escaped from trawl nets near the surface. To our knowledge, however, this is the first record of porpoises behaving similarly. Records of the depths to which porpoises descend are rare. Since porpoises presumably retain some air in their lungs when submerged, echoes of the same sort observed from fishes with sizable air bladders may be expected, in addition to echoes from the large surface area presented by a porpoise. Further observations with echo-sounders may add considerably to our knowledge of the depths to which these interesting mammals descend.

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14 March 1960

Osmotic Pressure and Aqueous Humor Formation in Dogfish

Abstract. Comparative chemical analyses show the aqueous humor of smooth dogfish has lower concentrations of urea and trimethylamine oxide than the blood plasma. Freezing-point depression determinations demonstrate that the aqueous humor has a correspondingly lower osmotic pressure. It would appear that water entering the aqueous humor from the plasma is moving against an osmotic gradient.

Current theories on aqueous humor formation in higher vertebrates generally contend that some ion (for example, sodium) is pumped across the ciliary epithelium from the plasma, and that water then follows by osmosis (*1*). Carbonic anhydrase appears to play an important role in this process, and its action has been compared to other water balance systems, such as that in the kidney (*2*). Aqueous humor usually has lower concentrations than plasma for those constituents which cross the ciliary epithelium more slowly than water—namely, urea, glucose, amino acids, and protein. Such substances do not attain a diffusion equilibrium since the rapid drainage of the fluid is nonspecific and allows all the constituents to be removed at the same bulk rate. Consequently, a steady-state distribution ratio (aqueous concentration: plasma concentration) is reached which is less than unity and is characteristic of the particular substance. Conversely, constituents which are pumped or secreted into the aqueous humor have distribution ratios greater than 1 (*3*).

As is well known, elasmobranch fishes retain huge quantities of urea and trimethylamine oxide in their plasma, thereby elevating the osmotic pressure of the plasma above that of sea water (*4*). The study reported here was undertaken to determine the distribution ratios of these substances, since a ratio of less than 1 would result in a potentially large osmotic pressure difference between the blood plasma and the aqueous humor. For this reason, it was thought that the operative mechanisms in the elaboration of aqueous humor might be exaggerated in these fishes to compensate for the lesser osmotic contribution of urea and trimethylamine oxide in their aqueous humor.

Table 1 gives the results of chemical and physical analyses on the aqueous humor and plasma of 90 smooth dogfish (*5*). Urea and trimethylamine oxide (TMAO) reach steady-state distribution ratios of 0.93 and 0.88, respectively, the combined deficit (plasma concentration-aqueous concentration) being approximately 34 mmole/kg of water. Theoretically this could represent a difference in osmotic pressure of about 580 mm-Hg. The only component of dogfish aqueous humor in great enough excess to be osmotically important is bicarbonate ion, whose distribution ratio averaged 2.5, representing a surplus of about 9 mmole/kg of water. The higher concentration of bicarbonate ion in the aqueous humor is nullified, however, by a lower chloride ion concentration, and it would appear that an exchange of these two anions occurs, as it apparently does in other carbonic anhydrase mediated systems. The *p*H is consistently high. Sodium ion attains a distribution ratio concordant with an estimated Donnan equilibrium. Analyses on other constituents failed to demonstrate any substance or substances in great enough surplus to balance the urea-trimethylamine oxide deficit, even though more than 98 percent of the respective dry weights were accounted for.

Table 1. Comparison of properties of aqueous humor and blood plasma in *Mustelus canis*.

Constituent	Fish (No.)	Concentrations			
		Av. (mg/100 ml)		Av. mmole/kg of H_2O*	
		Aqueous	Plasma	Aqueous	Plasma
Urea	11	1835	1898	320	342
TMAO	12	754	832	85	97
Sodium	12	621	613	279	288
Chloride	9	867	886	256	270
Bicarbonate	23			15	6
Potassium	4	27	31	7	8
Magnesium	4	7	7	3	3
Calcium	3	12	18	3	5
Phosphate	3	12	19	1	2
Sulfate	2	37	27	4	3
Glucose	8	120	210	7	13
Protein	6	22	2850		
Amino acids	8	15	56		
Ascorbic acid	9	2.4	.8		
Total		4331	7438	980	1037
Osmotic pressure (mm-Hg)	14			935	962
Average dry weight (mg)	4	4470	7510		
*p*H	8			7.86	7.33

* To the nearest millimole. Not corrected for Donnan effect.

