

The results of these studies are presented separately in this issue of the Bulletin (Forster, Hannafin, Shiffrin and Morad).

### Conclusions

Uptake of  $^{14}\text{C}$  taurine occurs *in vitro* against a strong concentration gradient when skate "hemi-atria" are incubated in a balanced isosmotic elasmobranch medium containing 0.1 mM taurine. The transport system is Na-dependent, and presumably carrier-mediated. The close structural analogue,  $\beta$ -alanine, reduces taurine uptake by 42% when present in 5-fold excess.  $\alpha$ -aminoisobutyric acid, and  $\gamma$ -aminobutyric acid (GABA) do not inhibit uptake. The system is energy-dependent and subject to  $\text{Na}^+ - \text{K}^+$  ATPase inhibition by ouabain. No evidence was found to indicate adrenergic stimulation of taurine transport, as has previously been shown in experiments on mammalian hearts. This work was supported by NIH Grant HL 04457-20.

Table 2

Taurine Uptake by Skate Atrium, Expressed as Percent of Control, as Affected by Various Agents Selected to Characterize the Transport System

Treatment	Percent decrease in uptake of taurine		
Na dependence			
0 mM Na	-76.22 ± 3.71%	(4)**	
35 mM Na	-56.27 ± 10.3%	(8)**	
70 mM Na	-49.71 ± 7.76%	(6)**	
140 mM Na	-30.35 ± 12.48%	(6)**	
Competitive inhibition			
$\beta$ -Alanine ( $10^{-5}\text{M}$ )	-42.40 ± 6.50%	(11)*	
$\gamma$ -Aminobutyric acid ( $10^{-5}\text{M}$ )	n.s.	(9)	
$\alpha$ -Aminoisobutyric acid ( $10^{-5}\text{M}$ )	n.s.	(7)	
Metabolic dependence			
Na azide ( $10^{-2}\text{M}$ )	-58.74 ± 1.27%	(3)*	
$\text{Na}^+ - \text{K}^+$ ATPase inhibition			
Ouabain ( $10^{-6}\text{M}$ )	-44.76 ± 2.89%	(4)*	
$\beta$ -adrenergic effect			
Isoproterenol ( $4 \times 10^{-7}\text{M}$ )	n.s.	(7)	
Dibutyl cyclic AMP ( $9.5 \times 10^{-6}\text{M}$ )	n.s.	(6)	

Value are means ± standard deviation. Number of fish per group is shown in parentheses. \*p < 0.001, \*\*p < 0.01, n.s. = not significantly different from control.

### EFFECTS OF UREA AND HIGH $\text{P}_{\text{O}_2}$ ON ACID SECRETION IN DOGFISH STOMACH

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The dogfish gastric mucosa, as mounted in an Ussing-type chamber and gassed with 95%  $\text{O}_2$  - 5%  $\text{CO}_2$ , is both hypoxic and hypocapnic, and increasing  $\text{P}_{\text{O}_2}$  to 1.9 atm and  $\text{P}_{\text{CO}_2}$  to 0.1 atm by a hyperbaric apparatus markedly increases secretory rate (Kidder, G. W., Bull. MDIBL 15:68, 1975). Since Hogben (Science 129:1224, 1959) has reported that in 95%  $\text{O}_2$  - 5%  $\text{CO}_2$  the inclusion of urea in the bathing solutions had no effect on the secretory parameters, urea was not used in the initial hyperbaric experiments. However, with the removal of  $\text{O}_2$ -limitation, it seemed desirable to reinvestigate this observation.

Accordingly, the gastric mucosa of freshly-killed dogfish was dissected free of the heavy muscle coat and mounted in a plexiglass chamber (3.14  $\text{cm}^2$  area). The serosal surface was bathed with the solution used by Hogben, containing (mM)  $\text{Na}^+$ , 239;  $\text{K}^+$ , 10;  $\text{Ca}^{++}$ , 5;  $\text{Mg}^{++}$ , 2;  $\text{Cl}^-$ , 244;  $\text{HCO}_3^-$ , 18; phosphate, 0.6 and glucose, 25. The mucosal solution was similar, but without glucose, phosphate or  $\text{HCO}_3^-$ , the latter two being replaced by NaCl. Urea-containing solutions were prepared by adding 350 mM urea to each of the above. All tissues were stimulated by carbachol at 0.25 mM on the serosal surface.

