the genito-urinary system of male skates ("sperm sac"), which E. K. Marshall had shown not to contain sperm or urine, but a "strongly alkaline solution apparently intented to protect the sperm from the acid urine."

The extraordinary composition of this liquid has gone unnoticed; it appears to have the highest HCO_3^- concentration of any biological fluid known. We have confirmed Smith's data for the "barndoor skate", R. stabuloforis; average of concentration (mM/L) of gland fluids of 2 fish were Na^+, 535; Cl^-, 339; HCO_3^-, 101; K^+, 9. In this species no carbonic anhydrase was found in the gland. In the "big skate," R. diaphenes (not reported by Smith) there was even more HCO_3^- but less chloride. Average of 2 fish showed Na^+. 619; Cl^-, 225; HCO_3^-, 291; K^+, 9. In this species there was a very high concentration of carbonic anhydrase; in fact more than we have found in any organ of any fish yet studied, 250 enzyme units/g. This is the same concentration as that of mammalian kidney.

The excess of measured cations is probably matched by $SO_4^=$, which at a concentration of 20-40 mM/L (Smith) is, like the other ions transported against a steep concentration gradient from plasma.*

This tissue appears to offer unusual opportunities for the study of ion transport, particularly of HCO_3^-. Further work is planned; it is also of interest to explore the suggestion that in one species HCO_3^- concentration is carried out without carbonic anhydrase while in the second, the same process (albeit quantitatively greater) utilizes the enzyme.

* Plasma composition. mM/L; Na, 260; Cl^-, 230; HCO_3^-, 6; K^+, 5; $SO_4^=$, 0.5.

Further Studies on the Physiology of the Alkaline Gland (Marshall's) in the Skate

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This is a continuation of the 1959 report on the same problem. The absence of carbonic anhydrase in *Raja stabuloforis* was confirmed, as well as the high CO_2 concentration of fluid (about 100 mM/L) taken from the sac of this gland, which is an appendage of the genito-urinary system in male skates. *Raja erinacea*, like *R. diaphenes*, however, had a high concentration of enzyme in the gland, and a very high CO_2 concentration (about 300 mM/L).

Pilot experiments were carried out in which acetazolamide or methazolamide was injected into *R. erinacea*. In one series, drug was given and one or two days later the fish was dissected and the gland contents analyzed for comparison with uninjected controls. In such experiments, the drug produced no change, despite its appearance in high concentrations in the plasma and gland fluid. This failure of effect was provisionally taken as indication that the gland fluid turns over slowly, and that under these conditions we were not analysing fluid formed during complete carbonic

anhydrase inhibition. In a second series, the fish were laparotomized and fluid was emptied from the sac. Drug or control solution was injected, and one or two days later the newly formed fluid was collected. In four out of seven cases the concentration of total carbon dioxide in the fluid formed during carbonic anhydrase inhibition was about 100 mM/L, i.e. substantially less than normal, and about the same as concentration in *R. stabuloforis*, where there is no enzyme. In three other fish there was no decrease in CO_2 concentration. Data thus far suggest that as in other physiological systems, carbonic anhydrase is not essential for bicarbonate accumulation, but that the presence of the enzyme may be associated with a somewhat higher gradient from plasma to gland fluid, than in its absence or inhibition.

Reflex Bradycardia in the Free Swimming Seal (Phoca Vitulina L.)

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In 1936 Irving and co-workers demonstrated that bradycardia occurs in the seal when breathing is arrested. Scholander and co-workers found that with artificial diving (tilting the seal's head under water) the blood flow in the periphery was reduced to a minimum. This has been referred to as the arterial constrictor response to diving. Nickels and Bradley reported that with simulated diving (occluded cone held over the seal's nose) bradycardia and cardiac arrhythmias, including fibrillation, occurred. In this study seals were monitored using subcutaneous needle electrodes on 50-foot teflon insulated electrocardiogram leads. They were allowed to swim freely in an aquarium and in the Bay on a 45-foot rope leash. During spontaneous diving there did not occur arrhythmias with the exception of an occasional failure of ventricular response to A-V conduction. When the seal was forcibly submerged and struggled a bradycardia occurred but was accompanied by inversion of P-waves, T-waves, the appearance of flutter fibrillation. The bradycardia accompanying struggling was not as pronounced as that found with normal diving. Atropine was found to block the reflex bradycardia of diving. One seal drowned after less than 3 minutes submersion following cessation of bradycardia. Since Scholander has demonstrated that seals can tolerate up to 15 to 20 minutes submersion while struggling, the occurrence of drowning in such a short period of time in an atropinized seal would suggest that the arterial constrictor response to diving was also obliterated. It was noticed that the seals would not dive for long periods while atropinized. These findings are in agreement with the observations in the rabbit by Forster and in the porpoise by Scholander. Bradycardia persisted while eating submerged. The bradycardia of normal diving differs from the Bradycardia of simulated diving when accompanied by struggling.