Freezing-point depression measurements made with a Fiske osmometer (sample size, 0.2 ml) showed that the osmotic pressure of the aqueous humor was about 25 milliosmoles lower than that of plasma, suggesting that the passage of water from the plasma into the eye of this fish is against an osmotic gradient. The smallest difference found in 14 fish tested was 14 milliosmoles. The measured osmotic pressures compared favorably with those calculated from the totaled results of chemical analyses when the effect of ionic strength on activities was considered.

It seemed important to find out how similar the aqueous formation in this species is to the process in higher vertebrates. It is significant that elasmobranchs are the only fishes which possess a ciliary body (*6*). The ciliary body contains carbonic anhydrase, as it also does in mammals, and the specific inhibitor of this enzyme, acetazoleamide (Diamox), can lower the distribution ratio of bicarbonate ion to less than 1 when it is injected intravenously in the dogfish (*7*). We regard the fact that most of the distribution ratios of substances measured were characteristic of higher vertebrates (namely, an ascorbic acid excess, very low protein, and so on) as circumstantial evidence that the aqueous humor formation in these fishes follows a pattern similar to that in mammals. In addition, penetration studies (plasma to aqueous) with Na^{24} showed that the turnover rate for this isotope is about 1 percent per minute, a value similar to values found for the rabbit (*3*).

The possibility that some sea water may be entering the eye across the cornea cannot be categorically rejected, since the aqueous humor is slightly hyperosmotic with respect to the sea water in which these fish were maintained. The composition of the fluid does not resemble sea water, however, and this would seem to indicate that any inward movement across the cornea would have to be essentially a movement of pure water and not of ions. For example, the concentration of magnesium ion is essentially the same in plasma and aqueous (3 mmole/kg), a value 20 times smaller than that for sea water (61 mmole/kg). A second possible explanation of these data could be based on the hypothesis that some constituent is reabsorbed from the aqueous humor, perhaps across the iris.

In the absence of evidence supporting either of the above two interpretations, the following tentative speculation is introduced: (i) in the dogfish, water is apparently moving from the plasma into the aqueous humor against an osmotic gradient; (ii) the additional work needed for this process is reflected in the unusually high bicarbonate ion concentration in dogfish aqueous humor; and (iii) carbonic anhydrase is instrumental in this process (*9*).

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5. The dogfish used in the analyses were *Mustelus canis*. Aqueous samples were taken from the pupillary region; blood samples, from the caudal vein.
6. G. Walls, "The Vertebrate Eye and Its Adaptive Radiations," *Bull Cranbrook Inst. Sci. No. 19* (1942).
7. T. H. Maren reported this effect in the spiny dogfish *(Squalus acanthias)* [*Federation Proc.* **17**, 391 (1958)]. We are indebted to him for his help in suggesting dosages and sampling times which enabled us to obtain similar results in our own fish. It should be noted, however, that Maren reports a somewhat lower distribution ratio (in untreated fish) for bicarbonate in the spiny dogfish.
8. The sea water in our tank had an osmolarity of 910 ± 5 milliosmoles.
9. This project was supported by the Office of Naval Research and by the Boston Lions Club Eye Research Fund. We wish to thank Alfred Marshak for suggesting certain aspects of this work, and Jin Kinoshita and Oliver Cope for the use of certain critical pieces of apparatus.

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23 February 1960

Control of Vocal Responding in Chickens

Abstract. Schedules of reinforcement were shown to control the rate of chirping by chickens in the same way as other motor responses in subhumans. Under a discrimination procedure, chickens responded selectively to the visual stimulus associated with food reinforcement for chirping. Control experiments demonstrated that food influenced the rate of responding because it was presented dependent on the chick's vocalizing and not because it had an innate eliciting or "emotionalizing" power.

