THE ABSORPTION AND EXCRETION OF WATER AND SALTS BY MARINE TELEOSTS

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It is a peculiar and interesting fact that marine teleosts excrete urine which is isotonic or hypotonic with respect to the blood, and therefore of considerably lower osmotic pressure than sea water (Rodier, 1899; Bottazzi, 1906; Dekhuyzen, 1905; Burian, 1908, 1910). From this, it appears that these animals are faced with the task of concentrating the surrounding ocean in order to obtain water for the formation of urine, a task which can only be performed at the expenditure of energy in osmotic work. This circumstance might be expected to result in profound differences in the water economy of marine fish as compared with fresh water and terrestrial animals to which water is available ad libitum.

The answer to the question of what tissue or tissues actually do the work of separating from the sea water the osmotically dilute solution destined to be excreted as urine appears to rest in the determination of whether the renally excreted water is initially absorbed from the intestine or enters the body through the gills. The work to be reported here leads us to the conclusion that water absorption occurs by way of the intestine; but, surprisingly, it is not in the intestine that the osmotic work is done, for the residue formed there as absorption proceeds is actually more dilute than the ingested sea water, and tends to approach the blood in tonicity. This osmotic dilution results from the fact that Na, K and Cl are absorbed from the intestine, along with a considerable fraction of the water, leaving behind only the poorly absorbed Mg and SO₄ in highly concentrated state. During this process of concentration some Mg and SO₄ are absorbed, to be subsequently excreted in the urine. The bulk of the Na, K and Cl, together with the bulk of the water absorbed from the intestine, never appear in the urine, however; they are excreted by routes other than the kidneys—

'This problem was recognized by Dekhuyzen (1905) who remarks "... la richesse relative en eau de cette urine prove plutôt que les poissons marine resorbent de l'eau, on si l'on préfère une solution diluée effectuant ainsi un travail contre la pression osmotique en consommant de l'énergie chimique."
probably by the gills. The salts and water excreted extrarenally, if considered together, would make up a solution hypertonic to sea water and thus compensate for the osmotically dilute urine and intestinal residue.

Thus, extrarenal (branchial?) excretion plays an active rôle in these animals in the regulation of body fluid composition, and relieves the kidneys of much their duty. The consequences of this fact are apparently in evidence in at least one respect: we believe it is the very small volume of urine excreted that is responsible for the absence of glomeruli in the kidneys of many strictly marine fishes.

Ingestion and absorption of sea water. That marine fish normally swallow large quantities of sea water is immediately suggested by the fact that there is always present in the intestine proper a considerable quantity of clear and usually colorless fluid. This fluid is frequently discharged from the anus with vigor when the fish is handled, and it can always be expelled into a catheter inserted into the anus by gently massaging the abdomen. Fluid is rarely present in any large quantity in the stomach of starved fish, but this may be due to the rapid passage of ingested water into the intestine where the major process of absorption occurs.

The ingestion of sea water can be readily demonstrated by adding an easily detected substance, such as a dye, to the aquarium water in which the fish are kept. We have found phenol red most satisfactory for this purpose.

In both the sculpin and the eel (in sea water) phenol red can be detected in the fluids removed from the stomach and intestine within 4 to 6 hours after the fish are placed in the tinctured sea water. After 24 hours, the intestine is filled with a bright red solution of the dye, considerably more concentrated than the ingested sea water. The red color is due to the alkaline reaction of the intestinal fluids, and the increased concentration of dye is due, as will be shown later, to the absorption of water from the intestine. The stomach contents are invariably acid in reaction and the dye has never been observed to be concentrated there.

The fact that phenol red is not readily absorbed from the intestine leads to its extensive concentration as water and salts are absorbed. Thus, 24 hours after immersion in the tinctured sea water the dye is usually 3 to 5 times as concentrated in the intestine as in the ingested sea water. This fact is of great importance in showing that in addition to swallowing sea water, these fish absorb a large fraction of the ingested water by way of the intestine. By adding alkali to develop the full color of the dye and centrifuging to remove the solid matter and the precipitated Mg, it is possible to determine the extent of concentration (i.e., of water absorption) that has occurred. The following is an example of a typical experiment: The intestine of an eel was emptied as far as possible of fluid by inserting a catheter through the anus and pressing upon the abdomen. The anus was then closed.
with a ligature to prevent the escape of any intestinal fluid and the urinary papilla was tied. Twenty hours after immersion in the tinctured sea water 2.3 cc. of fluid were removed from the stomach and intestine. To dilute the dye in this fluid to the same concentration as in the ingested sea water 10.0 cc. of water were required. Thus the fish must have swallowed 12.3 cc. of sea water and absorbed 10.0 cc. of water, during this period of time. A small amount of dye was present in the bile and urine; consequently the above figures for ingested and absorbed water are in error by being too small. If any sea water had been present in the gastro-intestinal tract at the beginning of the experiment this fluid would in addition undergo some absorption, and the residue should be deducted from the 2.3 cc. residue observed, so that errors from this source would likewise tend to reduce the figures for both the ingested and absorbed water. Consequently the above figures are by all considerations minimal for the conditions of the experiment.

Further evidence that sea water is normally swallowed by marine fish, and that water is absorbed from the intestinal tract will be given later in connection with the chemical composition of the gastro-intestinal fluids.

The validity of the above conclusions depends upon the exclusion of the possible though highly improbable alternative that the dye might have been absorbed by the skin or gills and excreted into the stomach and intestines. That this is not the case can be shown in Anguilla by ligating the pylorus to prevent the passage of sea water into the intestine. The body wall is opened opposite the lower end of the esophagus and the latter is tied in two places, a few millimeters apart, to insure complete obstruction. The incision in the body wall is then closed with ligatures and the fish placed in sea water heavily tinctured with phenol red. After 24 to 48 hours the fish is sacrificed and the stomach and intestinal contents tested for the dye by the addition of excess alkali. The invariable and complete absence of phenol red from the stomach and intestine in a large number of such experiments has convinced us that this dye gains access to the gastro-intestinal tract only in consequence of the passage of swallowed sea water through the stomach. If the phenol red remains for considerable periods of time and in a concentrated state in the intestine it may be absorbed, and it then appears in the bile as well as the urine, but this hepatic excretion occurs only after gastro-intestinal absorption, and never in the absence of dye from the gastro-intestinal tract. From a large number of experiments with starved but otherwise apparently normal fish we conclude that from 50 to 200 cc. per kilo per day of sea water are ingested by Anguilla and Myoxocephalus when these fish are immersed in sea water having a freezing point of $-1.8^\circ$C.

The extrarenal excretion of water. The collection of urine in the sculpin and the eel is facilitated by the fact that the bladder is readily accessible
through the urinary papilla. The bladder may be completely emptied by inserting a fine glass catheter through the papilla and expressing the urine by massaging the abdomen over the bladder. When it is desired to collect all the urine excreted over a period of hours, the bladder may be emptied and then the urinary papilla tied to prevent discharge. Collections over too long a period of time should be avoided because of the danger of over-distention of the bladder.

To continue with the eel experiment cited above, 2.3 cc. of urine were excreted during the 24 hours that the fish was in the tinctured sea water. Since 10.0 cc. of water had been absorbed from the intestine, it follows that either a, 7.7 cc. of water had been retained by the fish, or b, this quantity of water had escaped from the body by some route other than the kidneys and intestinal tract. The eel weighed 143.5 grams at the beginning of the experiment and 142.2 grams at the end; it had, therefore, lost 1.3 grams body weight, presumably as water. Adding this loss in weight to the 7.7 cc. unaccounted for above, it follows that 0.0 cc. of water had been excreted extrarenally and 2.3 cc. excreted by the kidneys. Data to be given subsequently furnish further evidence of the extrarenal excretion of water. Meanwhile reference may be made to table 1 which gives the results of several experiments with Anguilla and Myoxocephalus performed as above. These experiments show that these fish swallow from 50 to 200 cc. of sea water per kilo per day, of which they absorb roundly 75 per cent of the water. Of the absorbed water, as much as 90 per cent may be excreted by routes other than the kidneys.