Figure 1 shows the experimental results for one tissue. This tissue was mounted in urea-containing solutions and allowed to equilibrate for 1.5 hours at  $\text{P}_{\text{O}_2} = 1.9$  atm. The pressure was then released, the solutions changed to their urea-free equivalents, and the pressure reestablished, a process which takes about 10 minutes. After 1.5 hours in this condition, the tissue was returned to urea-containing solutions for 1.5 hours. In this particular experiment (but not generally) an additional 1.5 hours in urea was allowed. It seems clear that the secretory rate has decreased upon removal of the urea, and increases upon readdition of urea, although not to its initial level. In this experiment it would have been desirable to allow longer than 1.5 hours for initial equilibration; failure to do so tends to bias the results against finding any effect of urea.

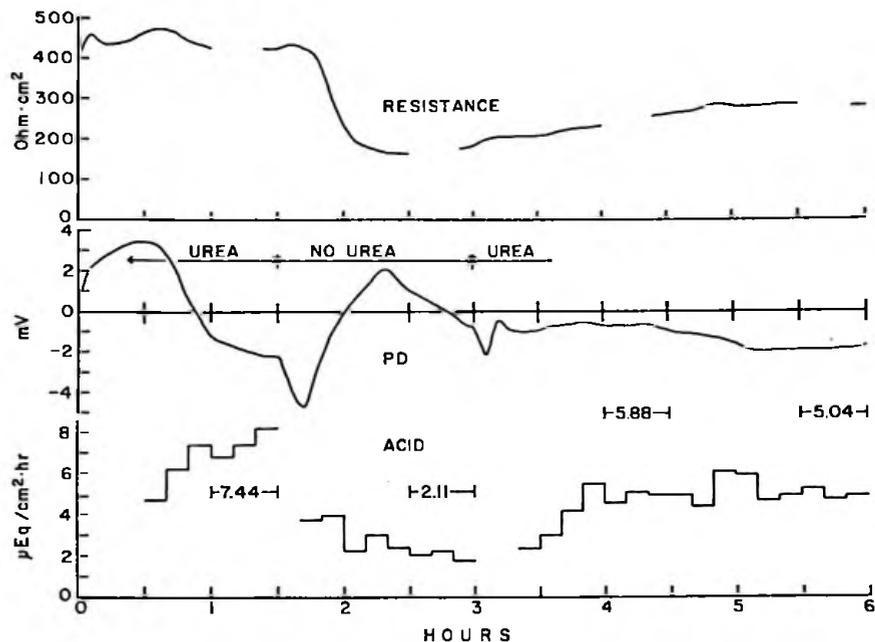


Figure 1. An experiment in which the effect of addition of 350 mM urea to both bathing solutions was tested. Secretory rate measured by pH-stat at pH 4.5; PD by KCl/calomel electrodes in the fluid streams; and resistance by  $\Delta V/\Delta I$  for 100  $\mu A$  (per 3.14  $cm^2$ ) pulse. Tissue maintained in hyperbaric conditions ( $P_{O_2} = 1.9$  atm,  $P_{CO_2} = 0.1$  atm) except lowered to 1 atm while solutions changed.

In a series of 6 tissues, the secretory rate in each condition was taken as the average of the last 30 minutes in that condition, and recorded as Table I. There is no significant difference between the means of the two groups as determined by the t-test. To remove some of the large variability between tissues, the rates in urea were expressed as percentages of the corresponding rate

TABLE I

Effect of 350 mM urea on the acid secretory rate of dogfish gastric mucosae in sufficient oxygen.

Date	UREA		NO UREA	
	$J_H^*$	$Z^{**}$	$J_H$	$Z$
8/15/78	4.93	151	3.26	100
8/16/78	12.94	130	9.95	100
8/17/78	7.98	150	5.30	100
8/18/78	4.51	95	4.76	100
8/21/78	11.98	151	7.91	100
8/24/78	7.44	353	2.11	100
Avg.	7.11	172	5.55	100
SEM	$\pm 1.69$	$\pm 37$	$\pm 1.19$	-

\* Secretory rate,  $\mu Eq/cm^2 \cdot hr$

\*\* Percent of rate without urea

TABLE II

Effect of hyperbaric  $O_2$  on acid secretory rate ( $\mu Eq/cm^2 \cdot hr$ )

