Two notes about the Echinoderms might be of interest. The starfish, **Asterias forbesi,** is usually considered to be of more southerly distribution than **A. vulgaris.** From the earlier surveys (1923-25) of the Salsbury Cove region, it would seem to have been rather uncommon. This summer, however, it was quite abundant - about 1 **forbesi** to 5-8 **vulgaris.** It is possible that this is a reflection of the increase in average summer temperature of waters along the north Atlantic coasts which some workers report. The sand-dollar, **Echinarachnius parma,** which is usually very common on mud flats near the laboratory, was rather rare this year. Thousands of tests of dead sand-dollars were found, indicating that the reduction in numbers had occurred shortly before.

The Effects of 6063 Injections On Urine and Plasma Composition in Fishes

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Some years ago W. W. Smith[1] reported from this laboratory that the pH of the urine in the dogfish and sculpin is fixed at a value of about 5.7 and cannot be changed by the administration of bicarbonate or alkaline phosphate, the latter at pH 7.4 to 7.7. She suggested that this fixation at an acid reaction might be related to the fact that, in the marine fishes, the urine contains large quantities of magnesium which precipitates as $Mg(OH)_2$, $Mg(NH_4)PO_4$, or, as Pitts[2] had previously shown, as $MgHPO_4 . 3H_2O$, if the urine is alkalinized beyond pH 6. She concluded that urine is acidified by the exchange of H^+ ions for B^+, as had first been inferred for mammals[3]. This exchange mechanism has subsequently received wide support in the mammalian studies reported by Pitts and Alexander[4], Pitts and Lotspeich[5] and Berliner, Kennedy and Orloff[6].

It was demonstrated by the investigators cited above that the acidification of urine in the mammal can be partially blocked by carbonic anhydrase inhibitors, the most powerful of which is the sulphanilamide 6063, recently studied by Ber-

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liner ,Kennedy and Orloff[6]. In view of the potency of this compound in blocking acidification in the mammal, it seemed of interest to determine its effect in marine fishes where the acidity of the urine is otherwise fixed.

Urine was collected by rubber balloons fastened to a retention catheter, the latter being firmly tied in the urinogenital papilla. Blood was drawn under oil from the caudal vein. The fishes studied were the following: dogfish (**Squalus acanthias**), sculpin (**Myoxocephalus scorpius** and **M. octodecimspinosus**), flounder (**Pseudopleuronectes americanus**), goosefish (**Lophius piscatorius**), catfish (**Ameirus nebulosus**) and bass (**Micropterus salamoides**). After several control periods the sodium salt of 6063 was administered intravenously in the tail vein as a 10 per cent aqueous solution in doses of 25 to 200 mg. per kg. of body weight, except in one instance when the dose was 1000 mg. per kg.

Urine and plasma were analyzed for pH (Cambridge pH meter with McInnes glass electrode), CO_2 content (manometric Van Slyke), urea[7], chlorides[8], potassium and sodium (internal standard flame photometer), titratable acidity, and freezing point. Bicarbonate, carbonic acid and CO_2 pressures were calculated from the CO_2 content and pH after correction for temperature and assuming a pK of 6.1 and an alpha - value of 0.0301 for plasma and 0.0309 for urine.

Injections of 6063 induced no significant changes in pH, total CO_2, titratable acidity or electrolyte excretion in the urine of the dogfish (10 experiments, 23 periods after 6063), sculpin (10 experiments), goosefish and bass (each 1 experiment). A slight increase in urine flow was usually observed after the injection.

These results indicate that the acidification of urine in these fishes does not involve a carbonic anhydrase system sensitive to 6063, and in this respect is different from that in the mammals. It may be, however, that the basic operation (i.e. the exchange of H^+ for B^+) is identical with that in the mammals.

In the fresh-water catfish (6 experiments, 18 periods), however, the urinary pH changed after 6063 from an average of 6.43 to 7.34, sodium excretion from 10.2 to 63.8 mEq/hour, potassium excretion from 1.75 to 9.5 mEq/hour, and urine flow increased from 0.9 to 2.2 ml/hour. Similar, though less significant changes were observed in the flounder (4 experiments, 11 periods).

The fact that 6063 is inactive in respect to alkalinizing the urine in the marine fishes but active in the fresh-water catfish may be related to the absence of the distal segment in the former, its presence in the latter. The situation in the dogfish is obscure since Kempton[9] describes a 'distal segment' in **Squalus acanthias.**

The changes in plasma composition induced by 6063 were essentially the same in all species. In the dogfish, 30 to 60 minutes after injection, pCO_2 increased from a mean normal value of 4.1 to a range of 10 to 20 mm. Hg, with a transient decrease in plasma pH. These changes were followed by a delayed increase in total CO_2 (mainly as bicarbonate) and a compensatory decrease in blood chloride during the next 24 hours. The plasma chloride, total CO_2 and pCO_2 tended to regress to normal values after 72 to 96 hours. No changes in the plasma cation concentration could be detected.

These effects can be explained by assuming that the drug inhibits erythrocyte carbonic anhydrase, and thereby reduces the efficiency of erythrocytic transport between the tissues and the gills. Less easy to explain, however, is the delayed increase in the total CO_2 content of the plasma, resulting mainly from the accumulation of bicarbonate. The plasma bicarbonate concentration in the dogfish after 6063 injections generally exceeded the values to be expected from the observed pCO_2 and the CO_2 dissociation curve of dogfish blood as recorded by Ferguson, Horvath and Pappenheimer[10]. It was therefore thought that 6063 might interfere with the excretion of bicarbonate. To test this hypothesis, various amounts of sodium bicarbonate (mostly 10 cc. of 10 per cent) were injected in several dogfish, with and without administration of 6063 (100 mg/kg. body weight). In control experiments in which no 6063 was given, the injected bicarbonate was rapidly excreted and normal blood concentrations were regained within 90 minutes, though without any notable changes in urine pH or bicarbonate excretion, the last confirming W. W. Smith's observations[1], as reported above. This demonstrates that bicarbonate can be excreted by the fishes by some extrarenal route, but no attempt was made to identify this excretory mechanism. The addition of 6063, however, led to a markedly delayed excretion of bicarbonate, suggesting that carbonic anhydrase is involved in the excretory process. This would require that this process involves the formation of carbon dioxide: presumably plasma bicarbonate could be converted to H_2CO_3 and then through the mediation of carbonic anhydrase, conceivably in the red cells but possibly elsewhere, to CO_2 for excretion.

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