Chemical composition of gastro-intestinal fluids. We have not succeeded in obtaining sufficient fluid from the stomachs of normal Anguilla and Myoxocephalus for analysis, but ample fluid can be obtained from the intestinal tract for this purpose. In a few instances sufficient quantities of fluid were obtained from single specimens to permit complete analysis by using 0.2 cc. samples. Otherwise composite samples were analyzed. Data on the osmotic pressure and inorganic composition of intestinal fluids are given in table 2, and also analyses of fluids removed from fasted and fed specimens of the goosefish, Lophius piscatorius. The fasted Lophius were specimens caught off Sandy Hook by the New York Aquarium's boat Sea Horse and kept in the Aquarium in New York Harbor water for 3 to 5 days without food. The stomachs were empty and contained only a small quantity of clear, colorless, mucilaginous fluid. The contents of the intestines were also clear, though sometimes bile stained. The fed specimens had been freshly caught off Sandy Hook by local fishermen and all proved on inspection to have food masses in the stomach in a partially digested state. The upper intestinal contents were cloudy with food residues, while the lower intestinal contents were for the most part clear, except for granules of a mucilaginous nature. The intestinal contents are
designated as "anterior" or "posterior" according to the region from which they were removed. All fluids were centrifuged to remove particulate matter prior to analysis, and in the case of the fed Lophius, trichloroacetic acid filtrates were prepared from the gastric and intestinal fluids to remove partially digested protein. Where urine and intestinal fluids were removed from the same fish this fact is indicated by corresponding numbers. (It may be noted with respect to these data that the failure of the inorganic cations to balance the inorganic anions is attributable in part to the presence of proteinates, bile salts, ammonia and other organic substances, and that some carbonate as well as bicarbonate is probably present in the alkaline intestinal fluids.)

It will be convenient to refer first to the analyses of the fluids from Lophius because here we have simultaneous analyses of the gastric and intestinal fluids from the same specimens.

In the gastric contents of both fed and fasted Lophius Mg and SO₄ are more dilute than in sea water. As these fluids pass down the intestine, Mg and SO₄ invariably become concentrated 6 to 10 times (by the intestinal absorption of water) and when the lower intestine is reached the concentrations present are several times greater than in the Harbor water itself. As the Mg and SO₄ increase, the Na, K and Cl initially present in the gastric fluid fail to increase, or may even decrease in concentration, so that in the end Mg and SO₄ come to make up the bulk of the residual salt in the alkaline intestinal residue.

The osmotic pressure of the gastric fluid is invariably less than that of the sea water from which the fish are removed, but greater by a considerable amount than the osmotic pressure of the blood. The osmotic pressure of the gastric fluid, and the relative dilution of Mg and SO₄ in it, are explicable by the assumption that this fluid is a mixture of swallowed sea water of high osmotic pressure and gastric juice of an osmotic pressure approximately equal to that of the blood. In addition, it may be supposed that when sea water is taken into the stomach, the higher osmotic pressure of the former would lead to a withdrawal of water from the blood into the gastric contents, the sea water thus becoming appreciably diluted.

The progressive decrease in osmotic pressure in the intestine indicates that either water is moving into the intestine from the blood, or salts are being absorbed. It was not possible for us to determine by direct experiments on Lophius which of these alternative processes obtains; but the answer is clearly indicated by the rapidly mounting concentrations of Mg and SO₄ and the disappearance of Na, K, Ca and Cl. Between the stomach and the lower intestine, Mg and SO₄ are concentrated 6 to 1, or more. These substances occur in only small amounts in the bile, indicating that they are not extensively excreted by the liver, and we have shown in Anguilla (in which an entirely similar situation obtains) that they are not
EXCRETION OF SALTS BY FISH

secreted in significant amounts by the intestinal mucosa (vide infra). Consequently the above facts indicate the absorption of both water and salts, other than Mg and SO₄, from the intestine. The decrease in osmotic pressure is due in part to the fact that it is primarily the univalent salts which are absorbed leaving the osmotically less active bivalent Mg and SO₄ behind. In addition, it is possible that actually more salt than water, in osmotic equivalents, is taken up by the intestine.

**TABLE 1**

*Phenol red experiments showing intestinal absorption and extrarenal excretion of water by starved Anguilla and Myoxocephalus in sea water*

"Water absorbed" was determined by diluting dye in intestinal residue to its original concentration in sea water.

<table>
<thead>
<tr>
<th>Cubic centimeters per kilogram per day</th>
<th>Per cent</th>
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<tr>
<td></td>
<td>Of sea water absorbed</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Swallowed</td>
<td>Absorbed</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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<td>54.4</td>
<td>46.0</td>
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<tr>
<td>Average</td>
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<tr>
<td>Myoxocephalus</td>
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<td>225.0</td>
<td>169.0</td>
</tr>
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<td>96.8</td>
<td>43.0</td>
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<td>Average</td>
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The entire picture corresponds, in brief, to our idea of what would inevitably happen to swallowed sea water; namely, osmotic and chemical dilution in the stomach by admixture with gastric juice, followed by absorption of salts together with the osmotically equivalent water during passage through the intestine, the poorly absorbed Mg and SO₄ largely remaining behind in the intestinal residue.

The analyses of the intestinal fluids removed from Anguilla and Myoxocephalus give results entirely similar to those obtained in Lophius; the Mg
# TABLE 2
Composition of gastro-intestinal fluids in marine teleosts

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<thead>
<tr>
<th></th>
<th>mM per liter</th>
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<td>Mg</td>
<td>Cl</td>
<td>SO₄</td>
<td>PO₄</td>
<td>CO₂</td>
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<td>2.5</td>
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<td>Gastric</td>
<td>1.03</td>
<td>108</td>
<td>3.0</td>
<td>6.2</td>
<td>30</td>
<td>235</td>
<td>17</td>
<td>6.5</td>
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<td>140</td>
<td>30.2</td>
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<td>72</td>
<td>150</td>
<td></td>
<td></td>
<td>59.0</td>
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<td>246</td>
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<td>Post. intest.</td>
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<td>140</td>
<td>11.4</td>
<td>6.0</td>
<td>99</td>
<td>81</td>
<td>114</td>
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<tr>
<td>Bile</td>
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<td>264</td>
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<td>14.3</td>
<td>11</td>
<td>26</td>
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<td>Cl</td>
<td>SO₄</td>
<td>PO₄</td>
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<td>PO₄</td>
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<td>87</td>
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* This sea water was used for no. 26 only, and for calculation of table 4. The other eels were kept in sea water differing but slightly from this sample.
and SO₄ contained in the swallowed sea water become concentrated by the intestinal absorption of water while the simultaneous absorption of Na, K and Cl results in a decrease in osmotic pressure so that the ultimate intestinal residue approaches the blood in tonicity.

The posterior portion of the intestine of Anguilla and Myxocephaslus is differentiated from the rest by being somewhat larger, and in the normal

