Everted gutsacs were prepared from consecutive intestinal segments of the shorthorn sculpin and Fundulus. The sacs were incubated in small flasks containing modified Ringer's solution with glucose, fructose or mannose as substrates. Calcium 45 Chloride or Ferrous 59 Sulphate were added to the bathing medium in several experiments in addition to the "carrier" calcium or iron. The flasks were equilibrated with 100% oxygen and shaken for 1-4 hours at 18-26° C. Subsequently the sacs were removed, drained and the concentration of calcium or iron was determined in the medium bathing the mucosal and the serosal surfaces. No net transport to the serosal surface or concentration gradients serosal/mucosal could be demonstrated for calcium or iron in these experiments.

Electrolytes of Cerebrospinal Fluid and Aqueous Humor of S. Acanthias: Effect of Carbonic Anhydrase Inhibition

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This is a continuation of work done in the summers of 1957 (Maren and Frederick) and 1958 (Maren and Fischer); the present report is chiefly concerned with Na⁺ and K⁺ concentrations in these fluids. Cerebrospinal (ventricular) Fluid: The finding of a considerable Cl⁻ excess (9%) in C.S.F. over plasma was confirmed; this excess is abolished by carbonic anhydrase (C.A.) inhibition, produced by intravenous acetazolamide (30 mg/kg). Na⁺, however, which is in slight excess (6%) in C.S.F. is not changed by C.A. inhibition. K⁺ also has higher concentration in C.S.F. than in plasma; this is unlike the mammalian relationship. This is probably not changed by C.A. inhibition. C.S.F. has a 5% osmotic excess over plasma. Aqueous Humor: There does not appear to be any substantial Cl⁻ excess in aqueous, or change following C.A. inhibition. Na⁺ however, has a 9% excess over plasma, and this is abolished by C.A. inhibition. There is also K⁺ excess in aqueous, but this is unaffected by C.A. inhibition.

It seems clear from these and our earlier studies that although carbonic anhydrase is involved in the secretion of these two fluids, their electrolyte properties are quite different, and injection of acetazolamide produces different results. A publication containing details of the work done on this project in the summers of 1957-1959 is being prepared.

Electrolytes and Carbonic Anhydrase Content of Marshall's Gland in the Skate

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Thirty years ago H. W. Smith (J. Biol. Chem. 81, p 407, 1929) reported the electrolyte composition of fluid obtained from an appendage of 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; C1⁻, 230; HCO₃⁻, 6; K⁺, 5; SO₄⁼, 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