constitute the "continuous" system.

The flow of seawater maintains both the temperature and oxygen content in the aquaria, which depends on the flow, temperature and other characteristics of the system. Some data on these characteristics are here reported.

### HEIGHTS

Both the pump requirements and the gravity flow from the tank depend on the relative heights in the system. These heights have been measured by rod and level, and are given in Table 1. It is clear that Marshall and Karnofsky laboratories are too high relative to the tank to be supplied from the intermittent (gravity) system with reasonable pressure.

Table 1.--Heights in the Seawater Distribution System

Location	Ft. above Mean Low Water	Ft. above tank bottom
Mean Low Water	0	-39.6
Mean High Water	10.6	-29.0
Pump level	15.5	-24.1
Top, cement legs under water tower	34.5	- 5.1
Bottom of water tank	39.6	0
Float switch on*	43.4	+ 3.8
Aquaria at		
Hegner	37.3	- 2.3
Union Sta.	36.3	- 3.3
Outside tanks nr. Inst. Shed	33.3	- 6.3
Kidney Shed	32.3	- 7.3
Neal	30.3	- 9.3
Halsey	29.3	-10.3
Gull Shed**	35.3	- 4.3
Marshall B'ment**	37.8	- 1.8
Marshall Deck**	43.3	+ 3.7
Karnofsky**	44.3	+ 4.7

\*Float switch which turns on intermittent pump, as currently adjusted

\*\*Currently on continuous pump system

### TANK DATA

The wooden storage tank is 7.75 ft in diameter and 7 ft deep, and is filled on each cycle to a depth of 6.6 ft, thus holding 2330 gal (8820 l.). When 990 gal (3748 l.) have been used, the float switch is activated and the tank is refilled; at this time 1340 gal (5072 l.) remains as a reserve. The water in the tank when full, weighs 19,445 lbs (8820 Kg).

### PUMPS

Three similar centrifugal pumps, each with a 5 HP electric motor, are mounted in the pump house. Each has an intake hose, strainer and foot valve, with water being drawn from about 6 ft below extreme low water level. One

pump is a spare which can be connected to either system, while one runs continuously and one is controlled by the float switch in the intermittent system tank. The spare pump has a 3-phase motor, and thus cannot be used with the float switch at present. When necessary, it can feed water to the storage tank, with the excess removed by an overflow slightly above the normal high limit of the tank.

Pump flow varies with the pressure across it, and thus depends not only on the difference in height between the pump and its output, and on the resistance of the lines, but on the varying vertical distance between the pump and the sea surface. For the intermittent pump, we have measured the output indirectly by determining the time ( $t_{off}$ ) required for normal usage to empty the 990 gal between the float switch trip points, which establishes the usage rate  $J_{use} = 990/t_{off}$  (gal/min). When running for time  $t_{on}$ , the pump must supply  $J_{use}$  plus fill the tank; this filling rate is  $J_{net} = 990/t_{on}$ . Therefore,  $J_{pump} = J_{net} + J_{use}$ , or  $J_{pump} = 990 (1/t_{on} + 1/t_{off})$ . This method was used to establish the pump rates reported in Figure 1, with sea level data obtained from the tide tables for Bar Harbor (difference to

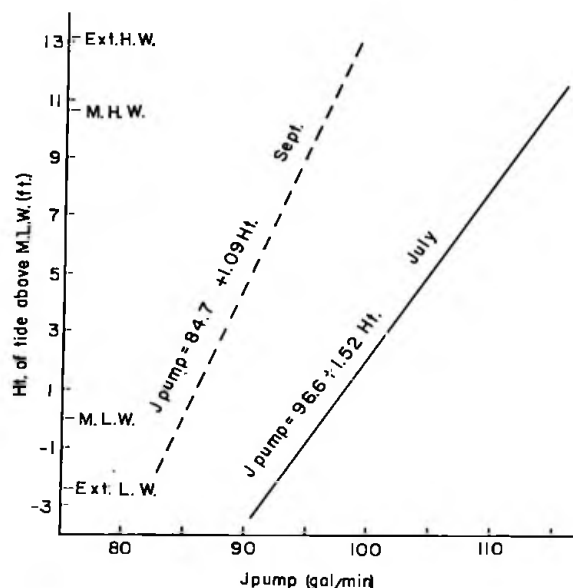


Figure 1.--Output ( $J_{pump}$ ) in gallons per minute of the intermittent pump as a function of height of tide (Ht.) in July (solid line) and in September (dashed line) as determined by the method described. In July, 1980 the delivery to the seawater tank at half-tide (5.3 ft.) was 105 gallons per minute. In September it was 90 gallons per minute.