| TABLE 3 |
|---|---|---|---|---|---|---|---|
| Composition of marine teleost urine |
| | °C | mM per liter |
| | K | Ca | Mg | Cl | SO₄ | PO₄ | CO₃ |
| 1 | Lophius, fasted | 0.69 | 1.9 | 11.8 | 63 | 172 | 40 | 7.1 | 1.5 |
| 2 | Lophius, fasted | 0.66 | 2.7 | 13.6 | 57 | 148 | 37 | 6.1 | 4.0 |
| 3 | Lophius, fasted | 0.72 | 2.7 | 15.3 | 74 | 173 | 41 | 6.1 | 2.0 |
| 4 | Lophius, fasted | 0.85 | 3.0 | 13.5 | 88 | 209 | 47 | 5.9 | 2.0 |
| 5 | Lophius, fasted | 0.77 | 0.6 | 15.5 | 73 | 195 | 38 | 6.3 | 1.1 |
| 18 | Lophius, fed | 0.61 | 1.9 | 16.8 | 64 | 171 | 29 | 4.2 | 1.5 |
| 19 | Lophius, fed | 0.76 | 2.1 | 9.3 | 79 | 181 | 40 | 2.8 | 1.0 |
| 20 | Lophius, fed | 0.64 | 7.6 | 14.8 | 75 | 161 | 36 | 14.7 | 1.2 |
| 21 | Lophius, fed | 0.80 | 4.3 | 15.2 | 68 | 207 | 36 | 2.9 | 1.3 |
| 22 | Lophius, fed | 0.71 | 2.3 | 13.3 | 100 | 206 | 47 | 9.8 | 1.0 |
| 26 | Anguilla, fasted | 0.66 | 5.7 | 7.5 | 75 | 124 | 52 | None | 4.0 |
| 27 | Anguilla, fasted | 0.75 | 2.7 | 17.3 | 91 | 214 | 34 | None | 4.0 |
| 28 | Anguilla, fasted | 0.80 | Tr. | 20.7 | 150 | 134 | 105 | 1.9 | None |
| 29 | Anguilla, fasted | 0.81 | Tr. | 18.9 | 167 | 203 | 105 | 10.4 | 0.5 |
| 30 | Anguilla, fasted | 0.68 | Tr. | 16.5 | 140 | 79 | 113 | None | None |
| 31 | Anguilla, fasted | 0.69 | Tr. | 17.7 | 150 | 84 | 125 | None | None |
| 37 | Myxocephaslus, fed | 0.78 | 0.1 | 23.6 | 114 | 129 | 54 | 16.0 |
| 38 | Myxocephaslus, fed | 0.76 | 0.1 | 39.0 | 142 | 165 | 61 | 78.0 |
| 39 | Myxocephaslus, fed | 0.75 | Tr. | 26.6 | 109 | 129 | 37 | 35.7 |
| 40 | Myxocephaslus, fed | 0.73 | 0.2 | 8.0 | 76 | 178 | 35 | 25.0 |
| 41 | Myxocephaslus, fed | 0.77 | Tr. | 30.7 | 125 | 191 | 34 | 21.7 | 4.6 |
| 42 | Myxocephaslus, fed | 0.83 | 0.1 | 31.4 | 125 | 231 | 32 | 23.6 | 3.9 |

animal is sometimes distended with fluid residue. This “rectum” is separated from the anterior intestine by a band of highly tonic muscle which apparently acts as sphincter. It seemed possible that water absorption might occur here to a greater extent than in the rest of the intestine and that the osmotic pressure of the intestinal residue might actually be raised to a value greater than the blood in this limited region only, as is the case in the cloaca of birds and reptiles. For this reason several composite samples of fluid were removed by catheter through the anus with
special care not to expel fluid from the upper intestine. Analyses of two such samples are given in table 2, nos. 26 and 27, and analyses of the composite urine collected at the same time in table 3, no. 26. (These eels had been in sea water for 10 days and were presumably thoroughly acclimatized.) The fact that the osmotic pressure in these samples is low indicates that the fluid in this portion of the intestine is more nearly isotonic with the blood than that removed from higher regions, and that this rectal portion, like the rest of the intestine, is unable to absorb water against osmotic pressure.

In none of the higher animals, so far as is known, is the intestine capable of absorbing water against osmotic pressure, and consequently it is not surprising to find that in fish a similar situation obtains, in that the intestinal residue is isotonic, or approaches isotonicity with the blood. Nor is there anything unusual in the interpretation of the data of table 2 in terms of the more rapid absorption of Na, K and Cl from the intestine leaving the less readily absorbed Mg and SO4 predominating in the residue; in this respect the fish again conforms with what is known about the higher animals (Goldschmidt, 1921). One might be content to let the matter rest with the above evidence, were it not that extraordinary conclusions necessarily follow from this interpretation and make it highly desirable to prove that no significant fraction of the Mg and SO4 in the intestinal residue come there by secretion of the intestine itself.

This proof was adduced by ligation of the pylorus as described above. After the operation the intestine was thoroughly rinsed of its immediate SO4 or Mg by forcing a NaCl solution (Δ = -0.8°C.) into the anus with a rubber nipple pipette. This rinsing must be repeated a number of times at hourly intervals, for the mucosa is literally saturated with both SO4 and Mg and these substances are removed with difficulty. The experiment was varied by using fresh water eels which had not been in sea water, and which had, therefore, less Mg and SO4 in the intestinal mucosa to begin with. Phenol red was always added to the sea water to detect leakage of the pyloric ligature.

If the intestine of an unoperated eel is rinsed, the anus closed and the fish then placed in sea water, from 300 to 900 × 10⁻³ mM of SO4 or Mg accumulate in the intestine in 24 hours due to the entrance and absorption of sea water. With the pylorus closed by ligature, no fluid whatever accumulates in the dry intestine, and the SO4 or Mg that can be recovered by washing at the end of 24 hours ranges from zero to 60 × 10⁻³ mM.

These experiments indicate that the Mg and SO4 normally found in the intestine of salt water fish come there by the ingestion of sea water, and not by secretion by the intestine following their absorption into the body through the gills or skin. Another, and somewhat more satisfactory, type of experiment leading to the same conclusion is based on the fact that
Anguilla (as well as other fish) when in fresh water does not swallow water, the urine under these conditions being derived from water absorbed by the esophageal or branchial membranes. Thus, if the fish is placed in a simple N 0.05 MgSO₄ solution (Δ = -0.15°C.), none of the solution is, as a rule, swallowed in 2 to 3 days and during this time the intestine remains free of both Mg and SO₄. If these salts were absorbed by the skin, etc., and secreted into the intestine, we see no reason why such absorption and secretion should not proceed from this solution as well as from sea water with the same concentration of MgSO₄.

Composition of urine. Turning now to the composition of the urine, given in table 3, it will be noted first that this is invariably isotonic or hypotonic to the blood, the freezing point of which in these fish varies from -0.6 to -0.8°C.

Thus, the osmotic pressure of the urine is considerably lower than that of sea water. This fact, as we remarked in the introduction, requires that the fish remove water from sea water against the osmotic pressure of the salts in the latter, a task which involves the expenditure of energy in the form of the physical-chemical work required to concentrate the corresponding quantity of sea water. To this urine there must be added the osmotically dilute intestinal residue, both of which must be obtained from the relatively concentrated sea water.

The mutually dilute nature of the urine and intestinal residue force us to the conclusion that osmotic work is being done by the fish elsewhere than in the kidneys and gastro-intestinal tract.

In regard to the inorganic composition of the urine, the most striking feature is the large amount of Mg and SO₄. These substances may be, and usually are, present in the urine 3 to 6 times as concentrated as in the sea water. This fact is also shown by the analyses of the urine of marine teleosts made by Sulze (1922), Edwards and Condorelli (1928) and Marshall and Grafflin (1928).²

² The intestinal fluid and frequently the urine of marine fish contains solid matter in suspension which consists largely of Ca carbonates and Mg (OH)₂ in the former and Ca phosphates in the latter. This particulate matter was centrifuged out and only the supernatant fluid analyzed. (Guitel, 1906, has noted the presence of calcium concretions in the ureters of marine teleosts.)

³ It is noteworthy that Lophius possesses a purely tubular kidney (Marshall and Grafflin, 1928), and the question is pertinent whether this fact makes any significant difference in the inorganic composition of the urine. It would appear that such is not the case, for Sulze's data show similar urine composition in the agglomerular Lophius and in Conger and Scorpaena which are predominantly glomerular (Marshall, 1929a). Edwards and Condorelli (1928) found Mg in amounts greater than in sea water in the urine of Lophius and of the agglomerular Synnatus and Hippocampus and of the predominantly glomerular Muraena. Add to these observations our similar findings in the glomerular Anguilla and Myoxocephalus and the conclusion is justified that the osmotic pressure and inorganic composition of normal urine is not significantly dependent on the pressure or absence of glomeruli.
The presence of large amounts of Mg and Ca in the urine of fish has been especially noted by Sulze (1922), who concluded that marine fish do not swallow sea water, since he did not observe it in the stomach; and that the Mg and Ca excreted in the urine must, therefore, have gained access to the body through the skin or gills. The fact that Mg and Ca occur in much larger amounts in the urine than Na, K or Cl Sulze explained by assuming that the former enter the body much more readily than the latter.