Date	Condition, atm $O_2$		
	0.9	1.9	2.9
8/28/78	3.28	7.11	6.23
8/29/78	0.74	6.83	7.04
8/30/78	0.00	6.53	4.09
8/31/78	1.78	6.79	7.18
9/1/78	-	6.28	6.09
Avg.	1.45	6.71	6.13
SEM	$\pm 0.82$	$\pm 0.16$	$\pm 0.62$

without urea. Unfortunately, again there is no significant difference ( $0.1 < P < 0.2$ ). However, the exclusion of the single highest value (353%) results in an average of  $135 \pm 11\%$ , which is different

from 100% at the 1% level of confidence. It would appear that the inclusion of urea usually has a small stimulatory effect on acid secretion with elevated  $P_{O_2}$ , and occasionally has a marked effect. Since urea is a normal constituent of elasmobranch plasma, its inclusion would appear justified even in the absence of entirely satisfactory statistics as to its effect, and it is included in the experiments to follow.

The apparatus used for the hyperbaric experiments did not previously allow pressures above 2 atm; thus, it was not known whether a  $P_{O_2}$  of 1.9 atm was sufficient to remove  $O_2$ -limitation, or whether high levels of  $O_2$  might be toxic. With a modified apparatus, a series of tissues mounted in urea-containing solutions was tested at  $P_{O_2}$  of 0.9 and 2.9 atm (at constant  $P_{CO_2} = 0.1$  atm) with the results shown in Table 2. Each condition was maintained for 1.5 hours, and the order of the conditions was random. The difference in acid secretory rate between 0.9 and 1.9 atm is significant, as expected from previous work, while the small difference between 1.9 and 2.9 atm is not significant ( $0.3 < P < 0.4$ ), and does not become so when a paired comparison is used. On this basis I conclude that the relief of  $O_2$ -limitation of acid secretion is complete at  $P_{O_2}$  of 1.9 atm or below, and that no toxic effects of  $O_2$  exist at this level. Supported by NSF PCM 77-03336.

#### THE CURRENT-VOLTAGE PLOT IN DOGFISH GASTRIC MUCOSA

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Transepithelial resistance is conventionally measured by passing a brief current pulse across the tissue, recording the resulting voltage transient. For proper choice of pulse length, the resistance is unchanged by minor variations in length, and seems to represent a composite of membrane resistances and pericellular shunt contributions. If none of these resistances were functions of current or voltage, a plot of voltage vs. current would be a straight line passing through the open circuit potential and having a slope ( $\Delta V/\Delta I$ ) equal to the tissue resistance. In a number of tissues with high pericellular shunt resistances, such I-V plots are multilinear; i.e., they consist of several straight line (constant resistance) segments which intersect at well-defined breakpoints, with short transition regions connecting the straight segments. Such is the case for the frog gastric mucosa (unpublished observations). I shall now report similar observations on the stomach of the dogfish, *Squalus acanthias*.

The gastric mucosa, stripped of its heavy muscle layers, was mounted as a flat sheet (3.14 cm<sup>2</sup> area) in a plexiglass chamber. Mucosal and serosal solutions were those of Hogben (Science 129:1224, 1959) with the addition of 350 mM urea to each (see previous paper). Voltage was recorded via KCl/calomel fiber junction "reference electrodes" inserted into the fluid streams, and displayed on a strip chart recorder (Bio-Rad 1310) with 99% response time of 0.35 seconds for half-scale deflection. Current was supplied by a constant current generator (ELS CS-1, courtesy of Dr. S. I. Helman) via Ag/Ag Cl electrodes remote from the tissue. Current pulses of 0.8 second duration were sent every 30 seconds, reversing the polarity between pulses, and increasing the current intensity stepwise after each pair of pulses. With slow chart speeds used, the voltage response appears as a "spike," and the recorded value is the maximum obtained within the limits of the recorder response time. Reported resistances are corrected for a chamber resistance of 10 ohms (31.4 ohm·cm<sup>2</sup>).

Like the frog gastric mucosa, that of the dogfish shows a multilinear I-V relationship of which Figure 1 is a striking example. The observed points clearly do not fall on a single straight line, nor on any reasonable higher-order curve, but are resolved into 3 straight line segments, with breakpoints at -22 mV (reference mucosal solution = 0) and +50 mV. There is a large difference in resistance across the mucosal-positive breakpoint and a smaller difference across the serosal-positive breakpoint;