The appropriate conditioning procedures for modifying subhuman vocalizing have, until recently, been little studied and poorly understood (*1*). It has not been clear whether respondent or operant conditioning techniques (*2*), or both, are suitable for the control of this behavior (*3*). The studies reported here (*4*) show that the rate of chirping by a hungry chicken can be controlled by schedules of presentation of food. The temporal patterns of vocal responding thus obtained are similar to those found with other operants under schedules of reinforcement, for example, key pecking in the pigeon and bar pressing in the rat, and a wide variety of human motor responses.

The subjects for these experiments were two Bantam chickens which were run individually at 80 percent of their ad libitum body weight (*5*) in sessions that lasted from 30 minutes to 6 hours. The experimental space consisted of an 8-in., cubical, sound-insulated compartment. An opening in one wall provided access to a food tray whose presentation was scheduled automatically. A microphone mounted in the ceiling of the compartment controlled a voice-operated relay which reacted to about 95 percent of the audible chirp responses by sending pulses to programming and recording equipment.

The rate of vocal responding was observed under two experimental and five control conditions. Figure 1 summarizes the data obtained from one bird; a second bird gave similar results. The first two studies were controls in which the rate of chirping was observed under conditions of no presentation of food ("operant level") and continuous presentation of food ("free-feeding") (*6*). The stabilized rate of responding under each of these two control conditions is shown in Fig. 1. Because chirping decelerated in the early stages of the operant-level session, it may be inferred that an average rate lower than 24 responses per minute would have been obtained for this condition if the session had been extended beyond its half-hour duration. The average rate during a 1-hour free-feeding session was 27 responses per minute. The chickens chirped both while pecking at the grain in the tray and while not eating.

In a third experiment the presentation of food was contingent on responding; a fixed-ratio 20 schedule of reinforcement was employed (*7*). Under this schedule, the food tray, containing meal, was presented for 4 seconds after every 20 chirps. An extremely high rate of responding was generated by this procedure; the average rate observed in a 1-hour sample after 10 hours of conditioning was 115 responses per minute. A typical sample of the performance obtained with the fixed-ratio 20 schedule is shown in Fig. 2, in which cumulative chirp responses are plotted as a function of time. The diagonal marks on the curve indicate the presentation of food. The interreinforcement time under this schedule averaged about 16 seconds.

In the fourth experiment, a control, food was presented with the same frequency and duration as obtained under

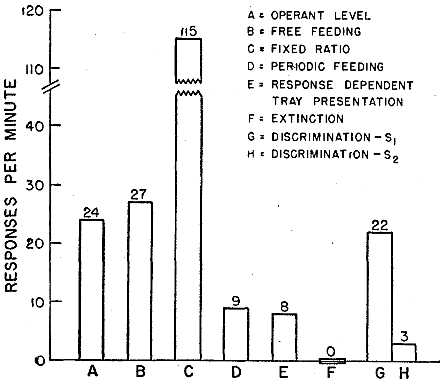

Fig. 1. Rates of chirping obtained under control and experimental conditions with one Bantam chicken.

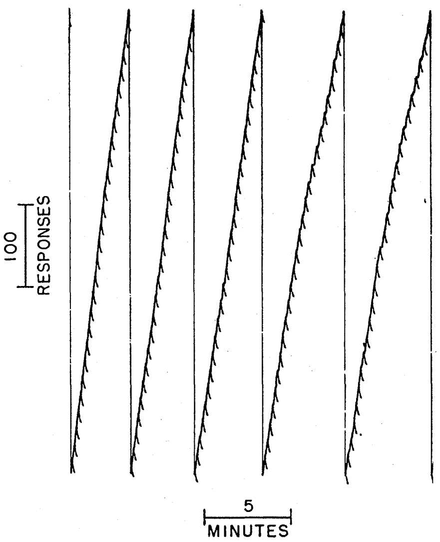

Fig. 2. Cumulative response curve for the chirp response of one chicken under a fixed-ratio 20 schedule of reinforcement.