A priori, there is much to argue against Sulze's view. There is not, in the first place, any evidence that Mg and SO₄ penetrate any tissue more readily than do Na, K and Cl. There is ample evidence to indicate that the reverse is true in higher organisms and that Mg and SO₄ are absorbed to only a very slight extent or not at all (Goldschmidt, 1921; Denis and Hobson, 1923; and Denis and Leche, 1925).

In refutation of Sulze's view it has been shown here that large amounts of sea water are swallowed by marine fish and that water, Na, K and Cl are absorbed into the body, leaving Mg and SO₄ in the intestinal residue. The failure, therefore, of Na, K and Cl to appear in the urine cannot be attributed to their failure to gain access to the body. Nor can the preponderance of Mg and SO₄ in the urine be attributed to preferential absorption from the intestine, for they are demonstrably absorbed less readily than Na, K and Cl. Setting aside, for the moment, the fate of the absorbed Na, K and Cl, and considering only the Mg and SO₄, it would seem that, in view of the enormous concentrations of these substances occurring in the intestinal residue as the result of the absorption of water and salts, some absorption of them must inevitably occur there. In this view it should be possible to exclude these salts from the urine by ligating the esophagus; but this operation in Anguilla effects only a moderate reduction in the urine concentration, or in the quantities excreted per day. The apparent reason for this is that with the ligature placed near the stomach (as it must necessarily be in Anguilla since the esophagus is not accessible higher up) the continued efforts of the fish to drink lead to marked distention and acute dilatation of the esophagus and in this dilated state, considerable quantities of sea water and its contained salts enter the body.

Since it is impossible to ligate the esophagus at the oral end without obstructing respiration, it is impossible to prevent absorption of Mg and SO₄ by operative procedures.

Evidence for the alimentary origin of the urinary Mg and SO₄ can be adduced, however, by using simple MgSO₄ solutions as described above. In 0.05 N MgSO₄ the urine flow is very great (from 40 to 100 cc./kgm./day) the water for the urine being absorbed elsewhere than from the stomach and intestine as demonstrated by the failure of the fish to drink tinted sea water. When kept in such solutions, Anguilla rarely swallows during the first 2 to 3 days. The Mg and SO₄ in the urine are very dilute and the total
amounts excreted per day are considerably less than when the fish is kept in sea water. The average results from several experiments of this kind are given in table 4. The basal Mg and SO₄ excretion in fresh water was determined on fresh water eels which had never been (to our knowledge) in sea water. In these experiments, because of the greater urine flow, the urine was collected by fastening a retention catheter with a 50 cc. rubber balloon attached in the urinary papilla. In the MgSO₄ experiments, the urine samples were collected only on the second day. The figures for sea water are based upon the excretion of fish which had been in sea water but 48 hours, to keep the conditions comparable with the MgSO₄ experiments. The results of these experiments show that after 48 hours in sea water Anguilla excretes in the neighborhood of ten times as much Mg as when immersed for the same period of time in 0.05 N MgSO₄ solution, and if

<table>
<thead>
<tr>
<th></th>
<th>Mg</th>
<th>SO₄</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mM per liter</td>
<td>mM per kgm. per day × 10³</td>
</tr>
<tr>
<td>1</td>
<td>Fresh water</td>
<td>0.7</td>
</tr>
<tr>
<td>2</td>
<td>MgSO₄ solution</td>
<td>2.19</td>
</tr>
<tr>
<td>3</td>
<td>Sea water (average)</td>
<td>150.0</td>
</tr>
<tr>
<td>4</td>
<td>Sea water (diuresis)</td>
<td>145.0</td>
</tr>
</tbody>
</table>

diuresis is produced by injecting sea water into the intestine through the anus, (vide infra) the difference may become still greater. The SO₄ excretion under the several conditions shows somewhat smaller differences, due apparently to the fact that less SO₄ was absorbed from the intestine during the experiment in sea water.

In another experiment of this same nature, after a control sample of urine was collected in distilled water, an eel was placed for three days in 0.01 N MgSO₄. When the total Mg and SO₄ excreted during the three day exposure to this solution was corrected by deducting the basal excretion, as determined during the control period, there remained 233 × 10⁻³ mM. of Mg per kgm. per day excreted over the basal, but no SO₄. In a corresponding quantity of the MgSO₄ solution there were 1490 × 10⁻³ mM. of Mg and 1639 × 10⁻⁵ mM. of SO₄. In short, the absorption of water in this solution had occurred in some place and in such a way that 90
per cent of the Mg and 100 per cent of the SO₄ had been excluded from the body.

These experiments leave no doubt that when immersed in MgSO₄ solutions in which the concentration of this salt ranges up to that of sea water, neither Mg nor SO₄ enter the body to any great extent unless the fish swallows some of the solution. If these substances entered the body of the fish in sea water through the skin and gills, as Sulze supposes, it is difficult to explain why they should fail to do so from MgSO₄ solutions. In view of the fact that both Mg and SO₄ are to some extent absorbed from the intestine (vide infra) and in view of the enormous concentrations of these substances in the intestines of normal fish, as shown by table 2, we are forced to set Sulze's interpretation aside and to conclude that apart from a moiety of SO₄ of metabolic origin, the urinary Mg and SO₄ of marine fish enter the body by alimentary absorption.¹

Having established that the intestinal Mg and SO₄ are derived from ingested sea water, and that the urinary Mg and SO₄ are derived from the alimentary absorption of these substances, we arrive at new evidence for the extrarenal excretion of water. For these substances are present in both intestinal residue and urine in greater concentrations than in sea water; this circumstance can only obtain if part of the water from the ingested sea water is disposed of elsewhere.

Extrarenal excretion of salts. With these facts established we can return to the fate of Na, K and Cl. From the preponderance of Mg and SO₄ in the intestinal residue it follows that relatively more Na, K and Cl are absorbed than Mg and SO₄; and from the preponderance of Mg and SO₄ in the urine it follows that less Na, K and Cl are excreted by this route than Mg and SO₄. The obvious inference is that the Na, K and Cl which are absorbed ahead of the Mg and SO₄ are being excreted by some route other than the kidney.

The absence of K from the urines of fed Lophius is particularly striking evidence of the extrarenal excretion of this substance. Lophius is a notorious meat eater, consuming in a single meal several pounds of fish. Though digestion is relatively slow in cold blooded animals (Riddle, 1909; Dobreff, 1927), it would be expected that the large amount of K ingested in a continued meat diet would lead to the preponderance of this substance in the urine, as is the case with mammals. K is demonstrably absorbed from the intestine of Lophius (table 2) yet no specimen of urine which we have

¹ Ca, on which Sulze places as much emphasis as Mg, we omit from consideration for the reason that this substance is largely precipitated as carbonates in the alkaline intestinal residue, and as phosphates in the urine. In our analyses, this precipitated Ca was centrifuged out, and consequently the amount of Ca left in solution, especially in urine, depends upon the acidity, phosphate content, interval between collection and centrifuging, etc., and has no significance.
examined has contained more than traces of this substance, even when the process of digestion is well advanced (table 3 "fed Lophius"). Since this appears to be the normal state of affairs, it does not seem likely that the absence of K from the urine is to be explained in terms of retention.

A simple calculation based on the total Mg and SO₄ jointly excreted in the urine and intestinal residue shows that large amounts of Na, K and Cl which are contained in the ingested sea water, and which are shown to have been absorbed from the intestine, remain unaccounted for in the urine. These substances have disappeared: either they are being retained by the animal in large amounts or they are escaping from the body by some route other than the kidneys. Since the analyses of the intestinal fluids and urines from fish obtained under perfectly natural conditions (Lophius and Myoxocephalus) agree in respect to the above facts, we conclude that retention is an inadequate explanation and turn to the other alternative, namely, that the univalent ions are being excreted by an extrarenal route. This fact becomes more significant if we recapitulate two previous conclusions drawn from the above data:

It has been shown by phenol red experiments with Anguilla and Myoxocephalus that water is excreted extrarenally. Further evidence of this fact is obtained in these fish and in Lophius by the increased concentrations of Mg and SO₄ in the urine and intestinal residue as compared with the ingested sea water.