Extreme spring high tide	= Ext. H.W.	= 13.1 ft
Extreme spring low tide	= Ext. L.W.	= 2.4 ft
Mean high tide	= M.H.W.	= 10.6 ft
Mean low tide	= M.L.W.	= 0

Salsbury Cove negligible) and a sine wave approximation for points between low and high tides. Some of the later data were obtained from a tide gauge mounted on the dock pier. The average time to use 1000 gallons was about 20 minutes. The operating lift (suction) from tide level to pump intake is  $9.2 \pm 5.3$  ft (average tides). The operating pressure/head from pump to tank is  $29.3 \pm 1.4$  ft. The total lift (tide level) to tank is  $38.5 \pm 6.7$  ft (average tide). The differential from an average low tide to a full tank is 45.2 ft.

Two sets of data are presented, taken in mid-July when the system was presumably clear, and in mid-September,

when growth of mussels had restricted some pipe diameters. The graph for July is based on 19 determinations at tides from -1.5 ft to +11.2 ft. The September graph is based on 6 determinations ranging from +2 to +10 ft. The difference between these sets is clear, amounting to about 10 gal/min at low tide. Therefore, the flow should be taken as about 80 gal/min for design purposes. Presumably the continuous pump shows similar characteristics, and the total system is therefore capable of 160 gal/min under worst-case conditions.

The pumps and their suction lines must be primed before they will function properly. At present, there is no simple way to achieve this, and restarting a pump which has lost prime is nearly impossible at low tide. For this reason, the spare pump cannot be considered as ready for immediate service. Both systems can be interconnected to be supplied by a single pump in which case some restriction of usage may be required.

#### AQUARIA

Aquarium racks situated outside laboratory rooms can accommodate 95 small (1 cu. ft.) aquaria with water supplied via 1/8" pet cocks. Some 4.5 cu. ft. rectangular tubs will also fit these racks. In addition to live cars at the dock, circular tanks are available 3' nominal diameter (6 cu. ft.), 4' (19 cu. ft.), 6' (55 cu. ft.) and 8' (121 cu. ft.). These are supplied with 3/4" valves.

The survey made July 31, 1980 (Table 2) showed 59 small aquaria and 39 larger ones in use with a capacity of

Table 2.--Aquaria in Use July 31, 1980

Nominal Size	Approx. Volume cu. ft.	No. in Use
Small	1	59
Tubs	4.8	19
3' diam	6	6
4' diam	19	10
6' diam	55	2
8' diam	121	1
6' x 10'	75	1

664 cu. ft. (approx. 5000 gallons) being supplied by 90 gals/minute.

#### SEAWATER USAGE

The flow at any point depends on the valve settings at that point and at other competing points. These were measured as adjusted on 7/21/80 by timing the filling of a 1 liter container where this could be done without interruption of experiments in progress, and estimated for other outlets. Table 3 shows the usage by laboratory. It should be emphasized that these are values as found, and the actual usage will vary, probably widely, with investigators' requirements, real or perceived. On this date, about 60% of the available capacity was being utilized.

#### TEMPERATURES

The temperature of the water supplied to each aquarium will depend on the pump inlet temperature as modified by frictional and atmospheric heating. An extensive series of temperature measurements was made between 6/18 and 8/20. Taking the temperature in the Gull Shed as representative of the continuous system, the data in Table 4 were obtained.

Table 3.--Saltwater Usage by Building July 21, 1980

Location	Flow gal/min
Continuous syst.	
Gull Shed	25.4
Marshall deck	trace
Marshall basement	20
Karnofsky	trace
Total	45.4
Tank system	
Neal	26.4
Halsey	2.6
Hegner	5.8
Union Station	3.0
Kidney Shed	trace
Outside tanks	9.4
Total	47.2
Overall total	92.6
Max. pump capacity	160.0
% utilization	58

Table 4.--Seawater Temperatures in Gull Shed\*

Month	Time of day**		Height of tide***	
	AM	PM	High	Low
June	12.42 $\pm$ 0.51 N = 6	12.15 $\pm$ 0.18 N = 4	11.73 $\pm$ 0.53 N = 3	12.56 $\pm$ 0.35 N = 7
July	13.41 $\pm$ 0.77 N = 8	12.78 $\pm$ 0.47 N = 11	12.57 $\pm$ 0.47 N = 13	14.08 $\pm$ 0.70 N = 6
August	14.01 $\pm$ 0.71 N = 4	14.23 $\pm$ 0.51 N = 9	13.51 $\pm$ 0.52 N = 7	14.97 $\pm$ 0.45 N = 6

\*Mean  $\pm$  SE. In no case is the difference within any month significant by Student's t-test; the increase in temperature from June to August is significant. There is a tendency for low tide temperatures (with intakes closer to surface) to be higher, while no such trend is observed as a function of time of day. One can conclude that the temperature at the outlet is probably a function of intake temperature, and that little warming occurs in distribution.