It is shown that osmotic work is being performed by some tissue or tissues other than the kidneys and intestine in all three species by the facts that the urine and intestinal residue are more dilute than the sea water from which they are derived.

Now comes evidence, in the absorption of Na, K and Cl from the intestine in quantities relatively greater than Mg and SO₄, and the excretion of these substances in the urine in quantities relatively less than Mg and SO₄, that indicates that NaCl and KCl are being excreted extrarenally.

It is conceivable that these three processes might be going on independently in separate places or even in the same place, but it is difficult to believe that such is the case. There are only two ways in which osmotic work can be done by the fish: 1, to extract water directly from sea water (say at the gills) to the exclusion of, and against the osmotic pressure of the salts in the latter; or 2, to secrete a hypertonic solution from the body into the relatively dilute sea water. If, for the moment, we admit the first of these alternatives, then we are faced with the extraordinarily complex phenomena of the organism a, extracting water from sea water for the formation of dilute urine and intestinal residue, b, excreting water into sea water (as shown the phenol red experiments and the excretion of Mg and SO₄), and c, excreting salts into sea water (as shown by absorption of Na, K and Cl from the intestine and the absence of these substances from the urine),
all simultaneously at some extrarenal and extra-intestinal point. If, now, we consider the second possibility, namely, that the fish is doing its extrarenal osmotic work by the secretion of a solution hypertonic to sea water, it is obvious that both salts and water must be available for this secretion; and the more water and salts thus secreted, the less will the absolute hypertonicity of the solution with respect to the external sea water have to be. Thus the extrarenal osmotic work, the extrarenal excretion of water and the extrarenal excretion of salts become simultaneously identified with a single process in this latter view. It seems to us that there can be no choice but to attribute the three processes to the secretion of a hypertonic solution.

**Extrarenal osmotic work.** If the absolute amounts of salt and water excreted extrarenally could be determined, one could calculate the osmotic pressure of this hypothetical solution. The absolute amounts of salts and water excreted extrarenally comprise the difference between the amounts ingested on the one hand, and the amounts excreted in the urine and intestinal fluid on the other. The average volumes of urine and intestinal fluids in the experiments given in table 1 are 1.5 and 1.9 cc. respectively; the latter figure is no doubt slightly large because of sea-water originally present in the intestinal tract at the beginning of the experiment. Making allowance for this error it would appear that about equal volumes of urine and intestinal residue are passed in any period of time.

The actual quantities of Cl and water excreted extrarenally can be calculated if we assume that no Mg is excreted extrarenally. The SO₄ is less reliable for this purpose, since a fraction of the urinary SO₄ is of metabolic origin. Assuming that all the Mg comes from ingested sea water it follows that for each liter of urine and each liter of intestinal residue excreted, an amount of sea water equal to \( \frac{mM. \text{Mg urine} + mM. \text{Mg intestine}}{mM. \text{Mg sea water}} \) in liters must have been swallowed. Of this swallowed sea water, only 2 liters of water (1 liter as urine and 1 liter as intestinal residue) are accounted for and the remaining water must have escaped from the body elsewhere.

When the unaccounted-for Cl is dissolved in the unaccounted-for water, the resulting solution is richer in Cl than the ingested sea water, as shown by the figures in table 5. Since NaCl or sea water solutions of equal Cl content have the same osmotic pressure, it follows that this hypothetical excretion is by so much hypertonic to sea water. As would be expected, the hypertonicity is relatively greater in the case of Anguilla and Myoxoccephalus in sea water than in the case of Lophius in Harbor water, for the osmotic pressure of the urine remaining the same, a relatively greater fraction of salt must be excreted into the sea water than into the more dilute Harbor water for each liter of urine formed.

**Demonstration of the extrarenal excretion of salts.** Since a relatively large
quantity of well aerated sea water is necessary to keep a fish alive it seems improbable that the extrarenal excretion of a hypertonic solution in sea water can be directly demonstrated. The extrarenal excretion of individual salts by Anguilla can be demonstrated by direct experiment, however, when this fish is kept in fresh water. The demonstration of this process in sea water is made difficult by the large amount of salts initially present, but in view of all the indirect evidence, as set forth above, pointing to the extrarenal excretion of salts in sea water, we consider its demonstration in fresh water to be of equivalent value. It may be remarked here that it is just those salts—Na, K and Cl—that fail to appear in the urine in sea water that are excreted extrarenally in fresh water, and, conversely, those salts

which from other considerations we believe to be excreted by the kidneys—Ca, Mg, and SO₄ and PO₄—that are excreted in fresh water entirely by the renal route.

In these experiments with fresh water, a normal solution of various salts was injected into the intestine through the anus with a blunt hypodermic needle and the anus closed by a ligature. The salt solutions were made up with about 0.1 per cent phenol red so that any leakage of the salt solution past the anal ligature could be detected readily. A retention catheter with rubber bag attached was fastened in the urinary papilla and the fish washed thoroughly in tap water for 1 to 2 hours to remove any of the salt solution from the skin. The fish was then placed in 2 liters of distilled water, aer-

| TABLE 5 |
| Calculation of the fraction of water and chloride and the osmotic pressure in the extrarenal excretion, based on the total Mg excreted in one liter of urine and one liter of intestinal residue |

<table>
<thead>
<tr>
<th></th>
<th>Water, liters</th>
<th>Cl, mM</th>
<th>QTY IN URINE</th>
<th>QTY IN INTESTINE</th>
<th>RELATIVE VAP</th>
<th>PER CENT IN URINE</th>
<th>CL PER LITER</th>
<th>H₂O EXCRETED</th>
<th>CL PER LITER</th>
<th>H₂O INGESTED</th>
<th>D.P. RELATIVE TO SEA WATER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lophius, average:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Water, liters</td>
<td>5.75</td>
<td>1.0</td>
<td>1.0</td>
<td>3.75</td>
<td>65</td>
<td>390</td>
<td>275</td>
<td>1.42</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl, mM</td>
<td>1,580</td>
<td>178</td>
<td>41</td>
<td>1,461</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Anguilla, no. 26 only:</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Water, liters</td>
<td>4.85</td>
<td>1.0</td>
<td>1.0</td>
<td>2.85</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl, mM</td>
<td>2,220</td>
<td>76</td>
<td>76</td>
<td>2,068</td>
<td>93</td>
<td></td>
<td></td>
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<tr>
<td>Myoxocephalus, average:</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water, liters</td>
<td>3.62</td>
<td>1.0</td>
<td>1.0</td>
<td>1.62</td>
<td>45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cl, mM</td>
<td>1,828</td>
<td>171</td>
<td>125</td>
<td>1,732</td>
<td>95</td>
<td></td>
<td></td>
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</tbody>
</table>
ated by compressed air, for 10 to 56 hours. At the conclusion of the experiment the water was evaporated and made up to 100 cc. This concentrated solution was then tested for phenol red and all experiments in which this dye was present in the water were discarded, since the presence of the dye showed that some of the salt solution had leaked past the anal ligature. In the absence of dye from the concentrated water, the latter, along with the urine removed from the catheter bag, was analyzed by the usual methods, using from 1 to 3 cc. samples. The quantities of salts present in the water and urine, as determined by multiplying the volume of these solutions by the concentration of each salt in them, are given in table 6 as millimols $\times 10^{-3}$.