\*\*AM = 0930 - 1300 EDST; PM = 1300 - 1700 EDST, to approximate solar time.

\*\*\*High =  $\pm$  3 hours of tabulated high tide; Low = otherwise.

A significant temperature increase is found as summer progresses, as expected. We were unable to account for the day to day variations by grouping values according to time of day or time of tide, although there is a consistent tendency for water drawn at low tide when the intakes are closer to the surface to be warmer than that from high tide. The temperature recorded in the Gull Shed and in the storage tank could be different by as much as 0.8°C, but the direction was not consistent, and the average difference ( $-0.045 \pm 0.116^\circ\text{C}$ ,  $N = 31$ ) is clearly not significant. Temperatures at other outlets likewise showed no obvious pattern of variation.

#### A COMPARATIVE STUDY OF ELASMOBRANCH CORNEAL AND SCLERAL COLLAGENS

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Vertebrate corneas are transparent, non-vascularized tissues of the eye, composed of three cell-types separated from one another by two basement membranes and an orthogonal gridwork of collagen fibrils. For most animals, if the cornea is transferred to an isotonic salt solution in vitro, it rapidly absorbs water, swells, and loses its transparency. However, the cornea of elasmobranchs and the primary spectacles of cyclostomes can be placed in isotonic saline, or even distilled water, without swelling or losing their transparency. Resistance to swelling by elasmobranch corneas and cyclostome spectacles is correlated with the presence of fibers (sutural fibers) which traverse the stroma perpendicularly to the collagenous plies and appear to connect its anterior and posterior faces. It is not known which corneal cells synthesize the sutural fibers, nor whether the fibers contain collagen. In the present study we have used collagen type-specific antibodies, together with indirect immunofluorescence, to localize the types of collagen within and surrounding the shark cornea and have used peptide fingerprint analysis to characterize the predominant collagen  $\alpha$ - and  $\beta$ -chains in shark cornea and cartilage.

Corneas, dissected from young and adult spiny dogfish sharks (*Squalus acanthias*), were prepared for transmission electron microscopy and immunofluorescence. In the latter case, tissues were fixed in formaldehyde solutions, sectioned with a cryostat, incubated with antibodies specific for collagen types I and II, and examined by indirect immunofluorescence. Collagen  $\alpha$ - and  $\beta$ -chains were separated by sodium dodecylsulfate-slab gel electrophoresis and characterized by two-dimensional mapping of  $^{125}\text{I}$ -labeled peptides generated by tryptic and chymotryptic digestion.

The corneal stroma, the sutural fibers which span the stroma, and the surrounding limbus were positive for type I collagen, as judged by immunofluorescence. The corneal stroma was negative for type II collagen. Scleral cartilage matrix was intensely positive for type II collagen, but was negative for type I. In confirmation of these results, slab gel electrophoresis revealed  $\alpha 1$ -, and  $\alpha 2$ -like bands from shark corneal stroma, but only an  $\alpha 1$ -like band from shark cartilage collagen. Two-dimensional peptide mapping revealed some degree of resemblance between the  $\alpha 1$  band of shark corneal stroma and the  $\alpha 1$  band of chick type I collagen. Likewise, the  $\alpha 1$  band of shark cartilage collagen somewhat resembled the  $\alpha 1$  band of chick type II collagen. The  $\alpha 2$ -like band of shark corneal stroma did not closely resemble the  $\alpha 2$  band of chick type I collagen. The most prominent  $\beta$  band of shark corneal stroma appeared to be a dimer composed of one  $\alpha 1$  chain and one  $\alpha 2$  chain. The collagen of shark corneal stroma was very susceptible to degradation by pepsin, whereas that from shark cartilage was much less susceptible. These results are presented in detail elsewhere (Exptl. Eye Res., (in press)). Supported in part by NIH EY 00952.