It will be noted that almost all the NaCl and KCl are excreted into the water around the fish, while Ca, Mg, SO$_4$ and PO$_4$ are excreted by way of the urine. The renally excreted SO$_4$ takes with it large amounts of Na into the urine, and Mg and Ca take with them large amounts of Cl. Several samples of slime, which is freely given off when the fish is held in a net, were analyzed and showed consistently that this slime is not the route of excretion of the above salts. It contains practically no Na, K, Cl or PO$_4$, a small quantity of Ca and Mg and, when fresh, a small quantity of SO$_4$. During bacterial fermentation and subsequent evaporation more SO$_4$ is split off, accounting for the consistent presence of small amounts of this substance in the water around the fish. (This bacterial production of SO$_4$ can be reduced to some extent by adding small quantities of boric acid to the water.) Nevertheless the quantity of SO$_4$ excreted in the urine after giving Na$_2$SO$_4$ is so large relative to the quantity in the water, that we conclude that SO$_4$, like Ca, Mg and PO$_4$, is excreted exclusively by the kidneys.

This direct demonstration that NaCl and KCl are excreted extrarenally when the fish is in fresh water is striking corroboration of the indirect evidence that these salts suffer a similar fate when the fish is in sea water. The fact that this extrarenal excretion occurs in fresh water as well as in sea water is an important one, for it indicates that this process is fundamentally common to fresh water as well as marine fishes, and probably constitutes the mechanism by which the composition of the blood is regulated in the former as well as in the latter.

_The site of extrarenal excretion._ The experiments of Bert (1871), Sumner (1905) and Scott (1913) show that the gills are the principal route by which osmotic changes are effected in fish placed in salt or fresh water. In apparent contradiction to this conclusion is the work of Portier and Duval (1922). These investigators found that scraping the slime from the skin of Anguilla resulted in rapid changes in osmotic pressure of the blood when this fish was placed in solutions of varying salinity. They draw the conclusion that the skin plays an important part in the water exchange, but their results, we believe, are open to another interpretation. Anguilla is a smooth skinned
fish lacking fully developed dermal scales, and by scraping the skin one
impairs, perhaps, the normal integumentary insulation and permits the
movement of water along the natural osmotic gradient to occur across the
skin. It may well be supposed that under these conditions the physiologi-
cal machinery normally operating to maintain the osmotic pressure of the
blood would be over-taxed. The fact that no dermal lymphatic system
exists in fishes, as in the Amphibia, supports the belief that the skin of the
former is largely a passive osmotic insulator. The facts that the
majority of fresh and salt water fishes are armoured with impervious scales,
and that this armoured condition was characteristic of the primitive ver-
tebrates, make it fairly certain that the skin was primarily entirely passive
in the regulation of water equilibrium, and that its participation is still
passive in those fishes in which, like Anguilla, the scales have been reduced
to subcuticular vestiges. The absence of salts in significant quantities from
the slime of this fish, as reported above, shows that the specialized dermal
glands are not involved in this problem, except in so far as they contribute
to the maintenance of the integumentary insulation.

The physiological problem of maintaining the osmotic pressure and salt
composition of the blood against the stress of fresh or salt water is one that
has been faced by the vertebrates since the time of their evolution. It is
clearly a major problem involving a significant amount of physiological
labor. It is to be expected that the tissues responsible for this task should
be in intimate relationship with the blood stream, and it is not improbable
that from the earliest history of aquatic animals, this task should go hand in
hand with the task of respiration. In this view, the enormous blood flow to
the gills with their extensive dispersion of blood internally and free exposure
to water externally, stands in marked contrast to any other integumentary
organ. These facts, supported by the experiments of Bert, Sumner and
Scott, lead us to believe that the gills constitute the tissues through which
osmotic regulation of the blood, by means of hypertonic salt secretion, is
effected.

Lastly, it may be remarked that from a chemical point of view it would
appear to be just as feasible for the animal to extract the water necessary
for the formation of urine directly from the sea water at the branchiae,
without going through the circuitous process of absorbing both water and
salts from the alimentary tract and then excreting the bulk of both by an
extrarenal route. So far as osmotic pressure is concerned the same amount
of work would apparently be involved in both methods, and in the former,
in addition to reducing the total salt handled, the organism could passively
exclude the Ca, Mg and SO₄ which, in the latter method, gain access to the
blood in consequence of the enormous intestinal concentration required and
which constitute an additional burden upon the kidneys. It cannot be
argued that the direct extraction of water from a salt solution is physiologi-
cally impossible, for this process apparently occurs in the cloaca of some reptiles and the birds, and in the tubules of the mammalian kidney. The answer to why the latter method prevails does not rest, apparently, upon any inherent advantage in the method, but rather upon the evolutionary history of the regulation of body fluid composition in the vertebrates. The fact that extrarenal excretion of salts occurs in fresh water as well as salt water indicates, as we have pointed out above, that the fundamental process of physiochemical regulation is qualitatively the same in fresh water and marine fish. If we think of the marine teleosts as having been derived from fresh water ancestors, then it seems possible that the direction of this cycle is determined by this fact; for the primitive fresh water forms were organized to hold salt in their blood in spite of the fresh water that bathed the gills, and the branchiae were thus oriented in the direction of being hypertonic to the external medium. The marine fish, in excreting a hypertonic solution through the gills, is continuing qualitatively in the mode of its fresh water ancestors, the only difference being an absolute elevation of the branchial osmotic level with respect to the blood.

Effects of marine habitat upon the kidney. It has been stated above that Anguilla when in fresh water does not swallow significant quantities of this water. Numerous observations on Cyprinus and Carrassius kept in fresh water tinctured with phenol red show that in most instances no water is swallowed within 24 to 48 hours. Ultimately the dye does appear in the gastro-intestinal tract, and becomes highly concentrated in the intestine by the absorption of water. But the fact that water is not swallowed in detectable quantities within the first 24 hours stands in marked contrast to the situation in marine fish which invariably "drink" within 2 to 3 hours after being placed in the tinctured sea water. In a series of experiments in which Anguilla was placed in diluted sea water for 24 hours to permit acclimatization, prior to the addition of phenol red, we found that water was ingested within the next 24 hours in solutions having the same freezing point as the blood, and in many instances in solutions somewhat more dilute. From these experiments we conclude that the cycle of water movement typical of the marine fish first becomes necessary at osmotic pressures (external) slightly below that of the blood.

In spite of the failure of fish in fresh water to ingest water, the urine flow is much larger than in marine fish. Thus we find urine flows (calculated as cc. per kgm. per day) ranging from 60 to 150 cc. in Anguilla, Cyprinus and Carrassius. Since this water is not absorbed from the gastro-intestinal tract it must enter the body of the fish either through the oral or esophageal membranes or through the gills, probably through the first.

In contrast to the large urine flows in fresh water fish, the volume of urine excreted per day is greatly reduced in marine fish. The following values (calculated as cc./kgm./day from the authors' data if not so expressed)
have been given by various investigators: Lophius, 20 to 30 cc., Scorpaena, 10 to 12 cc., Conger, 3 to 5 cc., (Burian, 1908); Lophius, 18 cc. Muraena, 5 cc. (Edwards and Condorelli, 1928); Lophius, 13 to 54 cc., Cryptacanthodes, 1.5 to 11 cc., Myoxocephalus, 3.2 to 57 cc., Opsanus, 0.6 to 9.4 cc., (Marshall, 1929b). We have obtained under conditions as nearly normal as possible, from 11 to 40 cc. in Myoxocephalus and 0.4 to 5 cc. in Anguilla. We regard these figures as probably all abnormally high, for the reason that we have found that introducing sea water into the intestine of Anguilla increases the urine flow, as compared with a control period, on an average 10 times, and may increase it 30 times. Unquestionably any increase in the quantity of water swallowed by the fish would have the same effect. Since a fish is always excited when it is handled or removed from water in a net, there is strong probability that removal from water leads to increased water ingestion and hence to increased urine flow. Grollman (1929) has noted

<table>
<thead>
<tr>
<th>SALT</th>
<th>HOURS</th>
<th>VOLUME, cc.</th>
<th>Na 10⁻³</th>
<th>K 10⁻³</th>
<th>Cl 10⁻³</th>
<th>Ca 10⁻³</th>
<th>Mg 10⁻³</th>
<th>SO₄ 10⁻³</th>
<th>PO₄ 10⁻³</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>56</td>
<td>Urine 41</td>
<td>41</td>
<td>287</td>
<td>308</td>
<td>24</td>
<td>24</td>
<td>119</td>
<td>97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water 2,000</td>
<td>1,530</td>
<td>420</td>
<td>1,950</td>
<td>B</td>
<td>84</td>
<td>(100)</td>
<td>Tr.</td>
</tr>
<tr>
<td>KCl</td>
<td>48</td>
<td>Urine 26</td>
<td>161</td>
<td>370</td>
<td>690</td>
<td>21</td>
<td>10</td>
<td>43</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water 2,000</td>
<td>2,120</td>
<td>1,900</td>
<td>2,360</td>
<td>B</td>
<td>27</td>
<td>(150)</td>
<td>Tr.</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>29</td>
<td>Urine 11</td>
<td>60</td>
<td>828</td>
<td>275</td>
<td>36</td>
<td>75</td>
<td>129</td>
<td>Tr.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water 2,000</td>
<td>183</td>
<td>600</td>
<td>B</td>
<td>24</td>
<td>(77)</td>
<td>Tr.</td>
<td></td>
</tr>
<tr>
<td>MgCl₂</td>
<td>30</td>
<td>Urine 30</td>
<td>510</td>
<td>51</td>
<td>2,600</td>
<td>89</td>
<td>1,500</td>
<td>273</td>
<td>250</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water 2,000</td>
<td>306</td>
<td>330</td>
<td>750</td>
<td>B</td>
<td>62</td>
<td>(62)</td>
<td>Tr.</td>
</tr>
<tr>
<td>Na₂SO₄</td>
<td>28</td>
<td>Urine 16</td>
<td>3,300</td>
<td>B</td>
<td>52</td>
<td>B</td>
<td>2.7</td>
<td>2.5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water 2,000</td>
<td>300</td>
<td>R</td>
<td>304</td>
<td>R</td>
<td>46</td>
<td>B</td>
<td>Tr.</td>
</tr>
<tr>
<td>Control</td>
<td>16</td>
<td>Urine 11</td>
<td>26</td>
<td>B</td>
<td>52</td>
<td>B</td>
<td>2.7</td>
<td>2.5</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Water 2,000</td>
<td>R</td>
<td>304</td>
<td>R</td>
<td>46</td>
<td>B</td>
<td>Tr.</td>
<td></td>
</tr>
<tr>
<td>Slime</td>
<td></td>
<td>B</td>
<td>17</td>
<td>B</td>
<td>15</td>
<td>17</td>
<td>25</td>
<td>Tr.</td>
<td></td>
</tr>
</tbody>
</table>

Edwards and Condorelli give much larger figures for Synnathus and Hippocampus, but since these fish are very small and since these investigators placed a ligature entirely around the body and subsequently collected the urine by cutting the bladder over a test tube, the figures must be set aside as questionable.
that the urinary N of Lophius urine obtained from fish freshly caught off
the Grand Banks is 2 to 3 times as great as in urines obtained from this fish
when kept in captivity (Marshall and Grafflin, 1928; Edwards and Con-
dorelli, 1928), indicating that in the latter case the fish are diuretic. It is
possible that starved fish swallow an excessive quantity of sea water, and
therefore have an excessive urine flow, as compared to fed fish. With these
facts in mind, we believe that the normal urine flow of marine fish should be
put at least as low as 5 per cent of that of fresh water fish, and possibly as
low as one per cent.  

This difference is attributable to the facts that in salt water, in addition
to the absence of any natural osmotic tendency for water to enter the body,
a large fraction of all the water absorbed from the alimentary tract is ex-
creted extrarenally. Furthermore, every liter of sea water handled by the
marine fish is procured at the expense of considerable osmotic work in the
extrarenal disposal of the contained salts, so we would expect the volume of
ingested sea water to be kept as low as possible for reasons of physiological
economy.

In the light of these facts it is significant that many fishes are known to
possess partly or completely agglomerular kidneys (Marshall, 1929a) and
that these agglomerular fish are all strictly marine. It seems probable that
this agglomerular condition is a consequence of alterations in water economy
incidental to the assumption of a marine habitat.

The portion of the kidney which has come, through a variety of evidence,
to be viewed as primarily involved in the excretion of water is the glomeru-
lus. Thus the modern theory of urine formation sponsored by Cushny
is based upon the primary filtration of colloid-free plasma through the capil-
lary tuft of the glomerulus, and the reabsorption of water and other constitu-
ents of this filtrate by the tubules. Though no incontrovertible evi-

4 At first sight it would appear that all the water excreted as urine in marine fish
might be accounted for as having a metabolic origin. Thus Magnus-Levy (quoted by
Rowntree, 1922) gives the following figures for metabolic water derived from 100 grams
/hr. as an average metabolic rate in active fish (Keastner and Plaut, 1924) and assum-
ing that 1 gram of fat requires 2019 cc. of O2 and 1 gram of protein requires 967 cc. of
O2 for combustion, one obtains from a pure fat diet 1.27 cc. of water, and from a pure
protein diet 1.02 cc. of water per kilo per day. To the above must be added 0.8 cc. of
water for each gram of body tissue degraded. These figures represent about the
minimal rate of urine excretion of Anguilla in sea water. But in spite of the apparent
adequacy of this explanation, there remain the facts that upwards of 50 cc. of urine
per kilo may be excreted by Anguilla, Myxocephalus and Lophius if intestinal
absorption is great, and that the Mg (and SO4) normally excreted in the urine of all
fish in which this has been analyzed requires the extrarenal excretion of an amount
of water equal to or several times as great as the urine itself. So that even under
conditions of minimum urine flow the metabolic water in starved fish can hardly play
a significant part in influencing the water economy as a whole.
dence is available to that effect, the volume of the urine is presumably
determined by the volume of the glomerular filtrate minus such water as
may be reabsorbed by the tubules.

Abundant evidence indicates that the early evolution of the vertebrates
occurred in fresh water, and that the marine fishes have migrated into the
ocean in comparatively recent times. (This evidence is in part reviewed
by Lull (1918) and other texts of biology and need not be detailed here.)
In this view the glomerular kidney may be considered to be the older
or fresh water type, and the degeneration of the glomeruli in marine fishes
may be viewed as a secondary specialization.

That the absence or degeneration of glomeruli is not attributable to the
extrarenal excretion of nitrogenous waste products is indicated by the fact
that in fresh water fish the glomeruli are fully developed, although the
greater portion of these substances are excreted by the gills (Smith, 1929).
Nor can the aglomerular condition be attributed to the extrarenal excretion
of salts. In fresh water fish, the renal salt excretion is primarily less than
in the marine fish because of a smaller salt intake, and since there is an
abundance of water available, these salts are excreted in very dilute solu-
tion. In the marine fish the salt excretion is greatly increased by virtue of
the salts which are constantly being absorbed from the intestinal tract, and,
moreover, a limited amount of water is available for their excretion. If the
glomeruli were related primarily to the excretion of salts, then one would
expect to find increased development of glomeruli, rather than reduction,
in marine fish, for it is upon them that the greatest burden of salt excretion
falls, both in respect to the absolute amounts of salts excreted and the
concentrations of them in the urine.

The remaining factor is the excretion of water. From evidence cited
above it appears that marine fish are faced with a permanent anuria relative
to their fresh water ancestors, and it seems probable to us that this fact is
responsible for the aglomerular condition.

If this interpretation is correct, aglomerular kidneys should never be
found in fresh water fish, or in fish which migrate for any length of time into
fresh water; and it may be supposed that it will be in those forms which
have been longest in the sea, and which are therefore most highly special-
ized in directions characteristic of a marine habitat, that the glomerular
degeneration will be most marked.

A study, similar to the above, of the salt and water equilibria in the
marine cyclostomes and elasmobranchs will be reported in the future.

SUMMARY

It is shown that marine teleosts swallow relatively large quantities of sea
water which subsequently undergoes absorption. Most of the ingested
water, Na, K and Cl are absorbed from the intestine leaving a residue
rich in Mg and SO₄ and approximately isotonic with the blood.
The salts of the urine consist primarily of Mg and SO₄, with only traces of K and small quantities of Na and Cl, the solution being isotonic or hypertonic to the blood.

It is shown that the intestinal Mg and SO₄ come from ingested sea water, and that the urinary Mg and SO₄ come from alimentary absorption of these substances.

Quantitative analysis of the composition of the ingested sea water, the intestinal residue and the urine lead to the following conclusions:

1. The osmotically dilute nature of the intestinal residue and urine elaborated from sea water shows that the marine fish is doing osmotic work at some point other than in the kidneys and gastro-intestinal tract.

2. The fact that Mg and SO₄ occur in greater concentrations in both the intestinal residue and the urine than in the ingested sea water from which these fluids are derived shows that part of the water absorbed from the alimentary tract is excreted by some route other than the kidneys.

3. The fact that relatively more Na, K and Cl are absorbed from the alimentary tract than Mg and SO₄, while less Na, K and Cl are excreted in the urine than Mg and SO₄ shows that a large part of the absorbed NaCl and KCl are being excreted by some route other than the kidneys.

By considering these three processes as identified with a single process, it is inferred that the marine fish excretes by some extrarenal route a solution of NaCl and KCl which is hypertonic to the ingested sea water and thus leaves part of the absorbed water free for the formation of the osmotically dilute urine and intestinal residue.

The extrarenal excretion of water is demonstrated in Anguilla and Myoxocephalus by the addition of phenol red to sea water and the quantitative, simultaneous determination of the quantity of water ingested and the quantity of urine excreted in a given time.

The extrarenal excretion of salts is demonstrated in the catadromous eel, Anguilla, when kept in fresh water. The greater part of the NaCl and KCl absorbed from the alimentary tract is excreted by the extrarenal route, while essentially all the Ca, Mg, SO₄ and PO₄ are excreted by the kidneys. In the absence of other salts, SO₄ takes Na into the urine with it, and Mg and Ca take Cl.

Reasons are given for believing that the extrarenal excretion of salts and water and the extrarenal performance of osmotic work are attributable to the gills.

The water for the formation of urine in fresh water fish is not absorbed from the stomach or intestine, but probably from the oral membranes.

The water cycle in marine fish is of such a nature as to reduce the quantity of water excreted as urine to a small fraction of that excreted in fresh water fish. It is suggested that this relative anuria is the principal causative factor in an evident tendency for the glomeruli to disappear in the kidneys of strictly marine teleosts.
METHODS. The above conclusions are based on analyses of urine and gastrointestinal fluids removed from fasted and fed fish under natural and experimental conditions supplemented by special experiments. All fluids were centrifuged to remove particulate matter, if present, prior to analysis.

Determinations of CO₂, Cl and freezing point were carried out directly upon the urine and intestinal fluids. CO₂ was determined by the manometric method of Van Slyke and Neill (1921) and Cl by the method of Van Slyke (1923). In the determination of Cl the nitric acid digest as well as the blanks and standards were routinely boiled for a minute or so over a microburner to expel all nitrous oxide fumes; they were then cooled under the tap and titrated at once. Midway of the titration 1 to 2 cc. of ether were added to cause the AgSCN precipitate to flocculate, the mixture was cooled if necessary and titration continued with vigorous shaking. With these precautions the end point is sharp and permanent.

A trichloracetic acid filtrate was prepared from the intestinal fluids of *Lophius* for the determination of PO₄ and SO₄; otherwise these substances were determined directly, SO₄ by Fiske's method (1921) after acidification to a pink color with methyl orange, or gravimetrically as BaSO₄ and PO₄ by Briggs' modification of the method of Bell and Doisy (1924).

Na, K, Ca and Mg were determined in redissolved aliquots after ashing with H₂SO₄, HNO₃ and superoxol. In the intestinal fluids from the fed *Lophus* the protein was first precipitated with trichloracetic acid, and the filtrate ashed. Otherwise the ashing was done directly. Na was determined by Kramer and Gittleman's method (1924), 0.5 cc. 10 per cent KOH being added prior to the addition of the pyroantimonate reagent to precipitate the Mg. K was determined by precipitation as the cobaltinitrite (Kramer and Tisdall, 1921) the reagent being prepared as described by these authors. When prepared by this method we find the reagent keeps indefinitely at room temperature, whereas the commercial sodium cobaltinitrite powder has invariably decomposed and otherwise proved untrustworthy in our experience. At least 4 hours were allowed for complete precipitation and where the K concentration is small, the mixture is allowed to stand overnight. The potassium cobaltinitrate precipitate was measured by diazotization (Briggs, 1923) which we find much simpler and more reliable than permanganate titration. Thirty minutes were allowed for the development of color after diazotization. In the earlier experiments Ca was determined by Clark and Collip's method (1925) and Mg was determined in the supernatant fluid by precipitation as MgNH₄PO₄ (Denis, 1922) and determination of the PO₄ (Briggs, 1924). Later, a modified method communicated to us by Dr. Kenneth Blanchard, was introduced. This method appears to precipitate the Ca more effectively and to make a cleaner separation of the Ca and Mg:

Three cubic centimeters of a solution consisting of 50 cc. N HCl, 1.66 grams oxalic acid and 13.3 grams ammonium oxalate made up to one liter with water are added to the unknown in a 15 cc. pyrex centrifuge tube. The mixture is heated in a boiling water bath for 30 minutes, cooled and 0.2 cc. 20 per cent sodium acetate are added. After one hour the Ca oxalate is centrifuged out and the supernatant fluid decanted into 30 cc. pyrex centrifuge tubes for the determination of Mg. The Ca oxalate is washed twice with 2 cc. of 0.5 per cent ammonia solution, the first washing being added to the Mg. The Ca oxalate is then titrated with 0.02 or 0.01 M KMnO₄ solution after the addition of 2 cc. N H₂SO₄. The Mg is precipitated from the combined supernatant fluid and first washing from the Ca as MgNH₄PO₄. The solutions are heated in the water bath for 10 minutes and 1 cc. of 4 per cent (NH₄)₂HPO₄ and 2 cc. of concentrated ammonia are added. After standing overnight the precipitated phosphate is centrifuged out, the supernatant fluid decanted, the precipitate washed three times
with 50 per cent ammonia solution and dissolved in a known volume of 0.1 N HCl. Phosphate is determined in an aliquot of this solution by Briggs' (1924) method.

Both Mg methods were checked in many cases by weighing the MgNH₄PO₄ obtained from duplicate samples. Freezing points were determined with a Beckmann thermometer in an apparatus designed to work with 1 to 3 cc. of fluid. The accuracy of the method was checked against NaCl solutions of known composition. The figures given are uncorrected for under-cooling, which may be excessive in these fluids, and an allowance of ±0.02°C. should be made for error from this source.

In the analyses of the fluids from Lophius 1 cc. or larger samples were used, except for CO₂ which was determined in a 0.2 cc. sample measured with a Van Slyke-Ostwald pipette. In most of the analyses of the fluids from Anguilla and Myxocephalus it was necessary to use 0.2 cc. samples, measured with standardized wash-out pipettes.

For experimental work we have used the marine sculpin, Myxocephalus octodecimspinus, the catadromous eel, Anguilla rostrata, the fresh water carp, Cyprinus carpio, and gold fish, Carassius auratas. The sculpin experiments were done at Salisbury Cove with fish caught off the laboratory wharf by hook and line; the eel, carp and goldfish experiments were done at New York with fish purchased in the local markets and kept in running fresh water or aerated tanks of salt water in the laboratory. The eels were placed in fresh water for a day or more after bringing them into the laboratory and then transferred to salt water in which they were kept for 5 to 10 days, to acclimatize them before use. The eel is a fairly hardy fish and stands transfer from fresh to salt water well. It may be noted that this fish migrates to mid-ocean to spawn, and therefore a salt water habitat is not unnatural for it. It is admirably suited to experimental work, and there is no reason to believe that results obtained upon it are not generally applicable to other marine or fresh water fish. It may be remarked, however, that the mortality in experimental work of this kind is very great, and because of the inherent difficulties involved, a larger fraction of well planned experiments may be defeated by mechanical failures than is generally the case.

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